

**DATA ANALYSIS OF COMMON DYSLIPIDEMIAS FOR BETTER
CLINICAL INTERPRETATION, CALCULATIONS OF
REFERENCE OR CUT-OFF LEVELS AND
RELATIVE PREVALENCE**



Thesis submitted to
UNIVERSITY OF CALICUT
For the degree of
DOCTOR OF PHILOSOPHY IN BIOCHEMISTRY
(Faculty of Science)

By
SHAIMA C. M.

under the guidance of
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Declaration

I, here by declare that thesis entitled “DATA ANALYSIS OF COMMON DYSLIPIDEMIAS FOR BETTER CLINICAL INTERPRETATION, CALCULATIONS OF REFERENCE OR CUT-OFF LEVELS AND RELATIVE PREVALENCE” is based on original work done by me under the guidance of Dr. Jose Jacob, Professor at Amala cancer research centre, Thrissur for the partial fulfilment for the award of Doctor of Philosophy in Biochemistry. No part of this work has formed the basis for the award of any degree. The contents of the thesis are undergone plagiarism check using iThenticate software at C.H.M.K. Library, University of Calicut, and the similarity index found within the permissible limit. I also declare that the thesis is free from AI generated contents.

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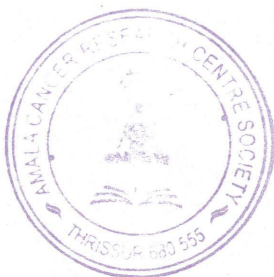
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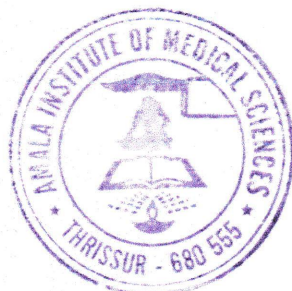
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Protocol Title	Data analysis of common dyslipidemias for better clinical interpretation, calculation of reference or cut-off levels and relative prevalence.
Principal Investigator	Shaima Rashid, Amala Institute of Medical Sciences, Thrissur
Guide	Dr. Jose Jacob, Professor and Head, Dept. of Biochemistry, Amala Institute of Medical Sciences, Thrissur
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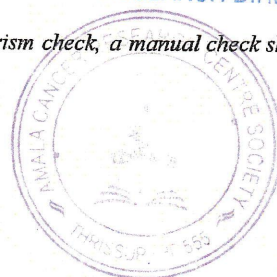
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This is to certify that Ms Shaima CM (U.O.No. 11899/2023/Admn) has completed her PhD research work under my guidance at Amala Cancer Research Centre, Thrissur. She has made the required corrections in the thesis entitled "DATA ANALYSIS OF COMMON DYSLIPIDEMIAS FOR BETTER CLINICAL INTERPRETATION, CALCULATIONS OF REFERENCE OR CUT-OFF LEVELS AND RELATIVE PREVALENCE" according to the suggestions and corrections pointed out by both the adjudicators.

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ABSTRACT

Dyslipidemias are characterized by abnormal concentrations of cholesterol and triglycerides in plasma lipoproteins, typically resulting from disorders in lipoprotein metabolism. In this study, we are collecting clinical biochemistry data related to dyslipidemia from hospital. The data are analyzed and interpreted clinically by dividing them into gender and age-specific groups. This partitioning helps determine **(a) proportion of patients with abnormal lipid profile, (b) gender-based differences, (c) reference intervals, and (d) the distribution patterns** among the various subgroups.

Lipid profiles were categorized into normal, isolated increases or decreases, and combined increases or decreases of four lipid components: serum triglycerides, total cholesterol, LDL-cholesterol, and HDL- Cholesterol. Our analysis includes assessing correlations, distributions, and variance to establish reference levels and the relative prevalence of common dyslipidemias observed in this hospital population.

Data analyses of **15,603 lipid profile samples** were done. Out of total 15,603 samples; there were 97 male samples and 86 female samples <18 years of age. There were 369 samples between age group 18 – 25 years. Out of which, there were 210 male samples and 159 female samples. The number of samples between 26 – 50 years was 5168. Out of which, there were 3247 male samples and 1921 female samples in the age group 26 – 50 years. The number of samples in age group >50 years was 9883. Out of which, there were 5925 male samples and 3958 female samples. Approximately 84% of general population in age group between 26 – 50 years, covered in this study have abnormalities in at least one of the lipid profile parameters analyzed. Most prevalent dyslipidemia is Isolated HDL- C decrease in males and females of all age groups except 26 – 50 years. In 26 – 50 years males, the most prevalent dyslipidemia is Isolated Serum triglyceride increase. But in females, Combined LDL-C and Total Cholesterol increase is the most prevalent dyslipidemia. Triglycerides >885mg/dL is the rare form of dyslipidemia. The proportion of patients with dyslipidemia is highest in males of 26 – 50 years. The healthiest lipid profile was observed in the age group females <18years.

The study employed statistical and graphical methods, including **Histograms, Box-Whisker plots, and Normal Q-Q plots**, to analyze and classify lipid profile

parameters such as Total Cholesterol (TC), Serum Triglycerides (TG), HDL Cholesterol (HDL-C), and LDL Cholesterol (LDL-C). Histograms and Normal Q-Q plots revealed a positive skew in Total Cholesterol and Triglyceride distributions, and Box-Whisker plots helped identify outliers, which were further categorized into near and far outliers. These graphical tools were used not only for statistical interpretation but also to clinically differentiate common, multifactorial dyslipidemias from rare, severe monogenic forms.

Correlation analysis showed that triglycerides positively correlated with glucose, Total Cholesterol, LDL-C, TSH, and BMI in adult males. While in females, Triglycerides positively associated with liver enzymes AST and ALKP, possibly due to sex-related hormonal differences. Triglyceride was inversely correlated with HDL-C in both sexes. Total Cholesterol positively correlated with LDL-C. The **reference intervals** for various lipid profile analytes across different gender and age groups were analysed. Due to the positive skewness of the data, the 90% reference interval calculation was deemed the most appropriate. For example, In female individuals aged 18 to 25 years, the 90% reference interval values were as: **Total Cholesterol:** 119.83 mg/dL (lower limit) to 235.55 mg/dL (upper limit); **Serum Triglycerides:** 32.75 mg/dL to 170.67 mg/dL; **LDL Cholesterol:** 62.08 mg/dL to 149.35 mg/dL; **HDL Cholesterol:** 23.59 mg/dL and 61.23 mg/dL These values reflect the range within which 90% of the population in this subgroup is expected to fall.

We had collected second set of fasting, 2-hr post breakfast and 2-hr post lunch blood samples (n=312) from healthy individuals of various age groups. Samples were analysed by partitioning and by comparing fasting, 2-hr post breakfast and 2-hr post lunch lipid data. Regarding fasting versus postprandial measurements, Total Cholesterol showed no significant difference, there were Significant mean difference between fasting and post lunch Serum triglyceride in all gender-age groups. HDL-C showed no significant variation with meal timing, whereas LDL-C demonstrated significant changes in both sexes aged 26–50 years. The study underlines the importance of **age- and gender-specific reference ranges** and shows how graphical and statistical techniques can effectively classify and analyse lipid disorders.

Key words: Box-Whisker plots; Serum triglycerides; Indirect Prevalence; Dyslipidemia; Reference interval; outliers; Post prandial.

സംഗ്രഹം

പ്ലാസ്മ ലിപ്പോപ്രോട്ടീനുകളിലെ കൊളസ്ട്രോളിന്റെയും ട്രൈഗ്ലിസറൈഡുകളുടെയും അസാധാരണമായ സാന്ദ്രതയാണ് ഡിസ്ലിപിഡീമിയയുടെ സവിശേഷത, ഇത് സാധാരണയായി ലിപ്പോപ്രോട്ടീൻ മെറ്റബോളിസത്തിലെ തകരാറുകൾ മൂലമാണ് ഉണ്ടാകുന്നത്. ഈ പഠനത്തിൽ, ആശുപത്രി രേഖകളിൽ നിന്ന് ഡിസ്ലിപിഡീമിയയുമായി ബന്ധപ്പെട്ട ക്ലിനിക്കൽ ബയോകെമിസ്ട്രി ഡാറ്റ ഞങ്ങൾ ശേഖരിക്കുന്നു. ലിംഗഭേദം, പ്രായപരിധി എന്നിവയെ അടിസ്ഥാനമാക്കിയുള്ള ഗ്രൂപ്പുകളായി വിഭജിച്ച് ഡാറ്റ വിശകലനം ചെയ്യുകയും ക്ലിനിക്കലായി വ്യാഖ്യാനിക്കുകയും ചെയ്യുന്നു. ഈ വിഭജനം (എ) പരോക്ഷ വ്യാപനം, (ബി) ലിംഗഭേദാധിഷ്ഠിത വ്യത്യാസങ്ങൾ, (സി) റഫറൻസ് ഇടവേളകൾ, (ഡി) വിവിധ ഉപഗ്രൂപ്പുകൾക്കിടയിലുള്ള വിതരണ രീതികൾ എന്നിവ നിർണ്ണയിക്കാൻ സഹായിക്കുന്നു. സെറം ട്രൈഗ്ലിസറൈഡുകൾ, മൊത്തം കൊളസ്ട്രോൾ, എൽഡിഎൽ-കൊളസ്ട്രോൾ, എച്ച്ഡിഎൽ-കൊളസ്ട്രോൾ തുടങ്ങിയ നാല് ലിപിഡ് ഘടകങ്ങളെ സാധാരണ നില, ഒറ്റപ്പെട്ട വർദ്ധനവ് അല്ലെങ്കിൽ കുറവ്, സംയോജിത വർദ്ധനവ് അല്ലെങ്കിൽ കുറവ് എന്നിങ്ങനെ തരംതിരിച്ചു. റഫറൻസ് ലെവലുകളും, ഈ ആശുപത്രി ജനസംഖ്യയിൽ കാണപ്പെടുന്ന സാധാരണ ഡിസ്ലിപിഡീമിയകളുടെ ആപേക്ഷിക വ്യാപ്തിയും കണക്കാക്കാൻ വേരിയബിളുകളുടെ പരസ്പരബന്ധങ്ങൾ, വിതരണങ്ങൾ, വ്യതിയാനം എന്നിവ വിലയിരുത്തുന്നത് ഞങ്ങളുടെ വിശകലനത്തിൽ ഉൾപ്പെടുന്നു.

പതിനയ്യായിരത്തി അറുനൂറ്റി മൂന്ന് ലിപിഡ് പ്രൊഫൈൽ സാമ്പിളുകളുടെ ഡാറ്റ വിശകലനം നടത്തി. ആകെ 15603 സാമ്പിളുകളിൽ; 18 വയസ്സിന് താഴെയുള്ള 97 പുരുഷ സാമ്പിളുകളും 86 സ്ത്രീ സാമ്പിളുകളും ഉണ്ടായിരുന്നു. 18 നും 25 നും ഇടയിൽ പ്രായമുള്ള 369 സാമ്പിളുകൾ ഉണ്ടായിരുന്നു. അതിൽ 210 പുരുഷന്മാരുടെയും 159 സ്ത്രീകളുടെയും സാമ്പിളുകൾ ഉണ്ടായിരുന്നു. 26 നും 50 നും ഇടയിൽ പ്രായമുള്ള സാമ്പിളുകളുടെ എണ്ണം 5168 ആയിരുന്നു. ഇതിൽ 26 നും 50 നും ഇടയിൽ പ്രായമുള്ള 3247 പുരുഷന്മാരുടെയും 1921 സ്ത്രീകളുടെയും സാമ്പിളുകൾ ഉണ്ടായിരുന്നു. 50 വയസ്സിനു മുകളിലുള്ള സാമ്പിളുകളുടെ എണ്ണം 9883 ആയിരുന്നു. അതിൽ 5925 പുരുഷന്മാരുടെയും 3958 സ്ത്രീകളുടെയും സാമ്പിളുകൾ ഉണ്ടായിരുന്നു. ഈ പഠനത്തിൽ ഉൾപ്പെട്ട 26-നും 50-നും ഇടയിൽ പ്രായമുള്ളവരിൽ ഏകദേശം 84% സാധാരണക്കാരായ ആളുകൾക്ക് വിശകലനം ചെയ്ത ലിപിഡ് പ്രൊഫൈൽ പാരാമീറ്ററുകളിലൊന്നിലെങ്കിലും അപാകതകളുണ്ട്. 26-50 വയസ്സ് ഒഴികെയുള്ള എല്ലാ പ്രായത്തിലുമുള്ള പുരുഷന്മാരിലും സ്ത്രീകളിലും ഒറ്റപ്പെട്ട എച്ച്ഡിഎൽ- കൊളസ്ട്രോൾ കുറയുന്നതാണ് ഏറ്റവും സാധാരണമായ ഡിസ്ലിപിഡീമിയ.

ഇരുപത്തിയാറിനും അമ്പതിനും ഇടയിൽ പ്രായമുള്ള പുരുഷന്മാരിൽ, ഏറ്റവും സാധാരണമായ ഡിസ്ലിപിഡീമിയ ഐസൊലേറ്റഡ് ട്രിജി വർദ്ധനവാണ്. എന്നാൽ സ്ത്രീകളിൽ, സംയോജിത എൽഡിഎൽ-സിയും ടോട്ടൽ കൊളസ്ട്രോളും വർദ്ധിക്കുന്നതാണ് ഏറ്റവും സാധാരണമായ ഡിസ്ലിപിഡീമിയ. ട്രൈഗ്ലിസറൈഡുകൾ $>885\text{mg/dL}$ ആണ് അപൂർവമായി കാണപ്പെടുന്ന ഡിസ്ലിപിഡീമിയ. 26 നും 50 നും ഇടയിൽ പ്രായമുള്ള പുരുഷന്മാരിലാണ് ഡിസ്ലിപിഡീമിയയുടെ വ്യാപനം ഏറ്റവും കൂടുതലുള്ളത്. 18 വയസ്സിന് താഴെയുള്ള സ്ത്രീകളിലാണ് ഏറ്റവും ആരോഗ്യകരമായ ലിപിഡ് പ്രൊഫൈൽ കണ്ടെത്തിയത്. ടോട്ടൽ കൊളസ്ട്രോൾ (TC), സെറം ട്രൈഗ്ലിസറൈഡുകൾ (TG), HDL കൊളസ്ട്രോൾ (HDL-C), LDL കൊളസ്ട്രോൾ (LDL-C) തുടങ്ങിയ ലിപിഡ് പ്രൊഫൈൽ പാരാമീറ്ററുകൾ വിശകലനം ചെയ്യുന്നതിനും തരംതിരിക്കുന്നതിനും ഹിസ്റ്റോഗ്രാമുകൾ, ബോക്സ്-വിസ്കർ പ്ലോട്ടുകൾ, ക്യൂ-ക്യൂ പ്ലോട്ടുകൾ എന്നിവ ഉൾപ്പെടെയുള്ള സ്റ്റാറ്റിസ്റ്റിക്കൽ, ഗ്രാഫിക്കൽ രീതികൾ ഈ പഠനത്തിൽ ഉപയോഗിച്ചു. ഹിസ്റ്റോഗ്രാമുകളും ക്യൂ-ക്യൂ പ്ലോട്ടുകളും ടോട്ടൽ കൊളസ്ട്രോൾ, ട്രൈഗ്ലിസറൈഡ് വിതരണങ്ങളിൽ പോസിറ്റീവ് വ്യതിയാനം വെളിപ്പെടുത്തി, ബോക്സ്-വിസ്കർ പ്ലോട്ടുകൾ ഔട്ട്‌ലൈയറുകളെ തിരിച്ചറിയാൻ സഹായിച്ചു. ഈ ഗ്രാഫിക്കൽ ടൂളുകൾ സ്റ്റാറ്റിസ്റ്റിക്കൽ വ്യാഖ്യാനത്തിന് മാത്രമല്ല, അപൂർവവും കഠിനവുമായ മോണിറ്റോറിംഗ് രൂപങ്ങളിൽ നിന്ന്

സാധാരണ മൾട്ടിഫാക്ടോറിയൽ തരം ഡിസ്റ്റിപിഡെമിയയെ ക്ലിനിക്കലിയായി വേർതിരിക്കുന്നതിനും ഉപയോഗിച്ചു.

പ്രായപൂർത്തിയായ പുരുഷന്മാരിലെ ഗ്ലൂക്കോസ്, ടോട്ടൽ കൊളസ്ട്രോൾ, എൽഡിഎൽ-സി, ടിഎസ്എച്ച്, ബിഎംഐ എന്നിവയുമായി ട്രൈഗ്ലിസറൈഡുകൾ പോസിറ്റീവായി ബന്ധപ്പെട്ടിരിക്കുന്നുവെന്ന് പരസ്പര ബന്ധ വിശകലനം കാണിച്ചു. സ്ത്രീകളിൽ, ട്രൈഗ്ലിസറൈഡുകൾ കരൾ എൻസൈമുകളായ എഎസ്സി, എഎൽകെപി എന്നിവയുമായി ബന്ധപ്പെട്ടിരിക്കുന്നു, പുരുഷന്മാരിലും സ്ത്രീകളിലും ട്രൈഗ്ലിസറൈഡ് എച്ച്ഡിഎൽ-കൊളസ്ട്രോളുമായി വിപരീതമായി ബന്ധപ്പെട്ടിരിക്കുന്നു.

വ്യത്യസ്ത ലിംഗഭേദത്തിലും പ്രായത്തിലും ഉള്ള വിവിധ ലിപിഡ് പ്രൊഫൈൽ അനലിറ്റുകളുടെ റഫറൻസ് ഇടവേളകൾ വിശകലനം ചെയ്തു. ഡാറ്റയുടെ പോസിറ്റീവ് ചാപല്യം കാരണം, 90% റഫറൻസ് ഇടവേള കണക്കുകൂട്ടൽ ഏറ്റവും അനുയോജ്യമാണെന്ന് കണക്കാക്കപ്പെട്ടു. ഉദാഹരണത്തിന്, 18 മുതൽ 25 വയസ്സ് വരെ പ്രായമുള്ള സ്ത്രീകളിൽ, 90% റഫറൻസ് ഇടവേള മൂല്യങ്ങൾ ഇപ്രകാരമായിരുന്നു: ആകെ കൊളസ്ട്രോൾ: 119.83 mg/dL (താഴ്ന്ന പരിധി) മുതൽ 235.55 mg/dL (ഉയർന്ന പരിധി); സെറം ട്രൈഗ്ലിസറൈഡുകൾ: 32.75 mg/dL മുതൽ 170.67 mg/dL വരെ; LDL കൊളസ്ട്രോൾ: 62.08 mg/dL മുതൽ 149.35 mg/dL വരെ; HDL കൊളസ്ട്രോൾ: 23.59 mg/dL മുതൽ 61.23 mg/dL വരെ. ഈ ഉപഗ്രൂപ്പിലെ ജനസംഖ്യയുടെ 90% പേരും ഏത് പരിധിക്കുള്ളിലാണ് വരുന്നതെന്ന് ഈ മൂല്യങ്ങൾ പ്രതിഫലിപ്പിക്കുന്നു.

വിവിധ പ്രായത്തിലുള്ള ആരോഗ്യമുള്ള വ്യക്തികളിൽ നിന്ന് ഞങ്ങൾ രണ്ടാമത്തെ സെറ്റ് ഉപവാസ രക്ത സാമ്പിളുകൾ, പ്രഭാതഭക്ഷണത്തിന് 2 മണിക്കൂർ ശേഷമുള്ള രക്ത സാമ്പിളുകൾ, ഉച്ചഭക്ഷണത്തിന് 2 മണിക്കൂർ ശേഷമുള്ള രക്ത സാമ്പിളുകൾ (n=312) ശേഖരിച്ചു.

ഉപവാസ ലിപിഡ് പ്രൊഫൈൽ ഡാറ്റ, 2 മണിക്കൂർ പോസ്റ്റ് ബ്രേക്ക്ഫാസ്റ്റ് ലിപിഡ് പ്രൊഫൈൽ ഡാറ്റ, 2 മണിക്കൂർ പോസ്റ്റ് ലഞ്ച് ലിപിഡ് ഡാറ്റ എന്നിവ തമ്മിലുള്ള വ്യത്യാസം ലിപിഡ് പ്രൊഫൈൽ അനലിറ്റുകളുടെ ശരാശരി വ്യത്യാസങ്ങൾ വിശകലനം ചെയ്യുന്നതിനായി, സാമ്പിളുകൾ ലിംഗ-പ്രായ വിഭാഗങ്ങളായി വിഭജിച്ച് വിശകലനം ചെയ്തു. ഉപവാസവും ഭക്ഷണത്തിനു ശേഷമുള്ള അളവുകളും സംബന്ധിച്ച്, മൊത്തം കൊളസ്ട്രോൾ മൂല്യങ്ങളിൽ കാര്യമായ വ്യത്യാസമൊന്നും കാണിച്ചില്ല. എന്നാൽ, എല്ലാ ലിംഗ-പ്രായ വിഭാഗങ്ങളിലും ഉപവാസത്തിനും ഉച്ചഭക്ഷണത്തിനും ശേഷമുള്ള ട്രൈഗ്ലിസറൈഡ് സാന്ദ്രതയ്ക്കും ഗണ്യമായ ശരാശരി വ്യത്യാസമുണ്ടായിരുന്നു. പ്രായ-ലിംഗ-നിർദ്ദിഷ്ട റഫറൻസ് ശ്രേണികളുടെ പ്രാധാന്യം ഈ പഠനം അടിവറയിടുന്നു, കൂടാതെ ഗ്രാഫിക്കൽ, സ്റ്റാറ്റിസ്റ്റിക്കൽ ടെക്നിക്കുകൾക്ക് എങ്ങനെ ലിപിഡ് ഡിസോർഡറുകളെ ഫലപ്രദമായി തരംതിരിക്കാനും വിശകലനം ചെയ്യാനും കഴിയുമെന്ന് കാണിക്കുന്നു

പ്രധാന വാക്കുകൾ: ബോക്സ്-വിസ്കർ പ്ലോട്ടുകൾ; സെറം ട്രൈഗ്ലിസറൈഡുകൾ; പരോക്ഷ വ്യാപനം; ഡിസ്റ്റിപിഡെമിയ റഫറൻസ് ഇടവേള; ഔട്ട്ലയറുകൾ; പോസ്റ്റ് പ്രാൻഡിയൽ.

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ABBREVIATIONS

µg	: Microgram
µL	: Microlitre
ABCA1	: ATP-binding cassette transporter A1
ABCG5/G8	: ATP-binding cassette subfamily G member 5/8
ADP	: Adenosine Diphosphate
ALP	: Alkaline phosphatase
ALT	: Alanine transaminase
AMI	: Acute Myocardial Infarction
APO	: Apolipoprotein
AST	: Aspartate transaminase
ATP	: Adenosine triphosphate
BMI	: Body Mass Index
CAD	: Coronary artery disease
CETP	: Cholesteryl ester transfer protein
CoA	: Coenzyme A
COP	: Coat Protein
COVID	: Corona Virus Disease
COX	: Cyclooxygenase
CV	: coefficient of variation
CVD	: Cardiovascular disease
Da	: Dalton
DHAP	: Dihydroxyacetone phosphate
DNA	: Deoxyribonucleic acid
EDTA	: Ethylenediaminetetraacetic acid
ER	: Endoplasmic reticulum
f/h-	: Family history of heart disease
FADH ₂	: Flavin adenine dinucleotide (reduced)
FFA	: Free Fatty acid
g/mL	: Gram/millilitre
HDL-C	: High Density Lipoprotein Cholesterol
HMG-CoA	: 3-hydroxy-3-methylglutaryl CoA
hr	: hour
HsCRP	: High sensitive C-reactive protein

ICU	: Intensive Care Unit
IDL	: Intermediate density lipoprotein
IP	: Inpatient
IQR	: Inter Quartile Range
kJ	: Kilojoule
LCAT	: Lecithin-Cholesterol Acyltransferase
LDH	: Lactate dehydrogenase
LDL-C	: Low Density Lipoprotein Cholesterol
LDLR	: Low density lipoprotein receptor
LIPA	: Lipase A, Lysosomal acid type
LPL	: Lipoprotein lipase
LXRs	: Liver X Receptors
mg	: Milligram
mg/dL	: Milligram/deciliter
min	: Minutes
mL	: Millilitre
mmol/L	: Millimoles per liter
mTOR	: mammalian target of rapamycin expression
MTP	: Microsomal triglyceride transfer protein
NAD	: Nicotinamide adenine dinucleotide
NADH	: Nicotinamide adenine dinucleotide
NADPH	: Nicotinamide adenine dinucleotide phosphate
NADPH	: Nicotinamide adenine dinucleotide phosphate hydrogen
NPC1L1	: Niemann-Pick C1-Like1
NSAIDs	: Nonsteroidal anti-inflammatory drugs
OT	: Outpatient
PAF	: Platelet-activating factor
PCSK9	: Pro-protein convertase subtilisin/kexin type 9
pH	: Potential of Hydrogen
PLTP	: Plasma phospholipid transfer protein
PTA	: Phosphotungstic acid
Q-Q plot	: Quantile-Quantile Plot
RBCs	: Red blood cells
SPSS	: Statistical Package for Social Sciences
SRM	: Standard reference material
S-W	: Shapiro-Wilk

T2DM	:	Type 2 diabetes mellitus
TAG	:	Triacyl glycerol
TAG	:	Triacyl glycerol
TC	:	Total Cholesterol
TC	:	Total cholesterol
TCA	:	Tricarboxylic acid
TG	:	Triglycerides
TSH	:	Thyroid-Stimulating Hormone
VLCFAs	:	Very long-chain fatty acids
VLDL	:	Very Low Density Lipoprotein
VLDL	:	Very low- density lipoprotein
w/v	:	Weight/volume
WHO	:	World Health Organisation

**DEDICATED TO,
MY FAMILY ...**

CHAPTER 1

INTRODUCTION

1.1. Introduction

Dyslipidemias are diseases characterized with abnormal concentrations of cholesterol, cholesterol esters and triglycerides in plasma lipoproteins. Dyslipidemias are commonly caused by disorders in lipoprotein metabolism. Dyslipidemias can be due to **hyper- or hypo-lipoproteinemia**. Hyperlipidemias maybe monogenic or polygenic and has been redefined on the basis of recent genetic data. **Primary dyslipidemia** is caused due to genetic abnormality in genes involved in lipoprotein metabolism pathways.

Secondary dyslipidemias may be due to underlying conditions like stress, hypothyroidism, metabolic syndrome, Type 2 diabetes mellitus, obesity, insulin resistance, positive energy intake, high fat diet, alcoholism, polycystic ovary, sedentary life style, postmenopausal disorders, renal disease, drugs such as corticosteroids and estrogen or a combination of these conditions.

General objective

Data will be generated from dyslipidemia cases by clinical history, family history, clinical examination and Biochemical evaluation of serum and urine samples. Data generated will be statistically analyzed for their heterogeneity, associations, differences, distributions, variances and stratifications. The best possible fit of data generated will be done to come to the best possible diagnosis. The samples may be grouped to analyze the reference levels, cutoff levels and relative prevalence.

1.2. Review of Literature

The topics reviewed were as follows. 1. Lipids, 2. Lipoproteins, 3. Role of liver in lipid transport and metabolism, 4. Dyslipidemia, 5. Indirect prevalence of dyslipidemia, 6. Biostatistics, 7. Reference interval.

1.3. Organisation of the thesis

The thesis comprises of 16 chapters. Chapters two to four are Objectives, Review of literature, and Materials and method, respectively. Fulfilment of the objectives as results were described in chapters 5 to 16.

This study includes both univariate and multivariate analysis. In the univariate analysis, a single variable such as Total Cholesterol, triglyceride, LDL Cholesterol or HDL Cholesterol were analyzed for its distribution, central tendency and spread or

range (Chapters 5 to 12). Multivariate analysis in the study were done by a) comparing the distribution of two or more variables such as Total Cholesterol and LDL Cholesterol or triglyceride and HDL Cholesterol. b) by analysis of correlation both graphically and by correlation coefficient (Chapter 13 and Chapter 16).

Result chapter 5 to 13

5. Histogram-Based Sub-Classification of Serum Triglycerides and proportion of Hypertriglyceridemia Across Gender and Age Groups: A Cross-Sectional Study.

Introduction: ICMR-INDIA B study-2014 and NCEP-2001 guidelines recommended a healthy cutoff of serum triglyceride as <150 mg/dL. This may be considered as the lower cut-off levels. ESC/EAS Guidelines-2019 have proposed an upper cut-off between moderate hypertriglyceridemia and severe hypertriglyceridemia at 885 mg/dL. There are no studies on the cut-off between, and prevalence of moderate and severe hypertriglyceridemia for the Indian population. These requirements are addressed in this chapter

Objective: i) To sub-classify serum triglyceride concentration using histogram regions. ii) To calculate the percentage of hypertriglyceridemia from histogram regions, below and above the upper cut-off level, in the gender and age groups.

Materials and Methods: This was an Observational Cross-Sectional study of healthy and dyslipidemia conditions. Serum triglyceride data was collected from the Clinical Biochemistry Laboratory, Amala Institute of Medical Sciences from September 2018 to March 2022. Samples were partitioned according to gender, and age groups of <18, 18 to 25, 26 to 50, and >50 years. Triglycerides were assayed by enzymatic method. Sample distribution before and after outlier removal were represented by histogram, and analyzed by Shapiro-Wilk and Jarque-Bera tests. Gender differences were analyzed by Mann-Whitney U test using SPSS software.

Results: The sample number increased as age increased. In the histogram, sample number decreased sequentially from the healthy to positively skewed and to outlier region in all age groups. The p-value of distributions before removal of outliers were

<0.05 in most of the gender-age groups. Males had higher mean triglycerides with p-value of <0.001 to 0.029, and had higher prevalence of hypertriglyceridemia in all groups. Using histogram, it was possible to differentiate between the highly prevalent multifactorial moderate hypertriglyceridemia from rare severe single gene defects.

Conclusions: The study partitioned triglyceride concentrations using histogram into healthy, positively skewed, and an outlier region. Histogram of hypertriglyceridemia can be clinically used to differentiate between the highly prevalent, multifactorial, moderate hypertriglyceridemia from the rare, severe, monogenic hypertriglyceridemia.

6. Box-Whisker plot used for partitioning serum hypertriglyceridemia according to quartiles and fences to identify gender and age-related differences and similarities – A Cross-Sectional study

Introduction: The Box–Whisker plot is a method of distribution of data series to give a graphical illustration of the dispersion, central tendency, and direction of skewness and the presence of outliers. Earlier we had used histogram (Chapter 5) as a method of sub classifying triglyceride concentration after partitioning the sample according to gender and age. The triglyceride concentration in each of the gender /age group was analyzed using Box-Whisker plot.

Objective: To evaluate the distribution, gender disparities, and the proportion of hypertriglyceridemia in relation to median, quartiles and fences using Box-Whisker plots

Methods: This observational cross-sectional study evaluated serum triglyceride concentrations in individuals with and without dyslipidemia. Samples were collected from the Clinical Biochemistry Laboratory through indirect sampling between September 2018 and March 2022. The large dataset enabled stratification by gender, followed by age groups (<18, 18–25, 26–50, and >50 years), and further division into statistical quartiles and outlier regions using Box-Whisker plots. Continuous multi-week sampling minimized selection bias across gender, age, and patient type (IP/OP). Triglyceride levels were measured using an enzymatic method on a dry chemistry

autoanalyzer. Data analysis was performed in SPSS, employing Shapiro-Wilk, Jarque-Bera, Mann-Whitney U tests, and Box-Whisker visualizations.

Results: Box-Whisker plot of serum triglyceride concentration in males and females of all four age groups showed positive skewing and confirmed by Shapiro-Wilk test. The compression of Box-Whisker plot was relieved by removal of outliers in repeated cycles. Number of samples collected rose with increasing patient age. In each distribution plot, the sample number decreased as the concentration of triglyceride increased. The least sample numbers were found in the outlier region. After removal of outliers, samples >18 years still showed positive skewing in all the groups indicating large number of moderate hypertriglyceridemia, especially in the male sample. Males showed higher triglyceride concentrations in the three groups up to 50 years. But in the age group >50 years the gender differences were low due to decrease in triglycerides in males and increase in females. The triglyceride concentration ranges in different quartiles were higher in males than in females in almost all age groups. The high triglyceride concentration far outlier sample percentage was higher in the male sample of all age groups.

Conclusions: A statistical and clinical method of classification of triglyceride concentration with quartiles, fences and outliers of Box-Whisker plot was proposed. Outliers were sub partitioned into near and far outliers. The concentrations greater than upper whiskers (a_3) are cases of hypertriglyceridemia. Box Whisker Plot of hypertriglyceridemia can be clinically used to differentiate between highly prevalent, multifactorial, moderate hypertriglyceridemia from the rare, severe, monogenic hypertriglyceridemia.

7. Histogram-Based Sub-Classification of Total Cholesterol and proportion of Hypercholesterolemia across Gender and Age Groups: A Cross-Sectional Study

Introduction: Family history of heart disease, metabolic syndrome, carotid and femoral plaque, coronary calcium score are some of cause leading to atherosclerotic CVD disease. There is a need for early intervention and management of dyslipidemia in the Indian population. In this study, lipid profile data was collected from Clinical

Biochemistry Laboratory and samples were partitioned according to age and gender. In each of the partitioned age and gender group, the Total Cholesterol distribution, percentage and number of samples in healthy, positively skewed and outlier regions of histogram were studied.

Objectives: To investigate the distribution of Total Cholesterol levels based on histogram, determine cutoff level between moderate and severe hypercholesterolemia and analyze the proportion of individuals with these dyslipidemic conditions in different age and gender groups.

Methods: This study, observational and cross-sectional in design, investigated Total cholesterol profiles under normal and dyslipidemic conditions at Amala Institute of Medical Sciences via indirect sampling. Biochemical assays were conducted using the Ortho Clinical Diagnostics Vitros 5.1 FS autoanalyzer. Cholesterol distribution was statistically examined using the Shapiro-Wilk and Jarque-Bera tests and visually assessed using histograms and Normal Q-Q plots. Gender-related differences were studied by means of student's t-test (for Gaussian distributed data) or the Mann-Whitney U test (for non-Gaussian data). All statistical computations were performed using SPSS software.

Results: Male and female samples of Total cholesterol of all age groups were positively skewed as seen in histogram, and deviating from normal distribution as seen by Shapiro-Wilk test. The Normal Q-Q plot and histogram also showed presence of outliers. The sample number increased sharply as age increased in males and females. As concentration of cholesterol increased, the sample number decreased sharply in the histogram from healthy region to positively skewed region and then to outlier region. There was no gender differences in the mean of total cholesterol observed in lower age groups before and after removal of outliers. But there were gender differences in the older age groups before and after removal of outliers with higher mean total cholesterol in the female sample (177.34 ± 48.27).

Conclusions: Histogram of fasting Total cholesterol samples were found to be positively skewed with the presence of outliers, indicated higher prevalence of hypercholesterolemia. Positive skewed region was contributed by moderate

hypercholesterolemia which was of common occurrence, and outlier region is contributed by highly increased cholesterolemia which was of rare occurrence and contributed by genetic abnormalities. Outliers were removed in repeated steps to identify and find the total number of outliers, and for graphically appreciating the positively skewed distribution pattern.

8. Box-Whisker plot used for partitioning serum hypercholesterolemia according to quartiles and fences to identify gender and age-related differences and similarities – A Cross-Sectional study.

Introduction: Total Cholesterol is an important biomarker for assessing cardiovascular risk. Understanding the distribution of Total Cholesterol levels in a population is essential for developing effective prevention and treatment strategies. In this study, Lipid profile data was collected from Clinical Biochemistry Laboratory and samples were partitioned according to age and gender. In each of the partitioned age and gender group, the Total Cholesterol distribution, percentage and number of samples in different regions of Box-Whisker plot were studied.

Objective: To evaluate the distribution, gender disparities, and the proportion of patients with hypercholesterolemia in relation to median, quartiles and fences using Box-Whisker plots

Methods: This cross-sectional observational study, based on indirect sampling, evaluated total cholesterol levels in both normal and dyslipidemic states at Amala Institute of Medical Sciences. The analyses were performed using the Vitros 5.1 FS autoanalyzer from Ortho Clinical Diagnostics. Distribution of cholesterol values was examined using Box-Whisker plots. Gender differences in lipid concentrations were tested by Student t-test or Mann-Whitney U test. Statistical analysis was conducted with SPSS software.

Results: Male and female samples of Total cholesterol of all age groups were positively skewed and vertically compressed as seen in Box-Whisker plot. The Box-Whisker plot also showed presence of near and far outliers. In each of the eight gender age groups, there were 0 to 1 sample in the Lower Near outliers ($F_1 - f_1$) and the Lower Far outliers ($<F_1$) region in the two lower age groups and in the fourth group above

50 years of age in both males and females. In the age group 26 – 50 years these two regions had sample numbers between 0 and 6. The sample number increased sharply as age increased in males and females. There were no gender differences in the mean of Total cholesterol observed in age groups below 50 years before and after removal of outliers.

Conclusions: Box-Whisker plot of fasting total cholesterol samples were found to be positively skewed with the presence of outliers. A statistical and clinical method of classification of Total Cholesterol concentration with quartiles, fences and outliers of Box-Whisker plot was proposed. Outliers were sub partitioned into near and far outliers. The concentrations greater than upper whisker (a_3) are cases of hypercholesterolemia. The a_3 concentrations range was from 242 mg/dL to 311 mg/dL in the four age groups. Box Whisker Plot of hypercholesterolemia can be used to differentiate between prevalent, multifactorial states from the rare, monogenic forms of the condition.

9. Histogram-Based Sub-Classification of LDL Cholesterol and proportion of Hypercholesterolemia across Gender and Age Groups: A Cross-Sectional Study

Introduction: Low-Density Lipoprotein (LDL-C) has been a significant focus of research due to its important role in determining cardiovascular disease burden. The available data suggests that LDL cholesterol prevalence is a public health concern in the Indian population. In this study, Lipid profile data was collected from Clinical Biochemistry Laboratory and samples were partitioned according to age and gender. In each of the partitioned age and gender group, the LDL Cholesterol distribution, percentage and number of samples in healthy, positively skewed and outlier regions of histogram were studied.

Objective: To investigate the distribution of LDL Cholesterol based on histogram, determine cutoff level between moderate and severe hypercholesterolemia and analyze the proportion of individuals with these dyslipidemic conditions in different age and gender groups.

Methods: This study, conducted at the Amala Institute of Medical Sciences, used an observational cross-sectional design and indirect sampling to investigate LDL cholesterol in normal and dyslipidemic individuals. Laboratory assessments were completed with the Ortho Clinical Diagnostics Vitros 5.1 FS analyzer. Statistical distribution was examined using the Shapiro-Wilk and Jarque-Bera tests and visualized with histograms and Q-Q plots. Differences between male and female lipid levels were tested by student's t-test or the Mann-Whitney U test. Spearman's method was used for correlation analysis, and SPSS software facilitated all statistical computations.

Results: In both males and females, sample number increased with advancing age. In each of the gender age groups, maximum sample number was seen in the healthy region and it decreased in the positively skewed region and further decreased in the outlier region. The mean LDL value increased in both males and females up to 50 years of age. Above 50 years of age LDL-C value appears to decrease. There were no gender differences in mean LDL-C value before 18 years and in the age group between 18 – 25 years. But in the age group 26 – 50 and >50 years, showed strong gender differences with higher cholesterol in the female sample. The positive skewing is due to moderate increase in LDL-C and outliers are due to very high increase in LDL Cholesterol.

Conclusions: A statistical and clinical method of classification of LDL Cholesterol concentration using histogram was proposed. Graphical analysis by histogram and Normal Q-Q plot showed presence of positive skewing and was confirmed by Shapiro-Wilk and Jarque Bera test. The mean LDL Cholesterol values were higher in female sample. Log transformation could not transform the distribution to Gaussian even after removal of outliers in higher age groups. This is due to increased proportion of hypercholesterolemia in the higher age groups. Hormonal influence of LDL Cholesterol concentrations resulting in gender differences in individuals between 18 and 50 years of age is another area for future study.

10. Box-Whisker plot used for partitioning LDL Cholesterol according to quartiles and fences to identify gender and age-related differences and similarities – A Cross-Sectional study.

Introduction: Increase in LDL Cholesterol is a significant risk factor for CVD in Indians. In this study, fasting LDL Cholesterol samples was collected from Clinical Biochemistry Laboratory and samples were partitioned according to age and gender. In each of the partitioned age and gender group, the LDL Cholesterol distribution, percentage and number of samples in different regions of Box-Whisker plot were studied. Visualizing the distribution of LDL Cholesterol levels can provide insights in to the characteristics of data, such as central tendency, variability and outliers.

Objectives: To evaluate the LDL Cholesterol distribution, gender disparities, and the proportion of hypercholesterolemia in relation to median, quartiles and fences using Box-Whisker plots

Methods: This cross-sectional observational study focused on normal and dyslipidemic LDL cholesterol levels, using indirect sampling, and was carried out at Amala Institute of Medical Sciences. Lipid profiles were assayed using the Vitros 5.1 FS analyzer. Data analysis was conducted with SPSS software, and distribution characteristics were visualized through Box-Whisker plots. Statistical comparisons of lipid concentrations between genders were done by student t-test or Mann-Whitney U test.

Results: Box-Whisker plot showed Positive skewing and presence of outliers. Box-Whisker plot of LDL cholesterol revealed an increasing trend in outliers with advancing age in both males and females. Samples in the lower near outlier regions may be due to abetalipoproteinemia below 25 years of age after removal of outliers; LDL-C was normally distributed. No gender differences were observed after outlier removal in < 18 years. In 18 – 25 years, 26 – 50 years and >50 years, there were strong gender differences with mean LDL Cholesterol value higher in females. The gender differences in samples >25 years of age was contributed by high mean LDL-C in female samples.

Conclusions: A statistical and clinical method of classification of LDL Cholesterol concentration with quartiles, fences and outliers of Box-Whisker plot was proposed. Outliers were sub partitioned into near and far outliers. The concentrations greater than upper whisker (a_3) are cases of hypercholesterolemia. The a_3 concentrations range from 177mg/dL to 319 mg/dL in the four age groups. Box Whisker Plot of hypercholesterolemia can help clinicians distinguish between common, multifactorial, moderate hypercholesterolemia and rare, severe, monogenic forms.

11. Histogram-Based Sub-Classification of HDL Cholesterol and proportion of Hyper/Hypoalphalipoproteinemia Across Gender and Age Groups: A Cross-Sectional Study.

Introduction: HDL cholesterol has important function in maintaining cardiovascular health, by extracting excess cholesterol from the circulation and carrying it to the liver for excretion. In this study, Lipid profile data was collected from Clinical Biochemistry Laboratory and samples were partitioned according to age and gender. In each of the partitioned age and gender group, the HDL Cholesterol distribution, percentage and number of samples in healthy, positively skewed and outlier regions of histogram were studied.

Objective: To investigate the distribution of HDL Cholesterol based on histogram, and analyze the proportion of low, normal, and elevated HDL Cholesterol levels across different age and gender groups.

Methods: An observational cross-sectional study on HDL cholesterol in normal and dyslipidemic conditions was conducted at Amala Institute of Medical Sciences using indirect sampling. HDL levels were assayed using autoanalyzer. Distribution was assessed using Shapiro-Wilk and Jarque-Bera tests, and graphically represented with histograms and Normal Q-Q plots. Gender differences were analyzed by student t-test or Mann-Whitney U test based on data distribution. All statistical analysis were done by SPSS software.

Results: There was significant gender difference in all the four age groups with mean HDL Cholesterol values higher in females than in males. Graphical analysis by histogram and Normal Q-Q plot showed presence of positive skewing and was

confirmed by Shapiro-Wilk and Jarque Bera test. In each of the gender age groups maximum sample number was seen in the low HDL Cholesterol region and minimum number in the outlier region (High HDL Cholesterol region). In all partitioned groups (except 18 – 25 years), the Normal Q-Q plot of HDL Cholesterol distribution showed, and very large deviation. There was Increase in positive skewing in males and females above 25 years of age. There was horizontal compression in the samples due to positive skewing and presence of outliers. In samples of age group between 18 – 25 years, 26 – 50 years and samples of age >50 years there were gender differences with Mann Whitney U test $P = <0.001$ after removal of outliers.

Conclusions: A statistical and clinical method of classification of HDL Cholesterol concentration using histogram was proposed. The mean HDL Cholesterol values were higher in female sample. The higher mean cholesterol and higher 95% confidence interval of HDL-C in females is a prominent gender difference in HDL-C indicating the possibility of lower risk for atherosclerosis and myocardial infarction in females.

12. Box-Whisker plot used for partitioning HDL Cholesterol according to quartiles and fences to identify gender and age-related differences and similarities – A Cross-Sectional study.

Introduction: HDL cholesterol has importance in maintaining cholesterol homeostasis and preventing atherosclerotic vascular disease. In this study, Lipid profile data was collected from Clinical Biochemistry Laboratory and samples were partitioned according to age and gender. In each of the partitioned age and gender group, the HDL Cholesterol distribution, percentage and number of samples in healthy, positively skewed and outlier regions of Box-Whisker plot were studied.

Objectives: To evaluate the distribution, gender disparities, and the proportion of hypo/hyperalphalipoproteinaemia in relation to median, quartiles and fences using Box-Whisker plots.

Methods: An observational cross-sectional study of HDL cholesterol in normal and dyslipidemic states was carried out at Amala Institute of Medical Sciences via an

indirect sampling approach. Chemistry analyses were done by autoanalyzer. The Shapiro-Wilk and Jarque-Bera tests were applied to assess distribution, which was further visualized by Box-Whisker plots. Gender differences in lipid concentrations were tested by Mann-Whitney U test or Student's t-test. Statistical analyses were performed by Statistical Package for Social Sciences (SPSS).

Results: Graphical analysis by Box-Whisker plot showed presence of positive skewing with large number of outliers with high HDL Cholesterol concentration particularly in older age groups. In each of the gender age groups maximum sample number was seen in the low HDL Cholesterol region and the mean HDL Cholesterol values were higher in female sample. Very few outliers were observed in two lower age groups. Positive skewing could be detected in the male sample <18 years and in the female samples 26 – 50 years. Samples in Lower near outlier region may be due to the presence of hypoalphalipoproteinemia. The gender differences were weak in the group <18 years after removal of outliers. Strong gender difference was seen in >18 years with Mann –Whitney U test - $P = <0.001$, after removal of outliers.

Conclusions: Higher number of outliers in the older age groups can be attributed to a greater number of individuals with elevated HDL Cholesterol levels (which are not known to increase the risk for atherosclerosis). Elevated triglyceride can lead to the exchange of cholesterol ester for triglyceride between VLDL and HDL, potentially resulting in decreased HDL Cholesterol levels, which may be prevalent in populations with high rates of hypertriglyceridemia. As mean HDL-C is higher in females; there is decreased risk of myocardial infarction in females below 50 years of age. For detecting near and far outliers, Box-Whisker plots are more useful, whereas histograms are better suited for assessing the degree of positive skewness in the data.

13. Correlation analysis of lipid profile analytes with Thyroid Function, Liver and Renal Function, Glucose levels and Body Mass Index.

Introduction: Lipid profile data was collected from Clinical Biochemistry Laboratory and samples were partitioned according to age and gender. Correlation analysis of lipid profile measurands with glucose, TSH, liver function and kidney

function test parameters were done.

Objective: To investigate the correlation of Lipid profile measurands with other biochemical and physical parameters like Glucose, Liver and Renal Functions, Thyroid Function and BMI in different age and gender groups.

Methods: This is an observational cross-sectional study of normal and dyslipidemia conditions at Amala Institute of Medical Sciences, utilizing an indirect sampling approach. Enzymatic dry chemistry assays were performed on the Ortho Clinical Diagnostics Vitros 5.1 FS autoanalyzer. Correlations were analyzed by Spearman's correlation for non-parametric data and Pearson's correlation for parametric data. Distribution characteristics were computed using SPSS.

Results: There is positive correlation of Triglycerides with fasting glucose in adult males and females. Triglycerides correlate positively with TSH in males of all age groups. Total Cholesterol correlates positively with TSH in males and females above 25 years. ALT positively correlated with Total Cholesterol, triglycerides and LDL-C in adult males. The positive correlation between AST, ALP and triglycerides in females but not in males may be attributed to sex-specific differences (estrogens) in lipid metabolism and liver enzyme activity. Triglycerides correlate positively with Total Cholesterol and negatively correlate with HDL-C in males and females of all age groups. There were Positive correlation between Triglyceride and BMI.

Conclusions: The correlation studies demonstrate significant relationship between biochemical parameters such as TSH, ALT, ALP and glucose with lipid profile analytes. These correlations highlight the interconnectedness of metabolic parameters and their association with cardiovascular risk. Comprehensive metabolic assessment and targeted interventions, including lifestyle modifications and pharmacological treatments are crucial for managing metabolic health and cardiovascular risk. Future research is needed to understand this relationship and develop effective management strategies, potentially enabling personalized medicine approaches that account for an individual's unique metabolic profile and treatment strategies.

14. Indirect Prevalence and Relative Prevalence of healthy and dyslipidemia

conditions in isolated and combined types of lipid profile

Introduction: Indirect prevalence of dyslipidemia is the percentage of dyslipidemia samples to the total sample presented at an institution for a particular duration of time.

Objective; To study the Indirect and Relative prevalence of dyslipidemia cases.

Methods: Lipid profile data was collected from Clinical Biochemistry Laboratory continuously for a fixed time period of time. Samples were partitioned according to age and gender. Indirect prevalence and relative prevalence of normal and dyslipidemia cases presented in hospital were studied. This is an Observational Cross-Sectional study of normal and dyslipidemia conditions presented at Amala Institute of Medical Sciences by **Indirect Sampling** method. Chemistry tests were done with Vitros 5.1 FS autoanalyzer from Ortho Clinical Diagnostics (OCD). Statistical Package for Social Sciences (SPSS) software was used for calculating distribution characteristics.

Results: Among all the adult individuals within different gender and age groups studied, dyslipidemia cases were more prevalent in males of the age group 26 – 50 years (83.71%). The females in age group <18 years were found to be the healthiest group without dyslipidemia (33.72%). The prominent dyslipidemia in the age group 18 – 25 years was isolated decrease in HDL Cholesterol with indirect prevalence of 30.82% in females. This is followed by isolated serum triglyceride increase with indirect prevalence of 14.76% in males. In the age group 26 – 50 years, the prominent lipid abnormality was combined LDL Cholesterol and Total Cholesterol increase (23.99% in females). With in age group >50 years, the highly prevalent lipid abnormality in the individuals was isolated HDL Cholesterol decrease (31.25% in males) followed by combined LDL Cholesterol and total cholesterol increase (17.79% in females). Among all dyslipidemia cases, the relative prevalence of isolated decrease in HDL Cholesterol was higher in all gender age groups. The relative prevalence of isolated HDL Cholesterol decrease was highest in females (45.61%) of age <18 years.

Conclusions: Most prevalent dyslipidemia is Isolated HDL- C decrease in males and females of all age groups except 26 – 50 years. In 26 – 50 years males, the most

prevalent dyslipidemia is Isolated TG increase. But in Females, Combined LDL-C and TC increase is the most prevalent dyslipidemia. Second Prevalent Dyslipidemia is isolated TG increase in males and females of <18 yrs, 26 – 50 yrs and in males of 18 – 25 yrs and >50 years. But females of age group 18 – 25 yrs and >50 years has combined TC and LDL-C increase as the second most prevalent dyslipidemia. Triglycerides >885mg/dL is the rare form of dyslipidemia. The prevalence of dyslipidemia is highest in males of 26 – 50 years. The healthiest lipid profile was observed in the age group females <18 years.

15. Reference interval calculation of lipid profile analytes

Introduction: A reference interval is set of values used to interpret laboratory results. It represents range of values expected for a healthy population, typically defined as central 95% of values from a reference population. Reference interval may vary by age, gender and population.

Objective: To calculate reference interval for lipid profile analytes in different age gender groups

Methods: lipid profile data was collected from clinical biochemistry laboratory and partitioned in to different age and gender groups. Samples having abnormal values or outliers for other diseases and for the lipid profile analytes were removed. The 90% and 95% reference interval was calculated for each individual lipid profile analyte in each age/ gender groups.

Results: The 90% Reference Interval for Total Cholesterol in adult males and females were 96 mg/dL – 246 mg/dL and 113mg/dL – 254mg/dL, respectively. The 90% Reference Interval for Serum Triglycerides in adult males and females were 55 mg/dL – 209 mg/dL and 48 mg/dL – 225 mg/dL, respectively. The 90% Reference Interval for LDL Cholesterol in adult males and females were 42 mg/dL – 169 mg/dL and 52 mg/dL – 180 mg/dL, respectively. The 90% Reference Interval for HDL-Cholesterol in adult males and females were 24 mg/dL – 56 mg/dL and 26 mg/dL – 64 mg/dL, respectively.

Conclusions: The 90% reference interval calculation was the most acceptable as data is positively skewed.

16. Distribution analysis and comparison of mean values of Fasting and Postprandial Lipid profile analytes.

Introduction: Family history, metabolic syndrome, carotid and femoral plaque, coronary calcium score are some of the risk factors for development of atherosclerotic cardiovascular disease. There is a need for early intervention and management of dyslipidemia in the Indian population. In this study, fasting and postprandial Lipid profile data was collected from apparently healthy individuals, distribution and mean value comparison of analytes were done.

Objective: To collect Fasting, 2-hour post breakfast, 2-hour post lunch blood samples and analyse for lipid profile data distribution. To Compare the means of Fasting and Postprandial lipid profile analytes

Methods: This study employed an observational cross-sectional design to investigate normal and dyslipidemic conditions in individuals at Amala Institute of Medical Sciences. Chemistry analyses were conducted using the Ortho Clinical Diagnostics (OCD) Vitros 5.1 FS autoanalyzer. Data distribution was analysed by Shapiro-Wilk and Jarque-Bera tests, alongside graphical methods including histograms, Normal Q-Q plots, and Box-Whisker plots. Gender differences and mean comparisons of lipid concentrations were analyzed by Student's t-test for parametric data or Mann-Whitney U test for non-parametric data. SPSS software was used to perform all statistical calculations.

Results: Total of 312 participants were there in the study. Fasting and Postprandial mean Total Cholesterol was higher in males of age group 18 – 25 and 26 – 50 yrs. But > 50 years it was higher in females. Fasting and Postprandial Mean Serum triglycerides were higher in males in all age groups. Fasting and Postprandial Mean HDL-C were higher in females of all age groups. No mean difference between fasting and post prandial Total Cholesterol values in all gender-age groups. Significant mean difference – between fasting and post breakfast TG in all gender - age groups (except female 18 – 25 yrs). There were Significant mean difference – between fasting and post lunch TG in all gender-age groups. No mean difference was observed between fasting and postprandial HDL Cholesterol values in all gender-age groups. There were

Significant mean difference between fasting and postprandial LDL Cholesterol values in both genders of age group 26 – 50 years.

Conclusions: There were significant mean differences between fasting and Postprandial Triglycerides in all gender - age groups.

CHAPTER 2

OBJECTIVES

2.1. Objectives of this study were,

1. Data will be generated from dyslipidemia cases by clinical history, family history, clinical examination and Biochemical evaluation of serum and urine samples.
2. Data generated will be statistically analyzed for their heterogeneity, associations, differences, distributions, variances and stratifications.
3. The best possible fit of data generated will be done to come to the best possible diagnosis. The samples may be grouped to analyze the reference levels, cutoff levels and relative prevalence.

CHAPTER 3
REVIEW OF LITERATURE

3.1. Lipids

Lipids are a diverse group of compounds found in body tissues. They are hydrophobic molecules that dissolve readily in organic solvents but are only sparingly soluble in water.

3.1.1. Lipids – functions: Lipids serve several crucial roles in living cells: (1) they act as metabolic regulators, such as hormones or hormone precursors; (2) they assist in the digestion and absorption of fat-soluble vitamins; (3) they provide a source of metabolic fuel and energy storage; (4) they form functional and structural components of biological membranes; (5) they function as electrical insulators in neurons; and (6) they cushion vital organs.

3.1.2. Lipids classification: Based on their chemical structure, lipids are broadly classified into five categories:

(1) Sterol derivatives, including cholesterol, cholesteryl esters, steroid hormones, bile acids, and vitamin D; (2) Fatty acids, which include short-chain (2–4 carbon atoms), medium-chain (6–10 carbon atoms), long-chain (12–26 carbon atoms), and prostaglandins; (3) Glycerol esters such as triglycerides, diglycerides, monoglycerides (acylglycerols), and phosphoglycerides; (4) Sphingosine derivatives like sphingomyelin and glycosphingolipids; and (5) Terpenes (isoprene polymers), including vitamins A, E, and K (Remaley et al., 2012).

3.1.2.1. Cholesterol

Cholesterol is the primary sterol found almost exclusively in animals. It plays a vital role in maintaining membrane permeability and fluidity, modulating transmembrane signaling pathways, and serving as a precursor for steroid hormones, bile acids, and vitamin D synthesis (Ikonen, 2008; Lingwood et al., 2010; Maxfield et al., 2010). Structurally, cholesterol is a high molecular weight solid alcohol with a tetracyclic perhydrocyclopentanophenanthrene ring system.

3.1.2.1.a. Cholesterol Absorption

Cholesterol is synthesized by most cells; however, the primary sites of cholesterol production are hepatocytes and enterocytes. The intestinal cholesterol pool is mainly influenced by endogenous sources, contributing approximately 800–1000 mg daily, whereas dietary intake accounts for only about 300–400 mg per day (Mok et al., 1979).

Cholesterol enters the intestinal lumen from three sources: dietary intake (primarily animal products), bile secretions, and the intestine itself through the turnover of mucosal cells. The duodenum and proximal jejunum are the principal sites of cholesterol absorption. Cholesterol gets absorbed in the stomach once dietary elements are exposed to enzymes produced in the mouth and stomach. The stomach also regulates the delivery of gastric chyme into the duodenum, where it mixes with bile and pancreatic secretions (Wang, 2003).

A critical step in intestinal lipid digestion—including triacylglycerols, phospholipids, and cholesterol esters—is their emulsification by bile salts (Mu et al., 2004; Phan et al., 2001; Iqbal et al., 2009; Pan et al., 2012). Due to their amphipathic nature, bile acids act as detergents and are essential for micelle formation. Emulsification enhances the substrates' accessibility for enzymatic hydrolysis by lipases present in the intestinal lumen. Almost all cholesterol present in the intestinal lumen exists as free (unesterified) cholesterol. Esterified cholesterol in the diet is rapidly hydrolyzed in the intestine to free cholesterol and free fatty acids by cholesterol esterases secreted from the small intestine and pancreas. Similarly, triglyceride hydrolysis yields free fatty acids (FFAs) and monoacylglycerols, while phospholipid hydrolysis produces FFAs and lysophospholipids. Enterocytes absorb monoacylglycerols and FFAs via diffusion and protein-mediated transport mechanisms (Iqbal et al., 2009; Pan et al., 2012; Abumrad et al., 2012).

For absorption, cholesterol which remains unesterified must be solubilized initially, which occurs by means of mixed micelles formation. Mixed micelles composed of fatty acids, conjugated bile acids, unesterified cholesterol, phospholipids, and monoglycerides. Mixed micelle formation facilitates cholesterol

absorption by solubilizing it and transporting it to the enterocyte surface, where active uptake is mediated by the NPC1L1 protein.

The presence of the ABCG5/G8 transporter on enterocytes restricts the uptake of cholesterol and plant sterols by promoting their active transport back into the gut lumen for excretion. Together, the regulatory actions of NPC1L1 and ABCG5/G8 critically control the amount of cholesterol reaching the lymphatic system from the intestinal lumen (Wang et al., 2017).

Once inside the intestinal mucosal cells, cholesterol undergoes esterification with fatty acids via the enzyme acyl-CoA: cholesterol acyltransferase 2, located in the endoplasmic reticulum membrane (Nguyen et al., 2012). The resulting cholesteryl esters, along with triglycerides, phospholipids, and specific apolipoproteins such as apo B-48, are further processed in the Golgi complex to form chylomicrons. These lipoproteins then enter the lymphatic system, draining into the thoracic duct and then into circulation.

The expression of NPC1L1 and ABCG5/G8 is regulated by cholesterol-sensing liver X receptor alpha and beta. Both receptors inhibit NPC1L1 expression and activate ABCG5/G8, promoting cholesterol excretion back into the intestinal lumen. Additionally, LXRs induce ABCA1-mediated cholesterol efflux to prevent excess cholesterol accumulation within enterocytes (Afonso et al., 2018).

3.1.2.1.b. Cholesterol Synthesis

Cellular cholesterol levels are dynamically regulated through de novo synthesis, dietary intake, storage, and elimination. Humans produce about 700–900 mg of cholesterol daily via endogenous synthesis, while dietary intake contributes roughly 300–500 mg. The liver accounts for about half of the body's cholesterol production. Both endogenous and dietary cholesterol are metabolized into bile acids at a rate of approximately 400 mg per day and into steroid hormones at around 50 mg per day; the remainder is excreted through feces and the skin (Russell, 1992).

Cholesterol is synthesized inside all tissues from acetyl-CoA. Approximately 90% of cholesterol synthesis takes place in the liver and intestines. The biosynthesis process occurs in three main stages. The first stage involves the formation of isopentenyl pyrophosphate from acetyl-CoA. This occurs in the cytoplasm, beginning with the synthesis of 3-hydroxy-3-methylglutaryl CoA from acetyl-CoA and acetoacetyl-CoA. Two acetyl-CoA molecules condense to form acetoacetyl-CoA catalyzed by cytosolic thiolase. Then, acetoacetyl-CoA condenses with another acetyl-CoA molecule, catalyzed by HMG-CoA synthase, to produce HMG-CoA (6 carbons). This intermediate is subsequently reduced to mevalonate by enzyme 3-hydroxy-3-methylglutaryl CoA reductase. Mevalonate is then phosphorylated sequentially by three ATP-dependent kinases, forming mevalonate 3-phosphate and 5-diphosphate. After decarboxylation and dephosphorylation, the active isoprenoid unit, isopentenyl diphosphate, is generated (Remaley et al., 2012).

The second stage involves the condensation of six isopentenyl pyrophosphate molecules to form squalene. It begins with isomerization of isopentenyl pyrophosphate to dimethylallyl pyrophosphate through a double bond shift. Isopentenyl pyrophosphate attacks an allylic carbocation formed from dimethylallyl pyrophosphate to produce geranyl pyrophosphate. This geranyl pyrophosphate is converted into an allylic carbocation, which is attacked by another isopentenyl pyrophosphate molecule to form the 15-carbon compound farnesyl pyrophosphate (Remaley et al., 2012). These condensation reactions are catalysed by the enzyme geranyl transferase. Following this, two farnesyl pyrophosphate molecules undergo a reductive tail-to-tail condensation catalyzed by squalene synthase in the endoplasmic reticulum, producing squalene.

Squalene is first converted into squalene epoxide (2,3-oxidosqualene) in an oxygen- and NADPH-dependent reaction. The 2,3-oxidosqualene is then cyclized to lanosterol by oxidosqualene cyclase. Lanosterol is further processed through multiple steps involving the removal of three methyl groups, reduction of one double bond by NADPH, and migration of another double bond, ultimately producing the 27-carbon cholesterol molecule (Berg et al., 2001).

3.1.2.1. c. Cholesterol Esterification

In the liver, cholesterol is incorporated into very low-density lipoproteins (VLDLs), which are then released into the bloodstream. Cholesterol undergoes esterification through a transesterification reaction, in which a fatty acid from the sn-2 region of phosphatidylcholine is transferred to free hydroxy group of cholesterol. This process is essential for enhancing the lipid-transporting ability of plasma lipoproteins and reducing the cellular toxicity caused by free cholesterol.

The enzyme lecithin: cholesterol acyltransferase (LCAT) plays a central role in this esterification process, converting free cholesterol into cholesteryl esters within plasma lipoproteins and playing a key part in high-density lipoprotein (HDL) metabolism. Within cells, a similar process is catalysed by acyl-CoA: cholesterol acyltransferase (Remaley et al., 2012).

LCAT is primarily formed in the liver and circulates in the plasma, where it temporarily associates with lipoproteins—mainly HDL. Apolipoprotein A-I (ApoA-I) is the most effective activator of LCAT, enabling the enzyme to convert free cholesterol into cholesteryl esters on the surface of HDL particles (kunnen et al., 2012).

3.1.2.1.d. Cholesterol Catabolism

Acid lipase in lysosome cleaves triglycerides and cholesteryl esters from lipoproteins taken into cells, producing free fatty acids and cholesterol. A mutation in the LIPA gene, which codes for this enzyme, causes these lipids to accumulate inside cells, leading to Wolman's disease.

In the liver, cholesterol can be excreted into bile, where it mixes with bile acids and phospholipids to form micelles. It may also be converted into bile acids or used in the synthesis of lipoproteins. About one-third of the body's cholesterol is converted daily into bile acids. Conversion of cholesterol to primary bile acids; cholic and chenodeoxycholic acids, involves shortening the side chain and adding hydroxyl groups to the steroid nucleus. The key regulatory step in this process is the hydroxylation at the 7-position, carried out by the enzyme 7 α -hydroxylase (Remaley et al., 2012).

Primary bile acids conjugate with taurine or glycine in liver to form more polar conjugated bile acids. Once secreted into the intestine, gut bacteria may deconjugate and further modify them, forming secondary bile acids. For example, cholic acid is changed to deoxycholic acid, and chenodeoxycholic acid becomes lithocholic acid. After being released into the intestinal tract, bile acids are reabsorbed in the ileum and recirculated to the liver. In the liver, secondary bile acids are reconstituted with glycine or taurine before being secreted again (Remaley et al., 2012).

3.1.2.2. Fatty Acids

Fatty acids are organic compounds consisting of a carboxyl group and a long hydrocarbon chain, represented chemically as RCOOH , where "R" denotes the alkyl chain. These molecules are amphipathic, meaning they contain both hydrophilic and hydrophobic regions. Based on the carbon atom number, they are categorized as short-chain (2–4 carbons), medium-chain (6–10 Carbons), or long-chain (12–26 Carbons), with the long-chain forms being most relevant to human nutrition and metabolism (Remaley et al., 2012).

Fatty acids are also classified by their saturation: saturated fatty acids which do not have double bonds, monounsaturated with one, and polyunsaturated with multiple double bonds. Carbon atoms in these chains can be numbered from the carboxyl end (Δ system) or the methyl end (ω or η system). Carbon next to the carboxyl group is the alpha (α) carbon, followed by the beta (β) carbon, while the terminal carbon is the omega (ω) carbon (Remaley et al., 2012). Saturated fatty acids, with no bends in their structure, can pack tightly together, giving them a high melting point and making them solid at room temperature. In contrast, unsaturated fatty acids occur in *cis* and *trans* forms depending on hydrogen positioning around the double bond. Naturally occurring unsaturated fatty acids in mammals are typically in the *cis* form, which introduces a bend in the chain, preventing tight packing and lowering the melting point—thus making them liquids at room temperature, such as in plant oils (Remaley et al., 2012).

Behenic acid, myristic acid, lignoceric acid, lauric acid, palmitic acid, arachidic acid, and stearic acid, are some of the saturated fatty acids. Palmitoleic acid, oleic acid, linoleic acid, α - linolenic (omega 3) acid, γ – linolenic acid, arachidonic acid, eicosapentaenoic acid (EPA) and docosahexaenoic (DHA) are examples of unsaturated fatty acids. (Gurr et al., 2002).

During catalytic partial hydrogenation, cis-unsaturated fats can convert to trans form, retaining unsaturation but adopting a more linear shape like saturated fats. This structural shift allows them to pack more tightly and remain solid at room temperature, raising their melting point. In physiological conditions (pH 7.0–7.4), fatty acids predominantly exist in their ionized form due to the carboxyl group's pKa of about 4.8. In the bloodstream, they are mainly found as esters with cholesterol or glycerol, or bound to proteins like albumin. Normal levels of free fatty acids in human plasma range from 0.30 to 1.10 mmol/L (8–31 mg/dL) (Remaley et al., 2012).

3.1.2.2.a. Fatty acid synthesis (lipogenesis)

Fatty acid synthesis primarily takes place in the liver, adipose tissue, kidneys, brain, and mammary glands. This biosynthetic process occurs in the cytoplasm and is catalysed by the fatty acid synthase enzyme complex. Acetyl-CoA, which originates in the mitochondria, is transported to the cytoplasm in the form of citrate via the tricarboxylic acid transporter in the mitochondrial membrane. Acetyl CoA carboxylase enzyme converts acetyl CoA to malonyl CoA. palmitic acid is synthesized from one acetyl CoA and seven malonyl CoA by fatty acid synthase enzyme complex (Vasudevan et al., 2011). This enzyme complex is a dimer composed of two identical subunits, with each subunit containing six functional enzymes—ketoacyl synthase, malonyl/acetyl transacylase, dehydratase, enoyl reductase, ketoacyl reductase, and thioesterase—along with an acyl carrier protein (ACP). The process of fatty acid synthesis (lipogenesis) requires cofactors such as NADPH, ATP, manganese ions (Mn^{2+}), biotin, and pantothenic acid. Insulin promotes lipogenesis, whereas glucagon acts as an inhibitor of the process (Vasudevan et al., 2011).

The formation of unsaturated fatty acids involves the enzymes desaturase and elongase, which are responsible for introducing double bonds and extending the

carbon chain length, respectively. Humans are capable of synthesizing saturated, monounsaturated, and certain polyunsaturated fatty acids from dietary amino acids. However, while humans possess $\Delta 4$, $\Delta 5$, $\Delta 6$, and $\Delta 9$ desaturase enzymes, they are unable to introduce double bonds beyond the ninth carbon atom from the carboxyl end (Botham et al., 2015). As a result, essential polyunsaturated fatty acids cannot be produced by the human body and must be obtained through the diet and are critical for maintaining proper health (Kaur et al., 2014).

3.1.2.2.b. Fatty Acid Catabolism

Fatty acid degradation in cells occurs through several oxidative pathways, namely alpha-, beta-, and omega-oxidation. Among these, beta-oxidation is the primary process for breaking down long-chain fatty acids in the mitochondria. In this pathway, two-carbon units are removed in sequential manner from the carboxyl end of the fatty acid molecule in the form of acetyl-CoA. Acetyl coA is oxidized to CO_2 and water via citric acid cycle. Transport of FADH_2 and NADH via the electron transport chain results in the synthesis of ATPs. When a 16-carbon fatty acid like palmitic acid undergoes beta-oxidation, it yields 8 acetyl-CoA molecules, 7 FADH_2 , and 7 NADH. The complete oxidation of one mole of palmitic acid generates approximately 106 ATP molecules, equating to about 3233 kJ of energy. Disruptions in fatty acid transport into mitochondria from the cytosol, or defects in beta-oxidation enzymes, can result in metabolic issues such as ketoacidosis, hypoglycemia, hyperammonemia, seizures, progressive neurological impairment, skeletal muscle weakness, and liver disorders (Vasudevan et al., 2011).

Very long-chain fatty acids (VLCFAs), typically those with 20 or more carbon atoms, first undergo partial beta-oxidation in peroxisomes before being shuttled to mitochondria for complete oxidation. A failure in this peroxisomal breakdown of VLCFAs leads to Zellweger syndrome. Meanwhile, odd-chain fatty acids are also oxidized in mitochondria, producing propionyl-CoA, which is transformed to D-methylmalonyl-CoA by propionyl-CoA carboxylase enzyme. This intermediate is then transformed into succinyl-CoA, which enters the TCA cycle and ultimately forms oxaloacetate, contributing to gluconeogenesis. In branched-chain fatty acids like

phytanic acid, the methyl group at the beta-carbon blocks beta-oxidation. These are broken down instead by alpha-oxidation within peroxisomes. Refsum's disease is a genetic disorder resulting from the absence of alpha-hydroxylase (phytanic acid oxidase), which is necessary for the alpha-oxidation of phytanic acid. Omega-oxidation, on the other hand, involves the oxidation of the terminal (omega) carbon through enzymes such as hydroxylases that require NADPH and cytochrome P450, and this pathway occurs in the endoplasmic reticulum.

3.1.2.2.c. Ketone body formation (ketogenesis)

During extended periods of starvation or in conditions where carbohydrate metabolism is severely disrupted, the body increases the breakdown of fat (lipolysis) and the oxidation of fatty acids. This leads to an excess production of acetyl-CoA. When acetyl-CoA accumulates beyond the available oxaloacetate, which is being redirected toward gluconeogenesis, it is instead funneled into an alternative mitochondrial pathway to generate ketone bodies: acetone, β -hydroxybutyric acid, and acetoacetic acid (Vasudevan et al., 2011).

Initially, two molecules of acetyl-CoA combine, under the action of acetoacetyl-CoA synthase, to form acetoacetyl-CoA. Another acetyl-CoA molecule then reacts with acetoacetyl-CoA to produce HMG-CoA. In the mitochondria, HMG-CoA is broken down by HMG-CoA lyase into acetoacetate and another acetyl-CoA molecule. Some of the acetoacetate is reduced to β -hydroxybutyrate, with the direction of this conversion influenced by the mitochondrial $[NAD^+]/[NADH]$ ratio. Additionally, a portion of acetoacetate spontaneously breaks down into carbon dioxide and acetone.

Ketone bodies act as an important energy source for extrahepatic tissues, particularly the heart and kidneys. Ketosis occurs when the synthesis of ketone bodies surpasses the capacity of peripheral tissues to utilize them, resulting in their accumulation in the bloodstream (ketonemia), excretion in the urine (ketonuria), and a characteristic acetone odor on the breath. Common triggers for ketosis include starvation and diabetes mellitus. If uncontrolled, ketosis can lead to metabolic acidosis, depletion of buffering capacity, deep labored breathing known as

Kussmaul's respiration, increased urine output (osmotic diuresis), sodium loss, dehydration, and even coma (Vasudevan et al., 2011).

3.1.2.3. Eicosanoids

Eicosanoids are bioactive lipid molecules that function similarly to local hormones, exerting their effects through autocrine or paracrine signaling. They are present in cells at very low concentrations and include compounds such as prostaglandins, thromboxanes, leukotrienes, and lipoxins—all of which are formed from arachidonic acid. Despite their minimal quantities, eicosanoids are highly potent and can trigger physiological responses at concentrations as low as 1 µg/L. Prostaglandins were first discovered in prostate secretions, though they are now known to be present in virtually all mammalian tissues and organs. Structurally, they contain a cyclopentane ring with a seven-carbon side chain at carbon 8 (R1) that extends below the ring and an eight-carbon chain at carbon 12 (R2) that extends above the ring. Their synthesis from arachidonic acid is catalyzed by cyclooxygenase (COX) enzymes, and this process is inhibited by NSAIDs (Ricciotti et al., 2011). Once produced, prostaglandins have a very short lifespan as they are rapidly broken down by enzymes such as 15 α -hydroxy-prostaglandin dehydrogenase and Δ 13-prostaglandin reductase.

One key prostaglandin, prostaglandin I₂ (also known as prostacyclin), is a potent vasodilator and inhibits the clumping of platelets. Thromboxane A₂, derived from platelets, promotes arterial contraction and encourages platelet aggregation. Leukotrienes, which are linear molecules with multiple conjugated double bonds, play critical roles in processes such as bronchiole constriction, smooth muscle contraction, leukocyte activation, and vasoconstriction. They are also key mediators of allergic and inflammatory responses. In contrast, lipoxins—tetraene structures synthesized by leukocytes—serve an anti-inflammatory function and work to dampen immune responses (Smith et al., 2008).

3.1.2.4. Glycerol Esters (Acylglycerols)

Acylglycerols are produced when fatty acids undergo an esterification

reaction with glycerol. The two main classes of glycerol esters are triacylglycerols and phosphoglycerides—the latter containing a phosphate group at the third (α') carbon of the glycerol backbone. Depending on how many fatty acid molecules are esterified to the hydroxyl groups of glycerol, the resulting compounds are grouped as monoacylglycerols, diacylglycerols, or triacylglycerols. If the fatty acid is attached to the first carbon atom of glycerol, the product is referred to as a 1-monoglyceride. Similarly, diglycerides can be either 1,2- or 1,3-diglycerides, depending on the positions of fatty acid attachment on the glycerol molecule (Remaley et al., 2012).

Triacylglycerols are the most abundant glycerol esters in the bloodstream and account for approximately 95% of stored fat in tissues. Triacylglycerols are stored in an anhydrous form, meaning they do not require water for hydration, and because they are highly reduced, they store more energy per gram than either carbohydrates or proteins. Triglycerides also have a low density, typically less than 1 g/mL. Plant-derived triglycerides, such as those from corn, sunflower, and safflower oils, are rich in cis-unsaturated fatty acids like linoleic acid. In contrast, triglycerides from animal sources are generally composed of saturated fatty acids. However, certain plant oils, such as coconut oil, are exceptions and contain high levels of saturated fats (Remaley et al., 2012).

Triacylglycerol biosynthesis: The liver and adipose tissue are the primary sites for triacylglycerol synthesis. Triacyl glycerol are synthesized by esterification of fatty acyl CoA with glycerol 3 phosphate. In the liver, glycerol-3-phosphate is derived directly from glycerol, while in adipose tissue, it is produced from dihydroxyacetone phosphate. Glycerol-3-phosphate is first converted into 1-acylglycerol-3-phosphate by the enzyme glycerol-3-phosphate acyltransferase, then into 1,2-diacylglycerol-3-phosphate by 1-acylglycerol-3-phosphate acyltransferase. This intermediate is subsequently acted upon by phosphatidate phosphatase and diacylglycerol acyltransferase to yield 1,2-diacylglycerol and ultimately triacylglycerol (Botham et al., 2015).

3.1.2.5. Phospholipids

Phospholipids are amphipathic molecules that serve as essential components of cellular membranes. They include two major types: glycerophospholipids and

sphingolipids. In glycerophospholipids, two fatty acids are joined to the C1 and C2 carbon atoms of glycerol 3 phosphate via ester bonds. Two fatty acid chains are attached to the first and second carbon atoms of glycerol-3-phosphate, typically through ester bonds, forming compounds like phosphatidic acid, cephalin, lecithin, cardiolipin, phosphatidylinositol, and phosphatidylserine. Alternatively, some variants such as plasmalogens and platelet-activating factor (PAF) have ether linkages. All glycerophospholipids are structurally derived from phosphatidic acid. Lecithin is the most abundant phospholipid in cell membranes, while cardiolipin is unique to mitochondria and is crucial for proper mitochondrial function (Botham et al., 2015).

Sphingolipids, on the other hand, are synthesized from the amino alcohol sphingosine. When a long-chain fatty acid binds to the amino group of sphingosine via an amide bond, it forms a ceramide. Adding a phosphocholine group to ceramide produces sphingomyelin, which is present in lipid rafts of cell membranes and the myelin sheaths that insulate nerve fibers. Glycosphingolipids result from the addition of polar sugar residues to the free hydroxyl group of ceramides. When a single sugar, such as glucose or galactose, is added, the compound is referred to as a cerebroside. Cerebroside is a glycosphingolipid located in membranes and is involved in cellular recognition, development, and signaling. Sulfatides are formed when a sulfate group is added at the 2-position of galactose in ceramide. If oligosaccharides are attached instead, gangliosides are formed. These complex molecules are abundant in the brain's gray matter and contribute to the organization of ion channels and paranodal structures in myelinated nerves. They also act as antigens and receptors, and their structure determines human blood groups (Hannun et al., 2018).

Both phospholipids and sphingolipids are associated with several medical conditions, including multiple sclerosis (characterized by nerve demyelination), infant respiratory distress syndrome, and a group of inherited disorders called sphingolipidoses. These lipid storage disorders, resulting from genetic enzyme deficiencies that impair the breakdown of sphingolipids, include Tay-Sachs disease, Fabry disease, Metachromatic leukodystrophy, Krabbe disease, Gaucher disease,

Niemann-Pick disease, and Farber disease (Merrill, 2008).

3.1.2.6. Terpenes

Polymers of the five-carbon isoprene unit termed as terpenes. Important terpenes in plant and animals include beta-carotene, limonene, squalene, lycopene and gibberellic acid. Terpenes form the backbone of fat-soluble vitamins.

3.1.3. Digestion and absorption of lipids

Triacylglycerols, cholesterol, and phospholipids represent the primary forms of dietary lipids. In the stomach, triacylglycerols containing short-chain fatty acids are partially broken down by gastric lipase—an enzyme stable at an acidic pH of around 5.4—resulting in the release of free fatty acids and diacylglycerols. Once in the small intestine, dietary lipids are emulsified into smaller droplets with the help of bile salts, phospholipids, and the mechanical action of peristalsis. The hydrophobic regions of bile salts insert into large fat globules and fragment them into smaller emulsified particles. Pancreatic lipase, along with its cofactor colipase, breaks down long-chain triacylglycerols to yield 2-monoacylglycerol. This intermediate is converted into 1-monoacylglycerol by monoacylglycerol isomerase, and further hydrolyzed to release glycerol and free fatty acids. Additionally, cholesterol esterase breaks down cholesterol esters into free cholesterol and fatty acids, while phospholipase A₂ hydrolyzes phospholipids into lysophospholipids and free fatty acids (Vasudevan et al., 2011). The major end products include, free fatty acids, 1-monoacylglycerol, glycerol, 2-monoacylglycerols, and cholesterol.

According to the Bergström theory, bile salts assist in the incorporation of lipid digestion products—including long-chain fatty acids, cholesterol, phospholipids, lysophospholipids, and monoacylglycerols—into organized spherical structures called mixed micelles. These micelles have a hydrophobic core and a hydrophilic outer surface, allowing them to facilitate the absorption of fat-soluble vitamins. Micelles move to the jejunal mucosa, where contents—such as fatty acids and monoacylglycerols—are absorbed into the intestinal cells via passive diffusion. Bile salts, are reabsorbed and transported back to the liver from ileum for reuse through

enterohepatic circulation. Inside the enterocytes, long-chain fatty acids and glycerol are re-esterified to form triacylglycerols. These newly formed triglycerides are then combined with cholesterol and apolipoprotein B-48 to assemble into chylomicrons, which are secreted into the lymphatic system via exocytosis (Satyanarayanan et al., 2016). In contrast, short-chain and medium-chain fatty acids bypass this process and are directly absorbed into the bloodstream without needing re-esterification.

3.2. Lipoproteins

Lipids circulate in the watery environment of plasma by forming large molecular complexes known as lipoproteins. These lipoproteins are spherical structures with a core made up of hydrophobic, nonpolar lipids such as triglycerides and cholesteryl esters. Surrounding this core is a surface monolayer composed of more polar or amphipathic lipids, including phospholipids and free cholesterol. Specific surface-associated proteins, termed apolipoproteins, are also present (Rifai, 1986).

In these lipoprotein particles, the outer monolayer consisting of phospholipids, cholesterol, and proteins interacts with the inner core lipids primarily through van der Waals forces and hydrogen bonding. These interactions are relatively weak, which enables the dynamic and spontaneous exchange of cholesterol among various lipoprotein classes and with cellular membranes.

A key protein involved in lipid exchange is cholesteryl ester transfer protein (CETP), which facilitates the reciprocal transfer of triglycerides and cholesteryl esters between different lipoproteins (Remaley et al., 2012). Another important lipid transfer protein is plasma phospholipid transfer protein (PLTP), which aids in transferring phospholipids from very low-density lipoproteins (VLDL) to high-density lipoproteins (HDL) (Cheung et al., 2002).

3.2.1 Lipoprotein classification

Lipoproteins are categorized based on their hydrated densities using ultracentrifugation, and include the following classes:

1. Chylomicrons with density of < 0.95 g/mL, a diameter greater than 70 nm, and a molecular weight ranging from 0.4 to 30×10^9 Da.
2. Very Low-Density Lipoproteins (VLDL): Density ranges from 0.95 to 1.006 g/mL, with diameters between 27 – 70 nm and molecular weights of 5 – 10×10^6 Da.
3. Intermediate-Density Lipoproteins (IDL): Density - 1.006 – 1.019 g/mL, a diameter of 22 – 24 nm, and a molecular weight of 3.9 – 4.8×10^6 Da.
4. Low-Density Lipoproteins (LDL): Their density lies between 1.019 – 1.063 g/mL, diameter ranges from 19 – 23 nm, and molecular weight is approximately 2.75×10^6 Da.
5. High-Density Lipoproteins (HDL): Density - 1.063 – 1.210 g/mL and a diameter of 4 – 10 nm, HDL particles have a molecular weight of 1.8 – 3.6×10^5 Da. HDL is further subdivided into two subclasses, HDL2 and HDL3, based on density.
6. Lipoprotein(a) [Lp(a)]: This class has a density between 1.040 – 1.130 g/mL, a diameter of 27 – 30 nm, and a molecular weight of 2.9 – 3.7×10^6 Da.

Lipoproteins can also be classified by their electrophoretic mobility. In this system, VLDL is referred to as pre- β -lipoprotein, LDL as β -lipoprotein, and HDL as α -lipoprotein. Using techniques like electrophoresis on agarose, cellulose acetate, paper, or polyacrylamide gels (Noble, 1968), lipoproteins can be separated based on their charge and size.

At an alkaline pH of 8.6:

- HDL moves with the α -globulin fraction,
- LDL moves with β -globulins,
- Lp(a) and VLDL occupy an intermediate position between α - and β -globulins, termed the pre- β -globulin region,

- IDL appears as a broad band between the β - and pre- β -globulin regions,
- Chylomicrons - at the origin (the point of application), showing no migration (Remaley et al., 2012)

3.2.2. Lipoproteins and their major lipid contents:

VLDL is the primary carrier of endogenous (internally synthesized) triglycerides, whereas chylomicrons are responsible for transporting exogenous (dietary) triglycerides. In a healthy fasting plasma sample collected in the morning, the majority of triglycerides are found within VLDL particles. However, following a meal (postprandial state), chylomicrons appear temporarily in the bloodstream and significantly contribute to the total plasma triglyceride levels.

LDL is the transporter of cholesterol in plasma, accounting for approximately 65–70% of the total cholesterol. High-density lipoprotein (HDL) carries about 20–30% of plasma cholesterol, while VLDL carries roughly 5–10%.

LDL particles contain minimal triglyceride content. It is estimated that about one-fifth of the triglyceride content (by weight) in VLDL corresponds to the amount of cholesterol in these particles. Based on this relationship, the Friedewald equation was developed to estimate total serum cholesterol (Remaley et al., 2012)

$$\text{Total Cholesterol} = \text{LDL-C} + \text{HDL-C} + (\text{Triglycerides} / 5)$$

3.2.3. Apolipoproteins

Apolipoproteins are the protein components of lipoprotein particles that associate with lipids. **Apolipoprotein A-I (apo A-I)** is the main protein constituent of high-density lipoproteins (HDL) (Frank et al., 2000). In chylomicrons, the apolipoprotein present is **apo B-48**, whereas **apo B-100** is found in very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), and low-density lipoproteins (LDL) (Packard et al., 1997). Apolipoproteins **C-I, C-II, C-III, and E** is present in various amounts across all lipoprotein classes except LDL.

3.2.3.1. Apolipoprotein A

Approximately 90% of HDL's total protein content consists of **apo A-I** and **apo A-II**, with a typical apo A-I to A-II ratio of about 3:1 (Breslow, 1995). **Apo A-I** serves as a cofactor for **lecithin-cholesterol acyltransferase (LCAT)**. It plays a key role in **reverse cholesterol transport**, facilitating the movement of cholesterol from cells to HDL particles (Frank et al., 2000). Studies shows **Apo A-II** may inhibit LCAT activity and activate **hepatic triglyceride lipase**. **Apo A-IV**, a component of newly secreted chylomicrons, has been shown in vitro to stimulate LCAT (Chen et al., 1985). **Apo A-V**, encoded by the **APOA5** gene on chromosome 11, is present in relatively small quantities and appears to regulate plasma triglyceride levels (Pennacchio et al., 2001).

3.2.3.2. Apolipoprotein B

Apo B is present on all atherogenic particles including **VLDL**, **IDL**, **large buoyant LDL**, and **sd-LDL**. Therefore, the plasma level of **apo B** serves as an indicator of the total number of atherogenic lipoproteins (Walldius et al., 2004).

Two isoforms of apo B exist in plasma: **apo B-100** and **apo B-48**.

- **Apo B-100** is the full-length version of the gene product, composed of over 4,536 amino acids. It is synthesized in the liver, assembled into VLDL particles, and secreted into circulation. During lipolysis, VLDL loses triglycerides and is gradually converted into LDL, retaining apo B-100 throughout this process. Each LDL particle contains one molecule of apo B-100, which functions as a ligand for **LDL receptors** in the liver and peripheral tissues, facilitating the uptake of LDL by cells (Javitt, 1995).
- **Apo B-48** is produced in the **small intestine** and is found exclusively in **chylomicrons**. It corresponds to the N-terminal portion of apo B-100 and is generated through a post-transcriptional editing. It consists of 2,152 amino acids.

3.2.3.3. Apolipoprotein C

Apolipoproteins C-I, C-II, and C-III are associated with **chylomicrons**,

VLDL, and HDL.

- **Apo C-I** has been shown to activate **LCAT** in vitro.
- **Apo C-II** plays a crucial role in lipid metabolism by activating **lipoprotein lipase (LPL)**, which helps hydrolyze triglycerides in chylomicrons and VLDL.
- **Apo C-III** inhibits LPL and thus reduces the breakdown of triglycerides (Remaley et al., 2012).

3.2.3.4. Apolipoprotein E

Apolipoprotein E - 34.5 kDa glycoprotein made of 299 amino acids. It is mainly synthesized in the **liver**, but other tissues like the **brain, kidney, adipose tissue, smooth muscle cells**, and **macrophages** also produce it. Apo E is present in **chylomicrons, VLDL, HDL**, and in **remnants** of chylomicrons and VLDL. As part of these lipoproteins, apo E acts as a ligand for receptors such as the **LDL receptor** and **LDL receptor-related protein**. It mediates the uptake and degradation of lipoproteins via **receptor-mediated endocytosis** (Li et al., 2014).

In HDL, apo E facilitates **cholesterol efflux** from **macrophage foam cells** in atherosclerotic lesions, contributing to **cholesterol homeostasis** in both plasma and tissues (Getz et al., 2018). Apo E is genetically **polymorphic**, with three common alleles: **ε2, ε3, and ε4**, which encode the isoforms **apo E2, E3, and E4** respectively (Seripa et al., 2007).

- **Apo E3** is the most prevalent form in the population.
- **Apo E2** is associated with elevated triglyceride levels and a higher risk for **type III dyslipoproteinemia**, a disorder linked to metabolic diseases (Smelt, 2003; Eto et al., 2013).
- **Apo E4** is linked to an increased risk of **cardiovascular disease** due to elevated **LDL cholesterol** levels (Bennet et al., 2007). Additionally, the **apo**

E4 allele has a strong association with **Alzheimer's disease** and other **neurological conditions** (Siest et al., 1995).

3.2.4. Lipoprotein Metabolism

Lipoprotein metabolism involves four major pathways: the exogenous pathway, endogenous pathway, the LDL receptor-mediated pathway, and the HDL reverse cholesterol transport pathway.

3.2.4.1. Exogenous Pathway

After dietary lipids are absorbed, nascent chylomicrons are formed in intestinal cells (enterocytes). Acyl-CoA synthetase activates Long chain fatty acids to form fatty acyl-CoA, which is then esterified with monoacylglycerols to produce triacylglycerols. In the rough endoplasmic reticulum, apo B-48 is synthesized and incorporated into nascent chylomicrons along with triglycerides, cholesterol, and phospholipids—lipid assembly primarily occurs in the smooth ER (Botham et al., 2015).

These nascent chylomicrons undergo glycosylation in the Golgi apparatus and are secreted into the extracellular space through reverse pinocytosis, entering circulation via the lymphatic system. They are rich in triglycerides (around 90% by mass), and contain apo B-48 and A-type apolipoproteins (about 2% by mass) (Gotto et al., 1986).

Once in circulation, chylomicrons acquire apo C and apo E from HDL. The presence of apo C-II and phospholipids on the chylomicron, activates lipoprotein lipase (LPL), which is located on the endothelium and anchored via heparan sulfate proteoglycans. LPL catalyses the breakdown of triglycerides into glycerol and free fatty acids. The fatty acids thus released are delivered to muscle cells, heart (as an energy source) or into adipose cells (for storage). During this process, some surface components—such as apo A, apo C, and phospholipids—are returned to HDL. The removal of 70–90% of triglycerides lead to the formation of chylomicron remnants, now enriched in cholesterol and cholesteryl esters (Botham et al., 2015).

Chylomicron remnants, which contain apo B-48 and apo E, are rapidly cleared by the liver through receptor-mediated endocytosis, primarily via apo E-dependent LDL receptors and LDL receptor-related protein-1. Hepatic lipase facilitates remnant uptake and hydrolyzes residual triglycerides. The cholesterol released may be used for bile acid synthesis, membrane incorporation, new lipoprotein formation, or stored as cholesteryl esters. In humans, chylomicrons are typically cleared from circulation within 1 hour (Botham et al., 2015).

3.2.4.2. Endogenous Pathway

In the liver, triglycerides and cholesterol synthesized from carbohydrates and fatty acids are encased along with apo B-100 into VLDL particles for secretion into the bloodstream. Apo B-100 is made on polyribosomes and lipidated with the help of microsomal triglyceride transfer protein (MTP), which assists in transferring triglycerides into the ER lumen to form VLDL2. This is then trafficked via COP II vesicles to the Golgi apparatus, where it merges with additional lipid-rich components to form VLDL1. These particles are then secreted into the space of Disse and pass through the fenestrated endothelium of hepatic sinusoids (Botham et al., 2015).

VLDL, comprising approximately 55% triglycerides, enters the circulation carrying apo B-100, minor quantities of C apolipoproteins, and apo E. Upon entering the bloodstream, it acquires additional C apolipoproteins from HDL (Remaley et al., 2012). Apo C-II activates LPL, which hydrolyzes VLDL triglycerides into free fatty acids. Compared to chylomicrons, VLDL binds less LPL and undergoes slower hydrolysis.

During this lipolysis, apo C proteins are returned to HDL, and VLDL remnants are formed. Some are taken up directly by the liver, while others convert into IDL (intermediate-density lipoproteins). About 50% of IDL is cleared by hepatic LDL (apo B-100/E) receptors. Surface lipids and apolipoproteins are transferred to HDL, and Cholesteryl esters are transferred from HDL to LDL by Cholesterol ester transport protein in exchange for triglyceride (Remaley et al., 2012)

IDL is further lipolyzed into LDL, which contains mostly cholesteryl esters and a single molecule of apo B-100. Most LDL is returned to the liver via LDL receptors. Excess LDL may infiltrate arterial walls, contributing to atherosclerosis (Remaley et al., 2012). LDL remains in circulation for approximately 3 days (Havel et al., 2001).

3.2.4.3. LDL Receptor Pathway

LDL receptors (LDLR) on liver cells bind LDL particles and remove them from plasma through **clathrin-mediated endocytosis**. Once internalized, the vesicles form **endosomes**; the acidic environment causes LDL and its receptor to separate. LDL receptors are recycled to the cell surface, and LDL undergoes **lysosomal degradation**, where **apo B-100** is broken down and **cholesteryl esters** are hydrolyzed to release **free cholesterol**.

When LDLR is bound to **PCSK9**, the complex is also internalized. However, due to increased binding affinity at acidic pH, this complex is sent to lysosomes for **degradation**, preventing receptor recycling (Wang et al., 2016; Wang et al., 2012; Park et al., 2014).

Outside the liver, about **30% of LDL** is sequestered by **extrahepatic tissues** via **non-receptor-mediated pinocytosis or scavenger receptors**. These processes are **unsaturable** and **not regulated**, particularly the scavenger receptor pathway, which is predominant in **macrophages**. These receptors recognize **oxidized LDL**, promoting foam cell formation and contributing to **atherosclerosis** (Remaley et al., 2012).

3.2.4.4. Reverse Cholesterol Transport Pathway

This pathway maintains cholesterol balance by carrying **excess cholesterol** from **peripheral tissues** to **liver** for excretion. HDL mediates this **anti-atherogenic process**.

The pathway starts with the synthesis and secretion of **lipid-poor apo A-I** by the intestine and liver. This protein associates with phospholipids to form **nascent pre- β HDL**. The transporter **ABCA1** facilitates the efflux of cholesterol and phospholipids to pre- β HDL, forming **discoidal HDL** enriched in **apo A-I** and free cholesterol.

LCAT, upon activation by **apo A-I**, esterifies free cholesterol by transferring fatty acids from phospholipids to cholesterol, producing **cholesteryl esters** and **lysophosphatidylcholine**. These esters migrate into the particle's core, forming spherical HDL.

Further cholesterol uptake from cells occurs via **ABCG1 transporters**, **SR-BI receptors**, and passive diffusion (Trajkoska et al., 2017). **HDL3** particles are formed and continue to accept cholesterol, eventually maturing into **HDL2**, which contains more cholesteryl esters.

Cholesteryl esters from HDL2 are transferred to **apo B-100-containing lipoproteins** (like LDL and VLDL) via **CETP**.

The **HDL cycle** involves interconversion between **HDL2** and **HDL3**. HDL2 is cleared by:

1. **Selective uptake of cholesteryl esters** through **SR-BI** in the steroidogenic tissues and liver.
2. **Hydrolysis of phospholipids and triglycerides** by **hepatic lipase** and **endothelial lipase**, returning HDL3 and free apo A-I to circulation.

3.3. Role of liver in lipid transport and metabolism

- a) Digestion and absorption of lipids is facilitated by production of bile within the liver.
- b) Fatty acid synthesis and its oxidation
- c) Synthesis of triacylglycerol and phospholipids
- d) Ketogenesis occurs in liver
- e) Synthesis and metabolism of lipoproteins: the synthesis of triacylglycerol and VLDL secretion in liver is enhanced by factors like fed state, feeding of high carbohydrate containing diet, high levels of circulating fatty acids as seen in Diabetes mellitus, ethanol intake, high insulin concentration and low glucagon levels (Botham et al., 2015).

Fatty liver: occurs when the rate of triacylglycerol synthesis surpasses its export in the form of VLDL. Nonalcoholic fatty liver disease is seen associated with diabetes and high fat diet intake. In uncontrolled diabetes and starvation there is excessive mobilization of fat and the capacity of liver to take up fatty acids from blood far exceeds its capacity for excretion as VLDL (Vasudevan et al., 2011; Schreuder et al., 2008).

The metabolic block in the production of lipoproteins also causes fatty liver. toxic injury to liver due to poisoning by compounds like arsenic, lead and viral infection can result in fatty liver. Deficiency of lipotropic factors like choline, lecithin, methionine, vitamin E, selenium, omega 3 fatty acids cause fatty liver. Alcoholic fatty liver is caused by alcoholism. Ethanol oxidation cause increase $[NADH]/[NAD]$ redox potential in liver which results in impaired fatty acid oxidation and increased lipogenesis. (Botham et al., 2015).

3.4. Dyslipidemia

Dyslipidemia are diseases with abnormal level of cholesterol and triglycerides in plasma lipoproteins and are commonly caused by lipoprotein metabolism disorders.

dyslipidemia can be hyper lipoproteinemia or hypo lipoproteinemia. Dyslipoproteinemia is diagnosed using plasma lipid and lipoprotein concentrations.

3.4.1. Primary versus Secondary dyslipidemia

Dyslipidemia is grouped into primary and secondary dyslipidemia. Primary dyslipidemias are caused by gene mutation and are genetically inherited. Primary dyslipidemias may be monogenic or polygenic and have been redefined on the basis of recent genetic data (Johansen et al., 2011; Johansen et al., 2011; Talmud et al., 2013). Secondary dyslipidemia is caused by unhealthy lifestyle factors, acquired medical conditions including underlying diseases and drug usage. (Yanai et al., 2021). Secondary dyslipidemias may be due to (1) endocrine and metabolic disorders : type 2 diabetes mellitus, hypothyroidism, hypopituitarism, lipodystrophy, insulin resistance, metabolic syndrome, polycystic ovary, stress (2) exogenous: drugs such as corticosteroids and estrogen, alcoholism, obesity , sedentary life style, post-menopausal stress, positive energy intake and high fat diet, oral contraceptives (3) renal diseases (4) Hepatic disorders (5) Storage disease (6) Acute and transient disorders: Burns, Hepatitis Acute trauma (surgery), Myocardial infarction, Bacterial and viral infections (7) Others Anorexia nervosa, Klinefelter's syndrome, Systemic lupus erythematosus, Idiopathic hypercalcemia, Progeria, Starvation, Werner's syndrome. (Remaley et al., 2012; Catapano et al., 2011; Reiner et al., 2011; Berglund et al., 2012; Yuan et al., 2007).

3.4.2. Dyslipoproteinemia classification

3.4.2.1. Fredrickson classification

Historically, Familial hyperlipidemias are classified by Dr D.S Fredrickson along with Robert Levy and Robert Lees. According to the **Fredrickson classification**, five phenotypes were described based on clinical features and pattern of lipoproteins on paper electrophoresis (Frederickson et al., 1965).

Table 3.1. Frederickson classification of dyslipidemia. (Wong et al., 2012).

Phenotype	Increased lipoprotein	Serum Total Cholesterol	Serum triglycerides	Plasma appearance
Type I	Chylomicrons	Normal to increased	increased	Creamy top layer
Type II a	LDL	increased	normal	clear
Type II b	LDL and VLDL	increased	increased	Clear/turbid
Type III	VLDL remnants and Chylomicrons	increased	increased	Clear/cloudy/milky
Type IV	VLDL	Normal to increased	increased	Clear/cloudy/milky
Type V	VLDL and chylomicrons	increased	increased	Creamy

(LDL- low density lipoprotein; VLDL very low-density lipoprotein)

3.4.2.2. Classification of hyperlipoproteinemia based on knowledge of concentrations of cholesterol and triglycerides (Table 3.2 and Table 3.3) (source: classification of hyperlipidemias and hyperlipoproteinemia, from www.ahajournals.org. WHO memorandum)

Table 3.2

Groups	Types	Cholesterol	Triglycerides
1 st group	Pure hypercholesterolemia/ hyper- β - lipoproteinemia.	High	Normal
2 nd group	Pure hyperchylomicronemia/ hyper pre- β -lipoproteinemia	Normal	High
3 rd group	Hyperlipoproteinemia (all major types)	High	High

Table 3.3. Dyslipidemia classification based on lipoproteins.

Type	Chylomicrons	LDL(β -lp)	VLDL (pre- β -lp)	Broad- β - Lipoproteins
Type I hyperchylomicronemia	High		Normal/slightly increased	
Type II a –hyper- β -lipoproteinemia		High	Normal	
Type II b –hyper- β -lipoproteinemia		High	High	
Type III- Broad- β -pattern				High
Type IV-Hyper pre- β -lipoproteinemia	Absent	normal	High	
Type-V-Hyper pre- β -lipoproteinemia	High		High	

3.4.2.3. Classification of hyperlipidemia based on examination of standing plasma electrophoresis. (Table 3.4)**Table 3.4**

Type	Standing plasma	Electrophoretic band appearance			
		Chylomicron band	α – HDL band	β – LDL band	Pre – β - VLDL band
Type I hyperchylomicronemia	Cream layer over clear infranatant layer	Heavy band present	Usually not visible	Usually not visible	Appear with diminished/ normal/ slightly increased intensity
Type II a –hyper- β -lipoproteinemia	Clear	Not visible	Normal band	Intensely stained band present	Absent/ if present, is of normal intensity
Type II b –hyper- β -lipoproteinemia	Clear or faintly turbid throughout	Not visible	Normal	Intensely stained band	Band - Increased in intensity
Type III- Broad- β -pattern	Usually turbid, frequently with a faint chylomicron “cream” layer	Faint band	Normal	On paper, agarose/ cellulo acetate, - Broad β band	On paper, agaroses or cellulose acetate a distinct pre- β band seen.
Type IV-Hyper pre- β -lipoproteinemia	Clear or turbid	Not visible	Normal or decreased	Normal or decreased	Band - Increased intensity
Type-V-Hyper pre- β -lipoproteinemia	Cream layer over turbid infranatant layer	Prominent band	Decreased	Decreased	Band is increased and trails to origin

3.4.2.4. Classification of hyperlipidemia based on Ultracentrifugation (Table 3.5)**Table 3.5.**

Type	Ultracentrifugation			
	Chylomicrons	HDL	LDL	VLDL
Type I Hyperchylomicronemia	Increased	Decreased	Decreased	Usually increased (as separation from chylomicron is incomplete)
Type II a –hyper- β -lipoproteinemia	Not increased	Normal	Increased	Normal
Type II b –hyper- β -lipoproteinemia	Not increased	Normal	Increased	Increased
Type III- Broad- β -pattern	Increased		Decreased (LDL- 1.010 – 1.019) Increased (LDL – 1.006 – 1.019)	Increased
Type IV-Hyper pre- β -lipoproteinemia	Normal	Normal or decreased	Normal or decreased	Increased
Type-V-Hyper pre- β -lipoproteinemia	Increased	Decreased	Decreased	Increased

3.4.2.5. Classification of dyslipidemia based on enzyme or protein defect in lipoprotein metabolic pathways

Lipoprotein disorders are grouped according to defects in the four metabolic pathways including exogenous, endogenous, LDL receptor mediated, and Reverse cholesterol pathway. Defects in these pathways can lead to (1) increased production of lipoproteins, (2) decrease in production or an increase in removal of lipoproteins, (3) defective cellular uptake of lipoproteins, (4) abnormal intravascular processing and eventually causing hyperlipidemia (Remaley et al., 2012)

3.4.2.5.a. Hyperlipoproteinemia: Dyslipidemia characterized by increased lipoprotein particles in blood (Table 3.6)**Table 3.6.** Hyperlipoproteinemia due to metabolic pathway defect

Hyperlipoproteinemia	Defect	Increased lipoprotein	Main symptoms Treatment	Genetic basis & Family history(f/h) Estimated Prevalence
Type I a familial hyperchylomicronemia or Buerger-Gruetz syndrome	Lipoprotein lipase (LPL) decrease	Chylomicron TAG >10 mmol/L (885 mg/dL)	Acute pancreatitis, hepatosplenomegaly lipemia retinalis, eruptive xanthomas,	Monogenic Autosomal recessive f/h-pancreatitis One in 1,000,000
Type I b Familial apoprotein C II deficiency	Altered ApoC2			
Type I c	LPL inhibitor in blood			
Type II a Familial hypercholesterolemia	LDL receptor deficiency	LDL TC > 200 mg/dL LDL > 130 mg/dL	Xanthelasma, increased risk for atherosclerosis, arcus senilis, tendon xanthomas Bile acid sequestrants, statins, niacin	Monogenic Autosomal codominant f/h –heart disease One in 500 (heterozygotes)
Type II b Familial combined hyperlipidemia	Decreased LDL receptor and increased ApoB	LDL and VLDL TC > 200 mg/dL TAG >10 mmol/L	Atherosclerosis and Coronary Heart Disease risk Statins, niacin, fibrate	Polygenic One in 100

Table 3.6. continuation

Hyperlipoproteinemia	Defect	Increased lipoprotein	Main symptoms Treatment	Genetic basis & Family history(f/h) Estimated Prevalence
Type III Familial dysbetalipo- proteinemia	Defect in Apo E 2 synthesis	IDL TC > 200 mg/dL TAG >10 mmol/L	Tubero eruptive xanthomas and palmar xanthomas Fibrate statins	Polygenic One in 10.000
Type IV Familial hypertriglyceridemia	Increased VLDL production and decreased elimination	VLDL TAG >10 mmol/L	Diabetes, obesity, can cause pancreatitis at high triglyceride levels Fibrate, niacin, statins	Polygenic f/h – type 2 diabetes, obesity, hypothyroidism One in 100
Type V Mixed hyperlipemia	Increased VLDL production and decreased LPL	VLDL and chylomicrons TC > 200 mg/dL TAG >10 mmol/L	Eruptive xanthomas, Lipemia retinalis, Pancreatitis, and abnormal glucose tolerance with Hyperinsulinism Niacin, fibrates	Polygenic
Hyperapobeta- lipoproteinemia	Overproduction of VLDL and apo B- 100	Increased LDL- apo B 100		

Table 3.6. continuation

Hyperlipoproteinemia	Defect	Increased lipoprotein	Main symptoms Treatment	Genetic basis & Family history(f/h) Estimated Prevalence
Familial Hypertriglyceridemia	The production of large VLDL with abnormally high triglyceride content	VLDL, LDL, apo B 100 –Normal in concentration.	Acute pancreatitis	Autosomal dominant 1:500 (common)
Familial Defective Apolipoprotein B-100	Mutation in apo B 100 gene and there by reduced affinity for LDL receptor	Increased LDL-C	Incidence of CHD	
Familial hyper alpha lipoproteinemia (Giammanco et al., 2021)	Mutation in CETP/HL/apo CIII, SR-B1 genes	Elevated HDL-C	Anti atherogenic beneficial	Polygenic
Familial lipoprotein (a) excess	LPA gene mutation	Lp (a) elevated Lp (a) > 50 mg/dL	Atherosclerosis, CHD, thrombosis	Polygenic

3.4.2.5.b. Hypolipoproteinemia: Dyslipidemia characterized by lower concentration of lipoprotein particles in blood. It may be caused by rare genetic conditions, due to underlying diseases or due to impaired adsorption or digestion of lipids from food.

- i) **Abetalipoproteinemia:** Defect - Mutation in MTP gene. Defect in chylomicron, VLDL assembly and transport, Autosomal recessive and characterized by Low/ absent LDL, VLDL. Main symptoms include Fat malabsorption, spinocerebellar degeneration, acanthocyte RBCs, and retinitis pigmentosa. (Syeda et al., 2022).
- ii) **Familial hypo beta lipoproteinemia:** Defect - Mutation in APOB gene, Autosomal dominant
- iii) **Familial alpha lipoprotein deficiency:** Characterized by Decreased HDL, patient exhibit increased risk of CAD.
- iv) **Hypoalphalipoproteinemia - Autosomal dominant**
 - a. **Tangier disease:** caused by Mutations in the ABCA1 gene on chromosome 9q3. It is characterized by severely reduced plasma HDL, abnormal HDL composition. There is accumulation of cholesteryl esters in tissues throughout the body symptoms include Hyperplastic orange tonsils, Splenomegaly, peripheral neuropathy (Remaley et al., 2012)
 - b. **Defect in synthesis of apo A1:** caused by Mutation such as rearrangement, deletion, insertion in apo protein gene locus. In Homozygotes, apo A1 is undetectable but in heterozygous condition, half normal concentration of serum HDL and apo A1 is present. Patients develop corneal clouding. There is increased risk for CHD (Assmann et al., 2001; Kwiterovich, 1990).
- v) **Fish eye disease or partial LCAT deficiency:** caused by Mutation of LCAT gene. Autosomal recessive disorder. There is Low serum level of HDL. Patients develop Corneal opacity

- vi) **Familial Lecithin cholesterol acyl transferase (LCAT) deficiency:** Mutation of LCAT gene on chromosome 16q22, Autosomal recessive disorder. It is characterized by Low serum level of HDL, and abnormalities in structure and composition of HDL, LDL particles. (Althaf et al., 2015).

3.5. Indirect prevalence of dyslipidemia

It is the percentage of dyslipidemia samples to the total sample presented at an institution/Hospital for a particular duration of time. Hospital based prevalence can be useful for understanding disease burden within the healthcare system or identifying trends in hospitalizations (Rothman et al., 2008; Szklo et al., 2019). In a study conducted by the ICMR-INDIAB, the percent prevalence and relative prevalence (n = 2042) for following conditions were: 1) Isolated and Combined Hypercholesterolemia (13.9% & 17.7%); 2) Isolated and Combined Hypertriglyceridemia (29.5%; 37.34%); 3) Isolated and Combined Low HDL Cholesterol (72.3%; 91.5%); 4) High LDL cholesterol (11.8%; 14.93%), respectively. The increase in prevalence of dyslipidemia cases is a major public health problem worldwide and it varies according to cultural, socioeconomic and ethnic characteristics.

3.6. Biostatistics

Biostatistical methods employed in this study are outlined and explained below (Reffenberg, 2012; Gurumani, 2005; Altman, 1991).

3.6.1. Measures of central tendency –Averages

It summarizes data by identifying a central position within the dataset. These metrics provide a concise overview and facilitate comparison across datasets. The primary types include:

3.6.1.1. Arithmetic mean

It is calculated by,

$$\text{Mean} = \frac{\text{sum of all the observations}}{\text{Total number of observations}}$$

$$X = \sum_{i=1}^n Xi/n$$

3.6.1.2. Median

It is the middle value of a data series when arranged in order:

$$\text{Median} = \frac{\text{value of the item } (n + 1)}{2}$$

3.6.1.3. Mode

In a dataset, if a value appears most frequently is called mode. A dataset may be unimodal, bimodal, or multimodal, and if no value repeats, it is considered amodal.

3.6.2. Measures of dispersion

A measure of dispersion quantifies the spread or variability in a dataset. The types used in this study are:

3.6.2.1. Range

The range is defined by the minimum and maximum values within the dataset. (Gurumani, 2005).

3.6.2.2. Standard deviation

It is the square root of the variance and measures the dispersion of data from the mean:

$$\text{Standard deviation (SD)} = \sqrt{\sum_{i=1}^n (X_i - \bar{X})^2}$$

Where, X_i - individual values; $\bar{X}$ - mean.

3.6.2.3. Coefficient of variation (CV)

$$\% \text{ Coefficient of variation} = \frac{\text{standard deviation}}{\text{mean}} \times 100$$

3.6.3. Kurtosis and Skewness

3.6.3.1. Kurtosis

Kurtosis measures the peakedness or flatness of a distribution relative to a normal distribution.

Coefficient of Kurtosis, $\beta_2 = \mu_4/\sigma^4$,

Where $\mu_4 = \Sigma (X - \bar{X})^4/n$ and $\sigma^4 = \{\Sigma (X - \bar{X})^2/n\}^2$.

If,

- $\beta_2 = 3$: Mesokurtic (normal distribution)
- $\beta_2 < 3$: Platykurtic (flat)
- $\beta_2 > 3$: Leptokurtic (peaked) (Gurumani, 2005).

3.6.3.2. Skewness

It is the degree of asymmetry of a distribution.

Positive **Skewness**: Mean > Median > Mode

Negative **Skewness**: Mean < Median < Mode

$$\text{Karl Pearson's coefficient of skewness} = \frac{(\text{Mean} - \text{Mode})}{\text{Standard deviation}} = (-1 \text{ to } +1)$$

3.6.4. Centiles

Percentile: It represents a value below which a specific percentage of observations fall. It reflects the cumulative frequency distribution of data.

Formula for Percentile:

To find the percentile of a value x , divide the number of values less than x (denoted as n) by the total number of values N , and then multiply by 100: **Percentile = $(n / N) \times 100$**

In simpler terms, the percentile tells what portion of the data lies below a particular value x

Example - Finding Specific Centiles: To determine the 5th and 95th percentiles in a dataset, first sort the data in ascending order. Then, calculate:

- 5th percentile = $0.05 \times N$
- 95th percentile = $0.95 \times N$

When the Position Is an Integer: If the resulting position is a whole number, the value at that position in the ordered list is the percentile.

When the Position Is Not an Integer: If the result is a decimal, identify the two closest ranks in the ordered data. Use the following interpolation method: Percentile value = Lower value + (Decimal part $\times$ (Upper value – Lower value))

3.6.5. Box-Whisker Plot

It is a graphical method used to summarize the central tendency, variability, and skewness of a continuous data distribution, while also highlighting potential outliers. Diagrammatic representation of data by means of Box Whisker plot gives visual information about dispersion, central tendency, outliers, and extent and direction of skewness (Gurumani, 2005).

3.6.6. 95% confidence interval of mean

Definition of Confidence Interval: It represents a range within which the true population value is likely to fall, with a certain level of confidence.

Confidence Interval Around the Mean: To construct this, values are taken above and below the estimated mean, based on a multiple of the standard error.

95% Confidence Interval Explanation: For a 95% confidence level, the standard error is multiplied by 1.96. This value is then added to and subtracted from the sample mean to define the interval.

Practical Interpretation: The 95% confidence interval provides a range that we can be 95% confident contains the true population mean. To calculate the 95% confidence interval, use the formula:

Mean $\pm$ (1.96 $\times$ Standard Error) This gives the lower and upper bounds of the interval.

Meaning of 95% Confidence: It means that if the same sampling procedure were repeated many times, approximately 95% of the intervals would include the true population mean.

3.6.7. Frequency Graphs

3.6.7.1. Histogram

A **histogram** is a type of graph used to show the distribution of a continuous dataset. The X-axis displays the actual class intervals (or ranges of values), and the Y-axis represents the frequencies of those intervals. The bars are adjacent and of equal width, representing equal class intervals, and their heights correspond to the frequencies. The histogram is useful for visualizing the shape and spread of continuous numerical data.

3.6.8. Gaussian distribution

Parametric statistical methods require that the data follow a normal distribution. This distribution is graphically represented by the normal curve, which is a symmetrical, bell-shaped curve centered around a single peak.

A normal distribution is a type of probability distribution that characterizes data which are symmetrically spread around a central value and have only one mode.

Key characteristics of the normal curve include:

- The mean, mode and median, all share the same value and are located at the center of the distribution.
- Perfectly symmetrical curve, with both sides mirroring each other.
- It is bell-shaped and conforms to strict parametric assumptions.
- The tails of the curve extend infinitely in both directions, getting closer to the X-axis but never touching it — a property known as being asymptotic.
- The total area under the curve represents the entire probability and is equal to 1 (or 100%).
- The skewness coefficient of a normal distribution is 0, indicating no asymmetry.
- The kurtosis (β_3) value is 3, reflecting the "normal" peak and tail thickness.

In a standard normal distribution (mean = 0):

- Approximately 68.27% of values lie within ± 1 standard deviation (SD) of the mean.
- Around 95.46% fall within ± 2 SD.
- Nearly 99.73% are within ± 3 SD.

3.6.8.1. Normal Quantile-Quantile plot

It is a graphical method used to assess whether a dataset follows a normal distribution by comparing its quantiles to those of a theoretical normal distribution. In this method, the quantiles of the sample data are plotted against the expected quantiles of a standard normal distribution, typically represented as $\pm z$ -scores. For normally distributed data, the plotted points may align closely along a straight line (diagonal), showing a linear relationship between the observed and theoretical quantiles. However, if the data deviate from normality, the points will stray from the straight

line, indicating departures from the normal distribution.

These plots can be drawn manually using normal probability paper, but modern statistical software like SPSS automatically generates Q-Q plots and visually displays how well the data fit the normal distribution.

It is a useful tool for comparing two datasets to see if they may have originated from the same distribution. In summary, a Q-Q plot helps determine how closely the **distribution of sample data** matches the **expected shape** of a normal distribution by comparing corresponding quantiles graphically.

3.6.8.2. Shapiro-Wilk Method for Tests of Normality:

It is a widely used method to test the normality of a dataset, first introduced in 1965 by Samuel Shapiro and Martin Wilk. This test applies the null hypothesis that the data come from a normal distribution and evaluates whether the sample significantly deviates from this assumption. It is specifically designed to detect deviations from normality and is considered one of the most powerful tests for small to moderate sample sizes. The p-value is > 0.05 , it suggests that there is no strong evidence against normality, and the sample can be treated as normally distributed

3.6.8.3. Transformations

When a dataset does **not follow a normal (Gaussian) distribution**, **data transformation** techniques can be applied to approximate normality and meet parametric test assumptions.

The most commonly used transformation methods include:

- **Natural logarithm transformation:** This involves applying the natural log (base e , where $e \approx 2.71828$) to each data point.
- **Logarithmic transformation:** In this method, the base-10 logarithm ($\log_{10}$) of each value is calculated to reduce skewness and improve symmetry.

- **Box-Cox transformation:** This flexible method applies a power transformation to the data using the formula X^λ , where X is the original value and λ (**lambda**) is a parameter selected to best approximate normality.

These transformation techniques help adjust **non-normal distributions** so that they more closely resemble a **normal distribution**, which is often required for certain statistical analyses. Choosing the right transformation depends on the type of **skewness or non-linearity** present in the data

3.6.9. Hypothesis testing

3.6.9.1. Student's t – Test (Parametric test)

It is a probability distribution that allows for reliable statistical inference about a population based on data from small samples. The t-distribution, often simply called the t-distribution, is the basis for a set of statistical tests known as t-tests (Altman, 1991), which are used to determine if there are significant differences between means. These tests are especially useful when working with small sample sizes, where the normal (Gaussian) approximation may not be appropriate. For the t-test to be valid, the data should follow a normal distribution (Gaussian) and the variances of the groups being compared should be equal. The t-test is a parametric test that assumes normality and homogeneity of variance, making it a standard choice for comparing means in small-sample research.

3.6.9.2. Mann Whitney U test (non-parametric test)

It is used to compare data from two independent groups when the assumption of normality is not met. It is used when comparing two unrelated samples, especially when the data are not normally distributed. The test works by ranking all observations from both groups together.

The core of the test is the calculation of a U statistic, which is used to assess whether one group tends to have higher or lower values than the other. The Mann–Whitney U test is more computationally involved than the t-test, as it requires assigning ranks and then using those ranks to calculate the U value. This method does

not rely on assumptions of normal distribution or equal variances, making it suitable for ordinal data or data with outliers.

$$U = n_1 n_2 + (n_1 + 1) - T$$

3.6.10. Correlation

This method is used to examine the potential association between two continuous variables. When two or more variables tend to change together, this relationship is referred to as a correlation. The term covariation is also used to describe how two variables vary in relation to each other.

Correlation analysis determines the direction and strength of a linear relationship between two continuous variables. If changes in one variable are systematically related to changes in another, they are considered to be statistically associated or co-varying. In statistical terms, when two variables exhibit a consistent pattern of movement, either in the same or opposite direction, they are said to be positively or negatively correlated.

3.6.10.1. Correlation types

Correlation can be classified as:

- **Simple correlation** refers to the relationship between two variables.
- **Multiple correlation** involves the relationship among three or more variables.

The direction of correlation between two variables can be positive or negative.

Positive correlation: When one variable increases, the other also increases; and as one decreases, the other decreases as well.

Negative correlation: When one variable increases, the other decreases; and as one decreases, the other increases.

3.6.10.2. Karl Pearson's Coefficient of Correlation (r)

It is a statistical measure that determines the direction and strength of the linear relationship between two variables.

The value of r ranges from -1 to +1:

- $r = +1$ - perfect positive linear correlation,
- $r = -1$ - a perfect negative linear correlation, and
- $r = 0$ - no linear relationship between the variables.

The formula for calculating Pearson's r is:

$$r = \frac{\sum(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum(x_i - \bar{x})^2 \sum(y_i - \bar{y})^2}}$$

where, $\bar{x}$ and $\bar{y}$ are the means of the x and y variables, respectively.

Two common methods for analysing the **presence, direction, and strength of correlation** between variables are:

- The **scatter plot**, which gives a visual representation, and
- **Karl Pearson's coefficient**, which provides a numerical measure (Altman, 1991).

Pearson's correlation coefficient is extensively used to assess how strongly two continuous variables are **linearly related** (Altman, 1991).

3.6.10.3. Spearman's rank correlation (non-parametric)

This method evaluates the direction and strength of the relationship between two variables based on their ranks, rather than their actual values. Spearman's correlation coefficient is equivalent to Pearson's correlation, but calculated using the ranked values of the data instead of the raw scores. When the data for either variable do not follow a normal (Gaussian) distribution, rank-based correlation methods like Spearman's are preferred.

3.6.10.4. Scatter diagram

A scatter diagram is a simple and effective graphical method used to examine the relationship between two variables. To create a scatter plot, the values of variable X are placed along the X-axis, and the corresponding values of variable Y are plotted along the Y-axis. Each pair of values (X, Y) is represented as a point on the graph, resulting in a collection of dots known as a scatter plot or scatter diagram. This pattern of dots helps visually assess the type and strength of correlation between the two variables. When the points group near a line that goes up from left to right, this suggests a positive correlation, meaning both variables increase together. On the other hand, If the scatter of points follows a line that goes down as you move right, this indicates a negative correlation. A strong correlation is evident when the points cluster tightly along a straight line —whether positive or negative.

3.6.11. Software Packages

1. SPSS - to compute distribution statistics. It also generates normality curves and box-and-whisker plots. Additionally, SPSS performs normality tests and hypothesis tests.
2. MedCalc - to calculate 95% reference intervals using parametric robust methods and for conducting the D'Agostino normality test. It also supports non-parametric (bootstrap) techniques for determining 95% reference intervals.
3. EViews software is applied to conduct the Jarque-Bera test for assessing normality.

3.7. Reference Intervals

Reference values are defined as quantitative data obtained from a single individual or group of individuals that correspond to a defined healthy state and it is useful for clinical diagnosis of disease (Horowitz and Jones, 2018).

The ‘Normal values’ term usually referred to healthy medical data that are compared with clinical data for disease diagnosis. The term “normal” has several related interpretations and meaning (Murphy, 1972), which leads to misinterpretations. In Statistics, “normal” is related to “Gaussian probability distribution”. The term normal distribution should be replaced by the term Gaussian distribution (Elveback et al., 1970).

In Epidemiology, the word “normal” refers to the data from healthy sample population as opposed to that from an unhealthy sample. In Clinical diagnosis, “normal” indicates the absence or decreased risks for certain diseases. Since the 1980s, the concept of reference values was implemented (Solberg and Grasbeck, 1989).

Defining the reference individual and selection of a group of reference individuals is the first step for establishing a reference value or reference interval. Population reference values/ intervals are difficult to calculate and therefore a sample group is selected to represent the reference population. As it is difficult to select a reference population, a subset population is selected random from the reference population.

Confidence intervals provide a range around the reference limit—derived from a percentile of the measurand—within which the true value is believed to fall, with a specified confidence level, most often 95%

The term reference distribution is used for the reference values in the sample of the reference individuals. The arithmetic mean ($\bar{x}$) and standard deviation (s_x) are measures of the distribution and location of the values (x) of the measurands. the capital alphabets X , $\bar{X}$ and S_x represent the population reference values, mean, and standard deviation. These population parameters of the measurand are difficult to obtain. Therefore, the sample parameters are taken from the reference sample population, as represented by small alphabets.

The reference interval is calculated from a Gaussian distribution of the measurand from a sample population. Inter percentile interval method is used to

estimate the reference interval. This method is simple; more commonly used and is recommended by IFCC. the typical reference interpercentile interval in 2.5-percentile to 97.5-percentile.

3.7.1. Method used to determine inter percentile intervals (Horowitz and Jones, 2018).

The inter percentile interval is determined using a parametric or a non-parametric method.

3.7.1.1. Parametric method

This method assumes a Gaussian distribution and is based on estimation of mean and standard deviation. In the parametric method from the Gaussian distribution, the interpercentile reference limits are calculated as values located two standard deviations below and above the mean

In cases where the reference distribution is non-Gaussian, data can be mathematically transformed to approximate normality, often confirmed through graphical methods. As an example, a positively skewed distribution may be made nearly symmetrical or Gaussian by use of data transformation such as reciprocal method or square-root method may be used rarely.

After transformation, the central 95%-interval is taken to represent the reference interval.

3.7.1.2 non-parametric method

This method does not depend on distributional assumptions or parameter estimates. Instead, it identifies percentiles by discarding the appropriate percentage of observations from each end of the ordered data. This excluded percentile is typically 2.5% at each end, taking the middle percentile. (Reference of IFVW, CSIL, 42, 15)

Reference intervals may be a general reference interval of adult population defined for the male sample and another reference interval of adult population defined for female sample. Sample considered in this group includes all apparently healthy

individuals above 18 years. for adolescent and children <18 years another reference interval may be calculated.

Another method of defining reference interval is the reference interval partitioned in to gender and age groups, where fasting and postprandial samples may be considered separate or together according to availability of sample number. According to this criterion of reference interval calculation there will be two genders and 4 age groups for the study (eight reference intervals)

In this study, we preferred to choose 90 % middle reference interval excluding 5% on either side to exclude the positively skewed regions and outliers with moderately or highly increased lipid profile analytes, respectively.

To calculate the parametric reference interval, the sample distribution should be evaluated for a normal or Gaussian distribution. If the distribution is not Gaussian then log transformation is done to convert the sample to Gaussian distribution.

In the non-parametric method, the 90% central reference interval is calculated from the normal or positively skewed sample using the percentile method for calculating the central 90% reference interval.

To further calculate a parametric base line reference interval, it may be required to exclude the outlier regions and then calculate the parametric reference interval before or after log transformation. If this method does not convert the distribution in to Gaussian distribution due to large number of moderately increased hyperlipoproteinemia leading to positive skewing, then the other methods such as reciprocal and square root methods are used for calculating the reference interval.

3.7.2. Reference interval for lipid analytes

It is a set of values used to interpret laboratory results. It represents range of values expected for a healthy population, typically defined as central 95% of values from a reference population. Reference interval may vary by age, gender and population. A study on Indian population suggested <200 mg/dL as a general reference guideline for Total Cholesterol. The reference interval for LDL Cholesterol

in the Indian population is 1.7 – 4.4 mmol/L. For other populations like Asians reference interval for LDL Cholesterol is 1.65-4.45mmol/L and 1.62 – 4.42 mmol/L in males and females, respectively. (Swaroop et al., 2018).

CHAPTER 4
GENERAL MATERIALS AND METHODOLOGY

4.1. Research Design

This observational cross-sectional study on dyslipidemia was conducted at the Amala Institute of Medical Sciences using an indirect sampling approach. Fasting lipid profile samples from both outpatient (OP) and inpatient (IP) departments were collected for the purpose of randomizing lipid profile data. Following systematic sampling, the data will be analyzed to assess distribution patterns, gender-based differences, and inter-parameter correlations. Dyslipidemia categories will be grouped to determine reference ranges and to estimate relative prevalence.

4.1.1. Organization of the Study

4.1.1.1. Observational, Cross-sectional study for establishing the reference interval of Lipid Profile analytes.

- a) Fasting blood samples will be collected from clinical Biochemistry laboratory.
- b) Lipid profile Analytes – Serum triglycerides, Total Cholesterol, LDL-Cholesterol and HDL Cholesterol and related parameters will be assayed in these samples.
- c) Analysis of the data for distribution characteristics will be done. If distribution is Gaussian, the data may be used for parametric statistical estimations and evaluations. If the data is not gaussian, then data is transformed by various methods like log transformation, square root and inverse methods before and after removal of outliers.
- d) Data will be analysed for heterogeneity in sample distribution (such as gender, age, groups).
- e) Lipid profile data will be analysed for correlation and Prevalence studies
- f) Final data analysis for reference interval calculation will be done by analysis of distribution according to criteria laid down by IFCC, CLSI and other internationally recognized organizations.

4.1.1.2. Observational, cross-sectional study for Distribution analysis and comparison of mean values of Fasting and Postprandial Lipid profile analytes

- a) The **Fasting, 2-hour post breakfast and 2-hour post lunch** blood samples will be collected from apparently healthy individuals of various age groups by direct sampling according to inclusion and exclusion criteria.
- b) Lipid profile Analytes – – Serum triglycerides, Total Cholesterol, LDL-Cholesterol and HDL Cholesterol will be assayed in these samples.
- c) Data were collected from these samples using assays for Alanine aminotransferase, fasting and postprandial glucose, TSH, Uric acid, Alkaline Phosphatase, serum creatinine, ferritin, may also be required to narrow down the differential diagnosis of a particular group of lipid profile.
- d) Parametric and non-parametric statistical calculations will be performed to analyse the significant mean value differences in fasting and Postprandial lipid profile analytes.

4.2. Permissions

Approval for conducting this study was obtained from the Institutional Research and Ethics Committees (Ref. AIMSIEC/42/2018, dated 19/10/2018), authorizing data collection through the Department of Biochemistry and the Clinical Biochemistry Laboratory. Informed consent, in written form, was secured from each participant prior to enrolment.

4.3. Study setting

The present study will be done at Department of Biochemistry, Amala Cancer Research Centre and Clinical Biochemistry Laboratory, Amala Institute of Medical Sciences, Thrissur.

4.3.1. Data collected from Clinical Biochemistry Laboratory

First set of data were collected from Clinical Biochemistry Laboratory by

Indirect sampling Method. Using inclusion and exclusion criteria, fasting lipid profile samples from OP and IP Departments and from the clinical Biochemistry laboratory database were collected from September 2018 to March 2022 continuously for randomization of lipid profile data.

The Reference Individuals were selected from the study population by **inclusion and exclusion criteria**. Samples were collected from age group above and below 18 years.

Inclusion Criteria:

Participants of both sexes (males and females), whether or not they had dyslipidemia or related complications, were enrolled in the study. Comorbid conditions among the subjects included diabetes mellitus, obesity, metabolic syndrome, hepatic steatosis, thyroid dysfunction, pancreatitis, renal disease, hypertension, alcohol consumption, smoking, various dietary habits, physical inactivity, and psychological stress,

Exclusion criteria:

All health conditions not listed in the inclusion criteria were considered exclusion criteria. Individuals with infections, COVID-19, post-operative status, physical trauma (e.g., fractures or injuries), malignancies, or those whose samples originated from emergency or intensive care units were excluded from the study.

4.3.1.1. Selection of Age Groups

Samples were stratified into four age categories: 0 to <18 years (newborns, children, and adolescents), 18–25 years (young adults), 26–50 years (middle-aged adults), and above 50 years (senior adults). Three months after study commencement, initial validation based on collected data showed: a) Each age group demonstrated gender-specific differences. b) There was a gradual increase in sample numbers across age groups.

The indirect sampling technique was employed instead of direct population sampling, owing to the need for large sample sizes to identify rare lipid abnormalities within a limited timeframe and with reduced financial burden (Horowitz, 2012).

4.3.1.2. Sample size requirement

Sample size estimation was based on a prior multicentric study (Qi et al., 2015), with assumptions of 24% prevalence, 20% allowable error, 5% significance level ($Z = 1.96$), and 80% power. This yielded a minimum required sample size of 304 per group.

$$n = \frac{Z^2_{(1-\alpha)}pq}{d^2}$$

($d = 20\%$ prevalence; $\alpha = 0.05$; $p = 24\%$; $q = 1 - p$; $Z_{(1-\alpha)}$ value = 1.96; power 80%; $n = 304.12$. Samples size for each group was rounded off to 304).

As the study evolved, a larger sample size was deemed necessary to accommodate subgrouping by gender and age ($2 \times 4 = 8$ groups), and further classification into three histogram categories— <150 mg/dL, positively skewed, and outlier values—resulting in 24 distinct subgroups (8×3). Thus, a total of 304×24 samples was required. Owing to insufficient sample availability in the <18 age group, the total was further doubled, giving a target of 14,592. The actual collected sample size of 15,603 satisfied the statistical demands for each subgroup analysis.

4.3.2. Data collected from participants

Second set of fasting and postprandial Lipid profile data were collected from apparently healthy participants directly with consent. Oral consent was obtained after describing about the research program and the method of sample collection. After including the participants, informed written consents were obtained, and then samples were collected. The participants were selected from the study population by **inclusion and exclusion criteria**.

Inclusion Criteria

Male and female adults with dyslipidemia and its complications but otherwise healthy, will be selected for the study. Individuals with defined physiological or pathological conditions such as diabetes mellitus, obesity, metabolic syndrome, hypertension, alcoholism, smoking, varying dietary habits, sedentary life style, fatty liver, thyroid dysfunction, may also be chosen for the evaluation.

Participants are advised to maintain their usual diet, sleep routine, and physical activity for three days prior to blood sample collection. They should arrive at the lab while fasting for 9 – 12 hours. A blood sample ranging from 4 to 10 ml is drawn and then centrifuged to separate the serum. The tests are performed immediately, and any leftover serum is preserved at -20°C for future use. Non-fasting samples are collected approximately 2 to 3 hours after breakfast or lunch.

Exclusion criteria

All disease conditions, except those defined above in the inclusion criteria, will be excluded from the study. Individuals with history of serious infections, pregnancy, lactation, Covid, psychiatry problems, fracture and injury involving tissue damage for six months are excluded. Individuals with malignancy will be excluded. Participant should not be on any medication or had any minor injury, infection or severe headache for a minimum of 2 weeks. Participants should not be stressed, such as overnight journey, preparing for examination, anxiety and loss of sleep. Participants with defined disease states, as stated in the inclusion criteria will be selected.

4.3.2.1. Selection of Age group and sample size requirement

The study included participants who were apparently clinically healthy and above 18 years of age. The sample size was determined by a statistician based on a previously published multicentric study on the prevalence of dyslipidemia (Qi et al., 2015).

$$n = \frac{Z^2_{(1-\alpha)}pq}{d^2}$$

Using the parameters:

- Estimated prevalence (p) = 24%
- Complement (q) = $1 - p$
- Acceptable error (d) = 20%
- Confidence level (α) = 0.05
- Z value at 95% confidence = 1.96
- Power = 80%

The calculated sample size was 304.12, which was rounded off to 304 for each group.

4.4. Sample collection and Sample Preparation for assays

Fasting blood samples were collected before 8:30 a.m., following an overnight fast and at least two hours after waking. Between 6 and 10 ml of blood was drawn into polypropylene tubes without clot activators. The samples were centrifuged immediately at 3000 rpm for 5 minutes to separate the cells before clotting could occur. The resulting supernatant was transferred into glass tubes to allow clotting, followed by a second centrifugation to separate the serum. Serum samples were either assayed immediately or stored in aliquots at -20°C (Haverstick & Patricia, 2018). For hemoglobin analysis, blood was collected in EDTA tubes and measured manually using Drabkin's method (Weinkove & McDowell, 2006)

4.5. Sample sizes and reference interval calculations

The sample sizes and reference interval calculations were done after removal of outliers, analysis of data distribution, and data transformations according to criteria laid down by International Federation of Clinical Chemistry and Laboratory Medicine [IFCC], and Clinical and Laboratory Standards Institution [CLSI] guidelines by using the software, MedCalc (Horowitz and Jones, 2018).

Lipid profile reference interval can be calculated using various methods.

1. Direct measurement: measuring lipid parameters directly in a healthy population
2. Statistical analysis.

It involves following steps

1. Data cleaning:
 - a) Check for outliers: identify and handle outliers that may affect the accuracy of reference interval
 - b) Verify data distribution: check if the data follows a normal distribution or if it is skewed
2. Descriptive statistics
 - a) Calculate the mean and median: Determine the central tendency of data
 - b) Calculate the standard deviation: determine the variability of data
 - c) Calculate percentiles: determine 2.5th, 5th, 95th, and 97.5th percentile to establish the reference interval
3. Reference interval calculation
 - a) Parametric method: if the data follows a normal distribution, calculate the reference interval using $\text{mean} \pm 2\text{SD}$.

If, we assume mean (M) and standard deviation (s) of data from a normal or apparently healthy population, we can estimate reference interval as (m-2s to m+2s)

Note- s x 2 was arrived at by round of 1.96 x s.

1.96 is used to round the reference interval values at 2.5% and 97.5%

- b) Non-parametric method: if the data is skewed, calculate the reference interval using 2.5th and 97.5th percentile.

Following steps are used for reference interval calculation using percentile ranking –

non parametric method

1. Rearrange values in ascending order to define the central 95% of the data as the reference interval.
2. Find the lower limit (2.5th percentile):
 - a) Multiply the value (n+1) (where n is the number of values in data set) by 2.5%
 - b) Use this number and count the first percentile (lowest value) by that amount. This will be the lowest limit
3. Do the same for the upper limit. Count down from the highest percentile to the 97.5 percentile
4. Confidence interval calculation: determine the 90% confidence interval for the reference limits to assess their precision
5. Interpretation: compare the calculated reference interval to existing literature or clinical guidelines to ensure they are accurate and relevant.

Statistical methods like Hoffmann and Bootstrap method can be used to estimate parameters and construct confidence intervals.

4.6. Indirect and relative prevalence calculation (Sterne and Kirkwood, 2003)

a) Prevalence of dyslipidemia % = $\frac{\text{Number of samples with dyslipidemia}}{\text{Total number of samples collected}} \times 100$

b) Prevalence of isolated/combined dyslipidemia cases %

$$= \frac{\text{Number of samples with isolated/combined dyslipidemia}}{\text{Total number of samples collected}} \times 100$$

c) Relative prevalence of a particular disease condition such as hyper beta lipoproteinemia or increase in LDL-C (%)

$$= \frac{\text{Prevalence of increased LDL-C condition}}{\text{prevalence of dyslipidemia}} \times 100$$

4.7. Measurement of Body mass index

Body mass index (BMI), calculated from an individual's weight and height, is widely recognized as a more accurate indicator of body fat and associated health risks than weight alone (WHO, 2008)

$$\text{BMI} = \frac{\text{Weight (kg)}}{\text{Height (metre)}^2}$$

4.8. Measurement of Waist circumference

Waist circumference (WC) is a useful measure for assessing health risks linked to excess fat accumulation around the abdomen. It is considered a more accurate indicator of central obesity than BMI (WHO, 2008). Measure waist halfway between the bottom of last rib and the top of hip bone. The measurement is taken while the individual stands upright without footwear, feet close together, arms relaxed at the sides, and after a normal exhalation. A non-stretch, flexible tape should be used, placed snugly around the waist without compressing the skin. In research settings, waist circumference is typically measured three times and recorded to the nearest 0.1 cm to ensure accuracy (WHO, 2008).

4.9. Autoanalyzer for Chemistry and Immunochemistry Parameters

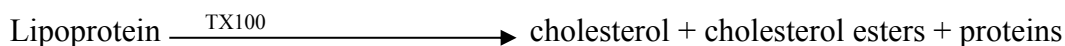
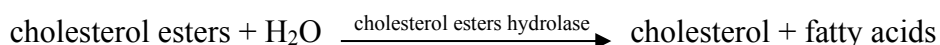
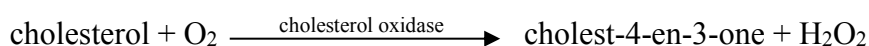
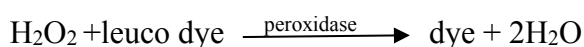
Biochemical analyses were performed using the Vitros 5,1 FS chemistry autoanalyzer (Ortho Clinical Diagnostics, USA), which includes both dry and wet chemistry capabilities. Immunochemical tests were conducted using the Access 2 analyzer (Beckman Coulter, USA) and the Liaison system (Diasorin, Italy)

4.10. Detailed Description of Assays

Assay of Cholesterol

Cholesterol assay was performed by enzymatic method in chemistry autoanalyser Vitros 5, 1 FS (OCD, USA). VITROS dry chemistry slides were used. A drop of sample (10 μ L) is deposited on the slide and the sample is evenly distributed by the spreading layer to the underlying layers. The Triton X-100 (TX100) surfactant in the spreading layer helps in the dissociation of cholesterol and cholesterol esters from lipoprotein complexes present in the sample. Cholesterol ester hydrolase catalyses the hydrolysis of the cholesterol esters to cholesterol. Free cholesterol is oxidized in the presence of cholesterol oxidase to form hydrogen peroxide and cholestenone. Finally, hydrogen peroxide then oxidizes a dye (leuco dye) in the presence of peroxidase to form a coloured dye. The density of the dye formed is proportional to the cholesterol concentration present in the sample and it is measured by reflectance spectrophotometry. This colorimetric method requires 37°C temperature and an incubation time of 5 minutes. The wave length used is 540nm.

Reaction sequence



Reagents

Triton X-100 - 0.81mg

Cholesterol oxidase - 0.4U(Nocardia or Cellulomonas)

Cholesterol ester hydrolase - 2.0U (Pseudomonas)

Peroxidase - 5.3 U (horseradish root)

2-(3, 5-dimethoxy-4-hydroxyphenyl)-4, 5-bis- (4-dimethylaminophenyl) imidazole (leuco dye) - 0.17mg.

Pigments, binder, buffer, surfactants, stabilizers and cross-linking agent

(Procedure manual, Ortho Clinical Diagnostics)

Assay of Direct HDL Cholesterol

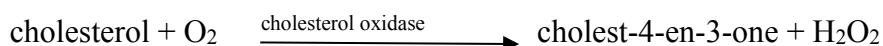
HDL (High Density Lipoprotein) cholesterol estimation was carried out in chemistry autoanalyzer Vitros 5, 1 FS (OCD, USA) by the use of VITROS dHDL slides. The VITROS dHDL slide is a multilayered, analytical element coated on polyester support. The method involves a non-HDL precipitation method which is followed by an enzymatic method. A drop of sample (10 μ L) is deposited on the slide. it is evenly distributed by the spreading layer to the underlying layers. Phosphotungstic acid (PTA) and magnesium chloride (MgCl₂) in the spreading layer aids the separation of HDL by the precipitation of non-HDL. In the spreading layer, the Emulgen B-66 surfactant is present which helps in the dissociation of cholesterol and cholesterol esters from HDL lipoprotein complexes present in the sample. cholesterol ester hydrolase catalyses the hydrolysis of the HDL- derived cholesterol esters to cholesterol. Free cholesterol is then oxidized in the presence of cholesterol oxidase to form hydrogen peroxide and cholestenone. Finally, hydrogen peroxide oxidizes a leuco dye in the presence of peroxidase to develop a coloured dye. The density of the dye formed is proportional to the HDL cholesterol concentration present in the sample and is measured by reflectance spectrophotometry. This colorimetric method requires 37°C temperature and an incubation time of 5 minutes. The wave length used is 670nm

Reaction sequence

HDL + non-HDL $\xrightarrow{\text{PTA/ MgCl}_2}$ high density lipoproteins + non-HDL

high density lipoprotein $\xrightarrow{\text{Emulgen B-66}}$ cholesterol + HDL cholesterol esters
+ proteins

cholesterol esters + H₂O $\xrightarrow{\text{cholesterol esters hydrolase}}$ cholesterol + fatty acids

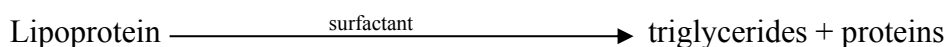
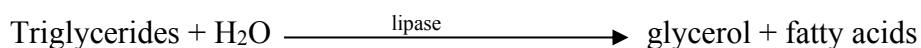
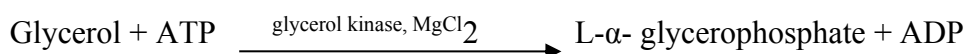
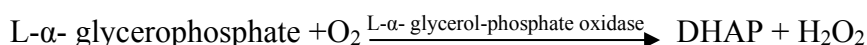
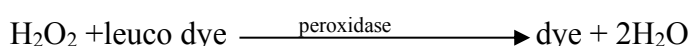


(Procedure manual, Ortho Clinical Diagnostics)

Assay of Triglyceride

Triglyceride estimation was done in chemistry autoanalyzer Vitros 5, 1 FS (OCD, USA) using the VITROS TRIG slides. The VITROS slide is a multilayered, analytical element coated on polyester support. This analysis is based on an enzymatic method. A drop of sample (5.5µl) is deposited on the slide. it is evenly distributed to the underlying layers by the spreading layer. The Triton X-100 (TX100) surfactant is present in the spreading layer. It helps in the dissociation of triglyceride from lipoprotein complexes which was present in the sample. The triglyceride molecules are then hydrolysed by lipase which result in formation of glycerol and fatty acids. Glycerol diffuses to the reagent layer, where it is phosphorylated by glycerol kinase in the presence of ATP. In the presence of L- α - glycerol-phosphate oxidase, L- α - glycerophosphate is then oxidized to hydrogen peroxide and dihydroxyacetone phosphate. The final reaction involves the oxidation of a leuco dye by hydrogen peroxide, catalysed by peroxidase, to produce a dye. The density of the dye formed is proportional to the triglyceride concentration present in the sample and is measured by reflectance spectrophotometry. This colorimetric method requires 37°C temperature and an incubation time of 5 minutes. The wave length used is 540nm.

Reaction sequence



(Procedure manual, Ortho Clinical Diagnostics)

Estimation of LDL Cholesterol

Both indirect and direct methods are used to measure LDL (Low-Density Lipoprotein) cholesterol. When the LDL cholesterol was below 400 mg/dL, **LDL cholesterol** was assayed by indirect method which is done using Friedwald equation.

Indirect method assumes that Total cholesterol is primarily composed of cholesterol of VLDL, LDL and HDL. LDL cholesterol is measured indirectly by using Friedewald equation. Here total cholesterol, triglyceride and HDL cholesterol are measured and LDL cholesterol is calculated from the primary measurement by using the empirical Friedewald equation:

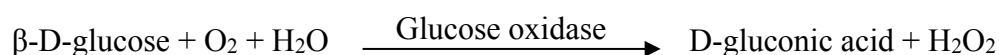
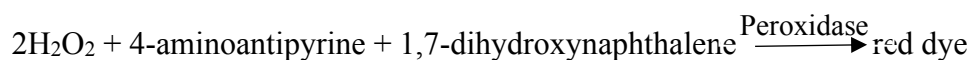
$$\text{LDL cholesterol} = [\text{Total cholesterol}] - [\text{HDL cholesterol}] - [\text{Triglyceride}]/5,$$
where all concentrations are given in milligrams per deciliter.

Triglyceride/2.22 is used when LDL cholesterol is expressed in millimoles per liter. The factor $[\text{Triglyceride}]/5$ is an estimate of VLDL cholesterol concentration and is based on the average ratio of triglyceride to cholesterol in VLDL (Remaley et al, 2012).

LDL cholesterol was directly assayed when LDL cholesterol was above 400 mg/dL. In lipemic samples cloudiness is cleared by cooling to 4°C overnight and centrifuging at 4°C at 10,000 rpm for 20 minutes (Remaley et al, 2012).

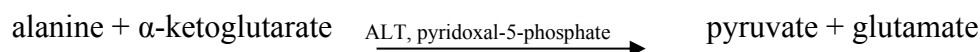
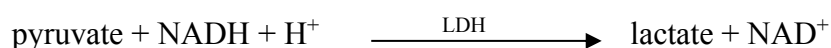
Assay of Glucose

This assay was performed using the Vitros 5,1 FS chemistry autoanalyzer (Ortho Clinical Diagnostics, USA), which features both wet and dry chemistry modules. The VITROS glucose slide is a multilayer analytical element coated on a polyester base. A 10 µL sample is applied to the slide, where it is uniformly distributed across the layers by the spreading layer. Glucose in the sample is oxidized by glucose oxidase to produce hydrogen peroxide and gluconate. Subsequently, in the presence of dye precursors, peroxidase catalyzes an oxidative coupling reaction that forms a colored dye. This colorimetric assay is carried out at 37°C with a 5-minute incubation period. The intensity of the dye is then measured using reflected light at 540 nm.



Assay of Alanine Aminotransferase

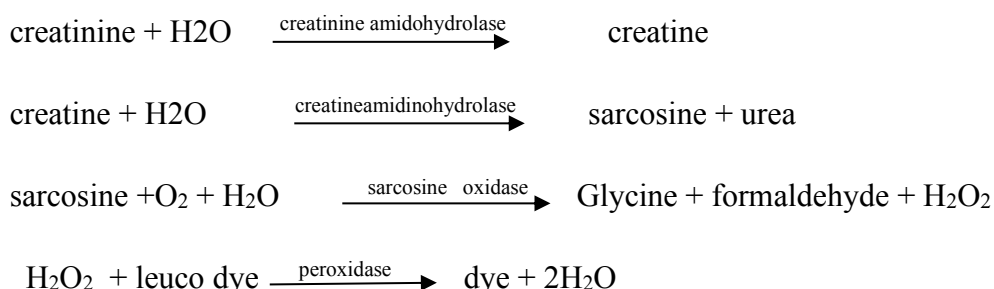
ALT estimation was carried out using the Ortho Clinical Diagnostics Vitros 5.1 analyzer with VITROS ALT slides. These slides are multilayer analytical elements coated on a polyester support. An 11 μL sample is applied to the slide, where it is evenly dispersed through the spreading layer to the underlying layers, followed by incubation at 37°C for 5 minutes. The spreading layer contains ALT substrates: L-alanine and sodium α -ketoglutarate. Alanine aminotransferase catalyzes the transfer of an amino group from L-alanine to α -ketoglutarate, forming pyruvate and glutamate. Subsequently, lactate dehydrogenase (LDH) catalyzes the conversion of pyruvate and NADH into lactate and NAD^+ . The oxidation rate of NADH is monitored via reflectance spectrophotometry. The rate of change in reflection density, measured at 340 nm, is directly proportional to ALT enzyme activity.



Assay of Creatinine

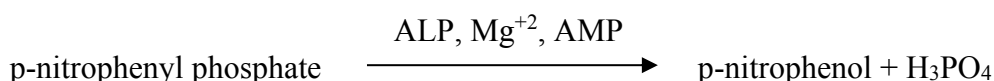
Creatinine levels were measured using the Ortho Clinical Diagnostics Vitros 5.1 analyzer with VITROS CREA slides. These slides are multilayer analytical elements coated on a polyester base. A 6 μL sample is applied to the slide, where it is uniformly distributed by the spreading layer across the underlying reagent layers. Creatinine diffuses into the reagent layer and is hydrolyzed to creatine, which is the rate-limiting step. Creatine is subsequently converted into sarcosine and urea by the enzyme creatine amidinohydrolase. In the presence of sarcosine oxidase, sarcosine is oxidized to glycine, formaldehyde, and hydrogen peroxide. The final step involves a peroxidase-catalyzed oxidation of a leuco dye, producing a colored compound. After the sample is applied, the slide is incubated at 37°C for 5 minutes. During the early phase of the reaction, endogenous creatine in the sample is also oxidized. The change

in reflection density is measured at two time points, and the difference is directly proportional to the creatinine concentration. The measurement is taken at a wavelength of 670 nm.



Assay of Alkaline phosphatase

A drop of the patient's sample is applied to the slide, where it is evenly distributed across the underlying layers by the spreading layer. This layer contains p-nitrophenyl phosphate and other necessary reaction components. Alkaline phosphatase (ALP) in the sample catalyzes the hydrolysis of p-nitrophenyl phosphate to p-nitrophenol under alkaline conditions. The resulting p-nitrophenol diffuses into the lower layers, where its formation is monitored by reflectance spectrophotometry. The rate of change in reflection density is used to calculate enzyme activity.



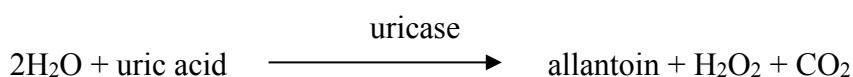
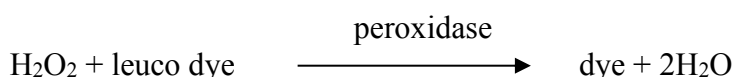
Assay of Ferritin

The Access Ferritin assay is a two-site immunoenzymatic ("sandwich") assay. In this method, the sample is mixed in a reaction vessel with a goat anti-ferritin alkaline phosphatase conjugate and paramagnetic particles coated with goat anti-mouse antibodies bound to mouse anti-ferritin complexes. Ferritin present in serum or heparinized plasma binds to the monoclonal anti-ferritin antibodies immobilized on the solid phase, while the enzyme-conjugated goat anti-ferritin binds to different antigenic sites on the ferritin molecule. After incubation, the solid-phase particles are captured using a magnetic field, and unbound substances are washed away. A chemiluminescent substrate (Lumi-Phos* 530) is then added, and the resulting light

emission is measured using a luminometer. The amount of light produced is directly proportional to the ferritin concentration in the sample. The ferritin level is determined using a stored multipoint calibration curve.

Assay of uric acid

A drop of the patient's sample is placed on the slide and uniformly spread by the spreading layer to the underlying layers. Uric acid diffuses into the reagent layer, where uricase catalyzes its oxidation to allantoin and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide then oxidizes a leuco dye to produce a colored compound. The intensity of this dye is measured by reflectance spectrophotometry.



4.11. Interconversion of Units of variables

The conversion between conventional and SI units is as follows: multiply the conventional value by the conversion factor to obtain the SI unit. For glucose, multiply mg/dL by 0.0555 to get mmol/L; for cholesterol, multiply mg/dL by 0.0259 to get mmol/L; for creatinine, multiply mg/dL by 88.4 to get $\mu\text{mol/L}$; for triglycerides, multiply mg/dL by 0.0113 to get mmol/L; and for ALT, units per liter are equivalent ($1 \text{ Unit/L} = 1 \text{ U/L}$).

4.12. Control of Quality

Both the immunochemistry and chemistry autoanalyzers met the quality criteria, with coefficient of variation (CV) and bias values below 4% across the range of data collected for the respective assays. Internal quality control was conducted twice daily, and an external quality assurance program (Biorad, USA) was performed monthly. Daily internal quality control data were evaluated using Westgard rules to determine the acceptance or rejection of analyte results. In cases of rejection,

corrective actions were taken to address issues related to instrument performance, reagent quality, storage conditions, or analyte calibration. The OCD Vitros chemistry calibrators for creatinine are traceable to the certified National Institute of Standards and Technology (NIST) standard reference material SRM 914a. First-party quality control materials were prepared following the guidelines outlined in “Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guidelines” (NCCLS Document C24, 2nd edition, 1999, NCCLS, Wayne, PA).

4.13. Lipoprotein Electrophoresis and staining:

Evaluation of the level of Lipoproteins by 1% agarose gel electrophoresis will be done. Lipids stained with Sudan black. Relative quantification will be done visually. (Noble, 1968).

Requirements

- Freshly prepared human serum
- Horizontal Electrophoresis apparatus and power pack.
- Microscopic slides and cover slides
- Agarose
- Buffer: Tris-glycine (0.05 M Tris and 0.10 M Glycine) – pH 8.4.
- Dye – Sudan Black
- Destaining solution (Methanol, Acetic acid and Distilled water)

Procedure

- a) Place two clean, square glass slides on a flat, even surface.
- b) Measure the required amount of dry agarose powder (1% w/v) and transfer it into a clean, dry test tube.
- c) Add Tris-glycine buffer to the test tube and heat it in a boiling water bath.
- d) Continue heating until the agarose dissolves completely in the buffer and forms a clear solution.
- e) Allow the solution to cool slightly, then use a pipette to carefully spread it over one of the square glass slides.
- f) Let the agarose set undisturbed for about 15 to 20 minutes to form a firm gel.
- g) Place a drop of undiluted human serum onto another clean glass slide, sprinkle a small amount of Bromophenol blue dye on it, mix gently with a cover slip, and press the cover slip onto one end of the gel to apply the sample as a thin, narrow line.
- h) Position the slide in the electrophoresis chamber so that the sample is placed closer to the cathode.
- i) Fill the buffer reservoirs in the electrophoresis setup with Tris-glycine buffer solution.
- j) Cut strips of Whatman No.1 filter paper, fold each strip by 1–2 cm, soak in buffer, and place one at each end of the gel slide, making sure the ends touch the buffer in the reservoirs.
- k) Connect the setup to a power supply and run electrophoresis at 100 volts for 1 to 2 hours, or until the tracking dye reaches the front edge (anode side) of the gel.
- l) Once electrophoresis is complete, cover the gel completely with Sudan black staining solution and let it sit for 15 to 30 minutes.

- m) Pour off the stain and apply the destaining solution onto the gel surface.
- n) After a few minutes, remove the destaining solution, and examine the gel to observe the visible lipoprotein bands

4.14. Statistical Analysis

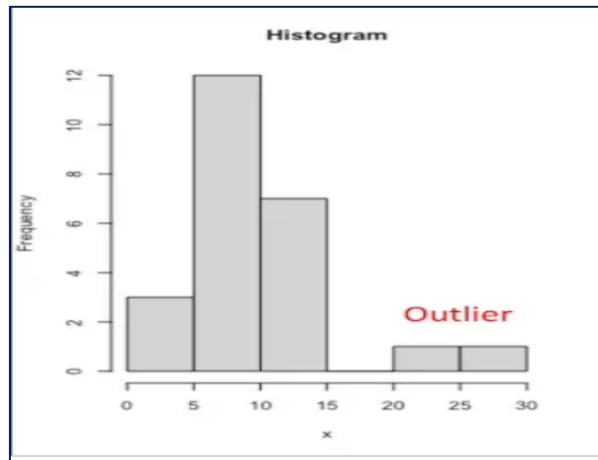
Statistical analysis and calculations were done **with SPSS, version 19.0 software, Eviews and Medcalc software**. Normality of distribution was estimated by graphical and numerical tests. Graphical tests used are Histogram, Box-Whisker Plot and Normal Quantile-Quantile plot. Normality of distribution was numerically estimated by Shapiro-Wilk test and Jarque Bera Test. Equality or homogeneity of variances of the groups compared was done by Levene's test. Log₁₀ transformations converted most of the positively skewed groups to Gaussian distribution and were of equal variances in the groups compared. When variables had Gaussian distribution (before or after transformation) and when there was equality of variance in the groups compared, parametric methods of analysis were used. Otherwise, non-parametric methods were used. The significant differences of variables of different groups were compared by 95% confidence interval of mean, two-tailed Student's t test, and by non-parametric Mann Whitney U test. (Klee & Westgard, 2012; Reffenburgh, 2012; Altman, 1991). The relationship between two variables is done by Pearson correlation, only if one of the variables had Gaussian distribution otherwise non parametric Spearman's Rank correlation was done. Correlation between the two variables was plotted graphically by X-Y scatter plot and was used to visually verify the correlations.

Histogram: It is a graphical data representation in which concentration of lipid profile analyte plotted against frequency of sample number (Fig.4.1).

How to identify outliers using histogram? (yang et al, Renesh Bedre's article)

- Data points far from main cluster: Values significantly higher or lower than most data points Isolated or sparse bars: Bars far away from main group, indicating unusual data points
- Asymmetrical distribution: skewed histograms may indicate outliers.

Figure.4.1. Histogram



Box-Whisker plot: Diagrammatic representation of triglyceride data by means of Box-Whisker plot gives visual information about dispersion, central tendency, outliers, and extent and direction of skewness (Gurumani, 2005). The reference Box-Whisker plot is symmetrically organized (Fig. 4.2).

Construction of Box-Whisker plot (Fig. 4.2)

- 1) Arrange the data series in ascending order
- 2) Calculate the Sample median, first quartile (Q_1), Third quartile (Q_3) and interquartile range (IQR).

$$\text{Median } (Q_2) = \text{value of item } (n+1)/2$$

$$\text{First Quartile, } Q_1 = \text{value of item } (n+1)/4$$

$$\text{Third Quartile, } Q_3 = \text{value of item } 3(n+1)/4$$

$$\text{Inter quartile range, IQR} = Q_3 - Q_1$$

- 3) Calculate the inner fence point f_1 and f_3

$$f_1 = Q_1 - 1.5 (\text{IQR})$$

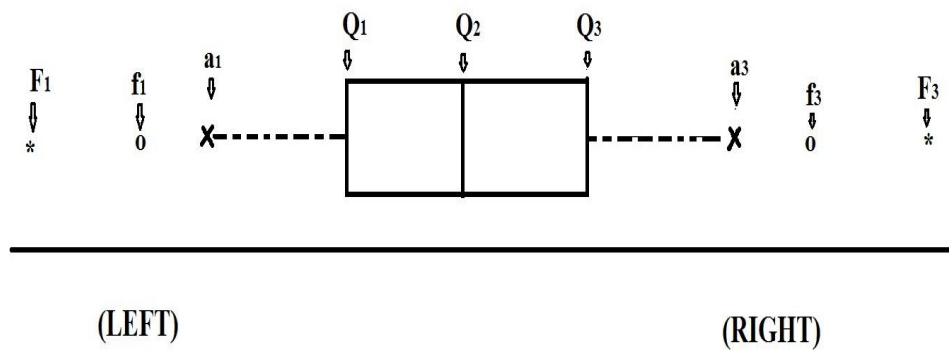
$$f_3 = Q_3 + 1.5 (\text{IQR})$$
- 4) 'Adjacent values' a_1 and a_3 are identified. The value that is closest to the f_1 but not less than f_1 is referred to a_1 and a_3 is the value that is nearest to f_3 but not above it.

- 5) Calculate the 'Outer fence' points F_1 and F_3

$$F_1 = Q_1 - 2(1.5) (\text{IQR})$$

$$F_3 = Q_3 + 2(1.5) (\text{IQR})$$
- 6) Construct box with ends at Q_1 and Q_3 . Draw a vertical line corresponding to the median in the interior of the box.
- 7) Indicate adjacent values, a_1 and a_3 by X, and connect to the box by dashed line.
- 8) $Q_3 - a_3$ is upper whisker (right)
- 9) $Q_1 - a_1$ is lower whisker (left)
- 10) Open circle indicates any value that fall between the inner (f_1, F_1) and outer fences (f_3, F_3). These represent near (mild) outliers.
- 11) Astericks represent the values in the data that lie beyond the outer fences. These represent far (extreme) outliers.

Figure 4.2. Schematic representation of Box Whisker Plot (Horizontally left – right orientation)



SPSS version 19 was used for calculating distribution by Shapiro-Wilk and Jarque-Bera tests, and graphical distribution by histogram before and after removal of outliers. The Shapiro-Wilk test is most appropriate for small sample sizes (≤ 50 samples) although it can handle samples size up to 2000 (Mishra et al., 2019; <https://statistics.laerd.com>) Jarque-Bera test is the most effective normality test for

large samples >2000 (<https://www.itl.nist.gov>). Jarque Bera test was performed using Eviews Software. Graphical data representation was done by histogram and Normal Q-Q plot. Gender differences in lipid concentrations were analyzed by Student t test or Mann-Whitney U test (Altman, 1991; Gao and Smith, 2003).

4.15. The confounding variables and their influences in sample collection:

Patients from obstetrics and Gynecology, post-operative cases, psychiatry, cardiology, patients after intervention procedures such as angiogram, endoscopy and all Intensive Care Units patients were excluded from the study. The contributions from any one disease on the lipid profile of the large data base (n = 15603) will be minor. These confounding influences were reduced by increasing sample number as far as possible (n = 15603) and by removal of outliers. Confounding influences such as type 2 Diabetes Mellitus, hypertension, BMI, smoking, alcohol consumption, history of myocardial infarction and stroke, physical activity, and diet, cannot be excluded completely. This is because such information was not available in the data base. Almost all samples collected are from morning fasting database but, some postprandial samples after breakfast may be included in the database. These can cause minor confounding influences individually and rarely in combination.

However, for the second set of data collected for evaluating fasting and post prandial differences (n = 312) all those confounding variables mentioned such as Diabetes, hypertension, BMI, smoking, Alcohol consumption, decreased physical activity, variations in dietary habits were excluded.

The other possible confounding influences in this study were due to the influence of various other diseases and their treatment on the lipid profile. The possibility of confounding variables influence on data collection were minimized by applying inclusion and exclusion criteria mentioned in the same section.

CHAPTER 5

HISTOGRAM-BASED SUB-CLASSIFICATION OF SERUM TRIGLYCERIDES AND PROPORTION OF HYPERTRIGLYCERIDEMIA ACROSS GENDER AND AGE GROUPS: A CROSS-SECTIONAL STUDY

5.1. Abstract

Introduction: ICMR-INDIA B study-2014 and NCEP-2001 guidelines recommended a healthy cutoff of serum triglyceride as <150 mg/dL. This may be considered as the lower cut-off levels. ESC/EAS Guidelines-2019 have proposed an upper cut-off between moderate hypertriglyceridemia and severe hypertriglyceridemia at 885 mg/dL. There are no studies on the cut-off between, and prevalence of moderate and severe hypertriglyceridemia for the Indian population. These requirements are addressed by use of histogram method of calculating cut-off levels and frequency distribution of hypertriglyceridemia which are unique to this study and not reported earlier.

Objective: i) To sub-classify serum triglyceride concentration using histogram regions. ii) To calculate the percentage of hypertriglyceridemia from histogram regions, below and above the upper cut-off level, in the gender and age groups.

Materials and Methods: This was an Observational Cross-Sectional study of healthy and dyslipidemia conditions. Serum triglyceride data was collected from the Clinical Biochemistry Laboratory, Amala Institute of Medical Sciences from September 2018 to March 2022. Samples were partitioned according to gender, and age groups of <18 , 18 to 25, 26 to 50, and >50 years. Triglycerides were assayed by enzymatic method. Histogram was divided into healthy <150 mg/dL, positively skewed, and outlier regions. Sample distribution before and after outlier removal were represented by histogram, and analyzed by Shapiro-Wilk and Jarque-Bera tests. Gender differences were analyzed by Mann-Whitney U test using SPSS software.

Results: The sample number increased as age increased. In the histogram, sample number decreased sequentially from the healthy to positively skewed and to outlier region in all age groups. The p-value of distributions before removal of outliers were <0.05 in most of the gender-age groups. Males had higher mean triglycerides with p-value of <0.001 to 0.029, and had higher prevalence of hypertriglyceridemia in all groups. Using histogram, it was possible to differentiate between the highly prevalent multifactorial moderate hypertriglyceridemia from rare severe single gene defects.

Conclusions: The study partitioned triglyceride concentrations using histogram into

healthy, positively skewed, and an outlier region. Histogram of hypertriglyceridemia can be clinically used to differentiate between the highly prevalent, multifactorial, moderate hypertriglyceridemia from the rare, severe, monogenic hypertriglyceridemia.

5.2. Introduction

ICMR-INDIA B study-2014 and NCEP-2001 guidelines recommended the healthy cutoff level of serum triglyceride as <150 mg/dL (Joshi et al., 2014; NCEP) and have been used in the present study. European Cardiology Society and European Atherosclerosis Society Guidelines-2019 (Mach et al., 2020) have proposed moderate hypertriglyceridemia as 170 mg/dL to 885 mg/dL (2 to 10 mmol/L). Moderate hypertriglyceridemia is common and is due to multifactorial genetic, and environmental etiology such as family history, prevalence of heart diseases, dietary habits, stress and sedentary lifestyle (Remaley et al., 2012). Severe hypertriglyceridemia above 885 mg/dL are rare and are due to single gene defects [Mach et al., 2020; Hegele et al., 2014; Drone and Hegele, 2020]. Familial chylomicronemia syndrome patients presenting with milky serum will have severe hypertriglyceridemia (Drone and Hegele, 2020).

The prevalence of hypertriglyceridemia reported were high from India at 38.6% to 22.8% from four regions (Joshi et al., 2014) and from China at 16.5% (Qi et al., 2015) South Asians develop cardiovascular disease at a younger age than American population, 9.7% of South Asian men develop first AMI at ≤ 40 years (Nammi et al., 2014). The postprandial triglycerides of coronary artery disease patients were elevated (Manochehri and Moghadam, 2016). There are no studies on the gender differences, cutoff and prevalence of moderate and severe hypertriglyceridemia for the Indian population, which is the rationale for this study.

The clinical objective of the study was to sub-classify serum triglyceride concentration using histogram regions, also to calculate the percentage of hypertriglyceridemia from histogram regions in the gender and age groups for upper cutoff level. Histogram method of calculating proportion of patients with hypertriglyceridemia is unique to this study.

5.3. Objectives

To investigate the distribution of serum triglyceride levels based on histogram, determine cut-off level between moderate and severe hypertriglyceridemia and analyze their proportion in different age and gender groups.

5.4. Materials and methods

This is an **Observational Cross-Sectional study** of hypertriglyceridemia at Clinical Biochemistry Laboratory, Department of Biochemistry, Amala Institute of Medical Sciences. Fasting lipid profile samples from OP and IP Departments were collected from September 2018 to March 2022 continuously for randomization of lipid profile data. This study was approved by Institutional Research and Ethics Committees (Ref. AIMSIEC/42/2018- dated 19/10/2018) for the Department of Biochemistry and for the data collection from Clinical Biochemistry Laboratory.

Inclusion Criteria: Males and females without and with dyslipidemia and its complications were selected for the study. These individuals may have conditions such as diabetes mellitus, obesity, metabolic syndrome, hypertension, alcoholism, smoking, varying dietary habits, sedentary life style, stressed individuals, fatty liver, thyroid dysfunction, pancreatitis, and renal dysfunction.

Exclusion criteria: All disease conditions, except those defined above in the inclusion criteria, were excluded from the study. Individuals with history of infections, COVID, post-operative, fracture, injury, malignancy, Emergency and ICU samples were excluded.

5.4.1. Selection of Age Groups

Samples collected were partitioned into newborn, children and adolescents of age range 0 to < 18 (<18 years), young adults (18 – 25 years), middle age adults (26 – 50 years), and senior adults (>50 years). After initiation of study for three months, preliminary validation of the age groups was done from the data obtained: a) all four age groups showed gender differences. b) Each of the four age groups showed a

sequential increase in sample number. All samples were enrolled by **Indirect sampling** method, which was preferred over direct sampling from the population, due to the large sample size requirements for detection of rare lipid profile disorders, in a shorter duration with less expenditure (Horowitz, 2012).

5.4.2. Sample size requirement

Sample size was calculated by statistician using an earlier report on prevalence of dyslipidaemia in a multicentric study (Qi et al., 2015).

$$n = \frac{Z^2_{(1-\alpha)}pq}{d^2}$$

d = 20% prevalence; $\alpha = 0.05$; p = 24%; q = 1- p; $Z_{(1-\alpha)}$ value = 1.96; power 80%; n = 304.12.

Samples size for each group was rounded off to 304.

But as the work progressed, it was observed that much larger sample sizes were required to support the partitioning according to the two gender and four age groups ($2 \times 4 = 8$ groups), and to further partition the samples in each of these eight groups into three histogram regions which were the <150 mg/dL, positively skewed and outlier regions ($8 \times 3 = 24$ groups). The total sample number required was (304×24). But there was decreased sample availability in the <18 years age group, requiring further doubling of the sample number ($304 \times 24 \times 2 = 14,592$). Thus, our sample size of 15,603 fulfilled the sample requirement of each of the subgroups.

5.4.3. Calculation of proportion of Hypertriglyceridemia in percentage.

Proportion of individuals with hypertriglyceridemia, %

$$= \frac{\text{Sample number with hypertriglyceridemia}}{\text{Total sample number in the group}} \times 100$$

The above formula has been adapted with reference to a hospital data.

(reference: Sterne and Kirkwood, 2003)

5.4.4. Methodology

- a) Collect fasting Serum Triglyceride sample data from the Clinical Biochemistry Laboratory
- b) Evaluate the Serum Triglyceride distribution by histogram before and after outlier removal in samples partitioned according to age and gender
- c) Calculate the cut-off value between moderate and severe hypertriglyceridemia.
- d) Analyse the number and percentage of samples in different histogram regions.

5.4.5. Assay for serum Triglycerides were done with Vitros 5.1 FS autoanalyzer from Ortho Clinical Diagnostics. Serum sample (5.5 μ L) deposited on the dry chemistry slide, was evenly distributed by the spreading layer to the underlying layers. Triton X-100 surfactant in the spreading layer dissociated the triglycerides from lipoprotein complexes, were then hydrolyzed by lipase to yield glycerol and fatty acids. Glycerol diffused to the reagent layer, where it was phosphorylated by glycerol kinase and ATP to form L- α -glycerophosphate, which in the presence of L- α -glycerol-phosphate oxidase formed dihydroxyacetone phosphate and H₂O₂. Peroxidase and H₂O₂ oxidized the leuco dye to a colored product which was measured by reflectance spectrophotometry at 540 nm (Vitros 5.1FS, OCD). The cutoff range for hypertriglyceridemia in this study was ≥ 150 mg/dL (Joshi et al., 2014).

5.4.6. Statistical Methods: SPSS version 19 was used for calculating distribution by Shapiro-Wilk and Jarque-Bera tests, and graphical distribution by histogram before and after removal of outliers. The Shapiro-Wilk test is most appropriate for small sample sizes (≤ 50 samples) although it can handle samples size up to 2000. Jarque-Bera test is the most effective normality test for large samples > 2000 . Gender differences in lipid concentrations were analyzed by Mann-Whitney U test (Refer General Materials and Methods).

5.5. Results

A. Sample number, partitioning, and distribution before removal of outliers

Sample number increased sequentially in males and females from the age group <18 years to the age group >50 years. The male sample numbers were higher than that of females. Normality testing in males by Shapiro-Wilk test ($p < 0.001$) and Jarque Bera test ($p < 0.001$) showed, non-Gaussian distribution before and after log transformation due to positive skewing and presence of outliers. However, the female samples showed normal distribution in the two lower age groups after log transformation due to the lower number of outliers. There was consistency of the results from Shapiro-Wilk test and Jarque Bera test. Male sample <18 years of age showed larger variations in serum triglycerides compared to the female sample (Table 5.1).

There were gender differences in the mean triglyceride concentration in the three age groups up to 50 years due to higher mean triglyceride in males. In the age group >50 years, the mean triglyceride was nearly equal with 125.53 ± 75.23 mg/dL in males and 124.87 ± 64.89 mg/dL in females. But the high sample number resulted in a $p = 0.022$. The mean triglycerides decreased in men >50 years (Table 5.1).

The histogram of triglyceride concentrations showed positive skewing when the sample number increased at the positive side of the distribution plot ≥ 150 mg/dL in males and females, and indicated the presence of large number of moderate hypertriglyceridemia. As the triglyceride concentration further increased in the outlier region, the sample number decreased due to their rare occurrence (Fig. 5.1 and Table 5.2).

The positive skewing of the male samples could not be detected (Fig. 5.1 A and C) or appreciated (Table 5.1 E) due to horizontal compression of the histogram resulting from the presence of high triglyceride concentration outliers, which had to be removed to overcome the compression for better graphical appreciation of the histogram pattern.

The cut off value for the last positively skewed sample for males were 1093 mg/dL (<18 years), 494 mg/dL (18 – 25 years), 924 mg/dL (26 – 50 years) and 588 mg/dL (>50 years). For the female sample, they were much lower at 211 mg/dL (<18 years), 260 mg/dL (18 – 25 years), 483 mg/dL (26 – 50 years) and 463 mg/dL (>50 years). These results indicated that cut off value was higher in males and there was no general trend of increase or decrease of cut off values between moderate and high hypertriglyceridemia as age increased in males or females (Table 5.2).

The percentage of samples in the healthy region (<150 mg/dL) was higher in females of all three age groups <50 years. But the percentage of samples of serum triglycerides in males and females >50 years were nearly equal in the healthy, positively skewed and outlier regions (Table 5.2).

When the proportion of patients with hypertriglyceridemia in adult sample population (>18 years) was examined 32.00% of males and 24.61% of females had hypertriglyceridemia indicating a higher prevalence in males (Table 5.2).

B. Sample number, partitioning, and distribution after removal of outliers

To appreciate the histogram and positive skewing in a better way, outliers were removed in repeated steps in both males and females of all four age groups.

There were gender differences with higher mean triglyceride concentrations in males of age group <18, 18 – 25, and 26 – 50 years. In the age group >50 years, males and females had similar mean triglyceride values (121.72 mg/dL in males and 122.43 mg/dL in females). But the p value for gender difference was 0.015 due to the very high sample number in males and females. The number of outliers removed, and the sample number before and after removal of outliers in various age groups are given (Table 5.3). The total number of outliers removed (n) in repeated steps increased as age increased: <18 (Male n = 2, Female n = 3), 18 – 25 (Male n = 11, Female n = 3), 26 – 50 (Male n = 56, Female n = 17), >50 (Male n = 45, Female n = 25). All age groups showed positive skewing before log-transformation. After log-transformation, the three lower age groups showed normal distribution with Jarque-Bera tests (Table 5.3).

The positive skewing of the histogram in the male and female samples indicated the presence of large number of increased triglyceride values ≥ 150 mg/dL, which was almost a third of the total sample. As outliers were removed in repeated steps of 1 to 4 cycles, the triglyceride concentration of the last positively skewed sample markedly decreased after removal of outliers (Fig. 5.2).

5.6. Discussions

The clinical cause of positively skewed distribution ≥ 150 mg/dL is the high prevalence of moderate hypertriglyceridemia due to multifactorial causes; the presence of outliers indicated the lower prevalence of severe hypertriglyceridemia due to single gene defects.

As the triglyceride concentration increased with age, the number of outliers also increased with age. Range between the last positively skewed triglyceride concentration and the last outlier was the reference range of severe rare hypertriglyceridemia for that particular group. The reasons for removal of outliers were as follows: a) to bring out the outliers embedded in the positively skewed region, b) identify the total number of outliers, c) to find out the cutoff between the positively skewed region and the outlier region, and d) to relieve compression of the histogram.

Diet, exercise (Torres et al., 2015, Wang and Xu, 2017), insulin resistance, and obesity (Klop et al., 2013)) cause biochemical changes that can influence the lipid profile, and glucose levels (Ormazabal et al., 2018). There was a close correlation between insulin resistance and triglyceride concentration (Ma et al., 2020). Hypertriglyceridemia increase LDL-Cholesterol and decrease HDL-Cholesterol (Remaley et al., 2012). The prevalence of type 2 diabetes mellitus has been reported to be high in the India population (Pradeep and Mohan, 2021). Studies in four different regions showed highest hypertriglyceridemia in Chandigarh (38.6%) and least in Maharashtra (22.8%). The percentages were higher in urban compared to rural areas in Jharkhand and Chandigarh (Joshi et al., 2014) Kerala study-2018, on hypertriglyceridemia prevalence in rural and urban areas were 29.7% and 36.5% in men >30 years, 16.4% and 17.1% in women, respectively (Harikrishnan et al., 2018). In the present report, prevalence of fasting hypertriglyceridemia was at 33% in males

and 24.61% in females by indirect sampling. There were studies on the prevalence of hypertriglyceridemia in Russia (Karpov and Khomitskaya, 2015) and in India (Joshi et al., 2014), where there was no mention about the positive skewing, the range of outlier concentrations, or prevalence in the different regions of the histogram or age groups.

The sample number decreased to one sample in the outlier region of the groups <18 and 18 – 25 years of age in males, justifying the need for sample number of 15,603. Sample number increased sequentially from <18-year to >50-year groups due to increased patient visits to the hospital and increased laboratory tests as age increased.

The three existing classifications of dyslipidemia are the increase or decrease in concentrations of lipoproteins, apolipoproteins and the cut off levels for individual lipids. The major individual lipids are Triglycerides, Total Cholesterol, LDL-Cholesterol, and HDL-Cholesterol (Remaley et al., 2012). There were studies on isolated and combined increase in individual lipids (Joshi et al., 2014; Remaley et al., 2012); the present study differs by clinically partitioning the triglyceride distribution into three histogram regions of healthy, moderate and severe hypertriglyceridemia. The clinical significance of this partitioning is the differentiation between moderately prevalent multifactorial and rare single gene defects. The major source of serum triglycerides in the fasting sample is VLDL and in the postprandial sample, there is also a contribution by chylomicrons (Remaley et al, 2012). The fasting sample lipid concentration was more stable as there were no influences by dietary lipids. Thus, the fasting sample reduced the variations in serum triglycerides.

The onset of menopause is around 50 years. Males and Females >50 years showed similar triglycerides concentrations. The higher mean triglycerides in men <50 years may be related to sex hormones (Wang et al., 2011).

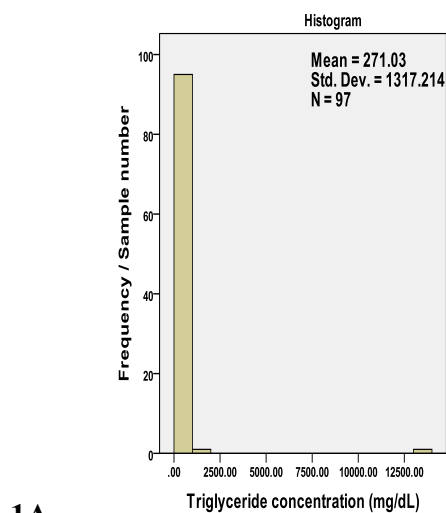
5.7. Conclusions

A statistical and clinical method of classification of triglyceride concentration was proposed. Histogram of hypertriglyceridemia can be clinically used to differentiate between highly prevalent, multifactorial, moderate hypertriglyceridemia from the rare, severe, monogenic hypertriglyceridemia. Genetic and DNA sequence data analysis of the multifactorial and single gene defects are not done, and are topics for future studies. Hormonal influence of triglyceride concentrations resulting in gender differences in individuals between 18 and 50 years of age is another area for future study.

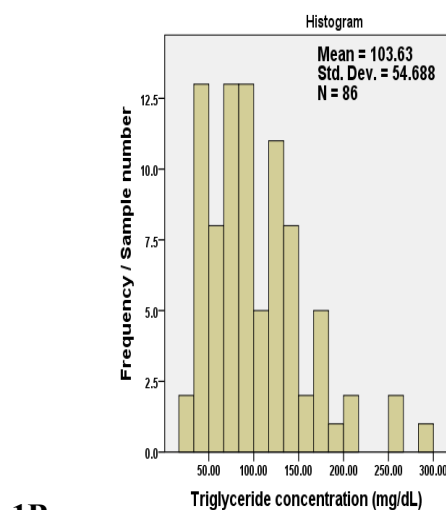
A. Sample number, partitioning, and distribution before removal of outliers**Table 5.1.** Shapiro-Wilk/Jarque Bera methods of calculation of Triglyceride (mg/dL) distribution before and after log transformation, and the Gender difference before removal of outliers.

Samples number, (Total n = 15603)	Triglyceride Mean±SD Min –Max	Triglyceride distribution Shapiro – Wilk (Jarque Bera) tests, P		Gender Difference, Mann- Whitney U Test (P)
		Log transformation		
		Before	After	
Age group < 18 years				
Male (n = 97)	271.03±1317.21 26 – 13053	<0.001 (<0.001)	<0.001 (<0.001)	0.029
Female (n = 86)	103.63±54.69 28 – 299	<0.001 (<0.001)	0.521 (0.537)	
Age group 18 - 25 years				
Male (n = 210)	170.72±532.06 27 – 7749	<0.001 (<0.001)	<0.001 (<0.001)	<0.001
Female (n = 159)	102.85±61.53 25 – 388	<0.001 (<0.001)	0.359 (0.400)	
Age group 26 – 50 years				
Male (n = 3247)	170.50±145.04 32 – 3922	<0.001 (<0.001)	<0.001 (<0.001)	<0.001
Female (n = 1921)	126.57±82.01 16 – 1264	<0.001 (<0.001)	<0.001 (<0.001)	
Age group> 50 years				
Male (n = 5925)	125.53±75.23 13 – 1116	<0.001 (<0.001)	<0.001 (<0.001)	0.022
Female (n = 3958)	124.87±64.89 30 – 1131	<0.001 (<0.001)	<0.001 (<0.001)	

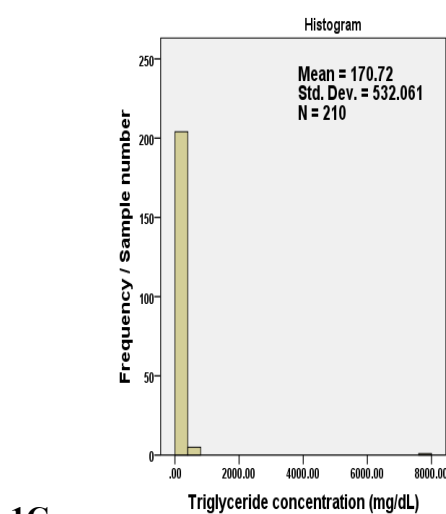
Figure 5.1. Histogram of the sample distribution of serum triglycerides (mg/dL) in various gender-age groups before outlier removal. (X axis: Triglyceride concentration (mg/dL), Y axis: Frequency of sample number)



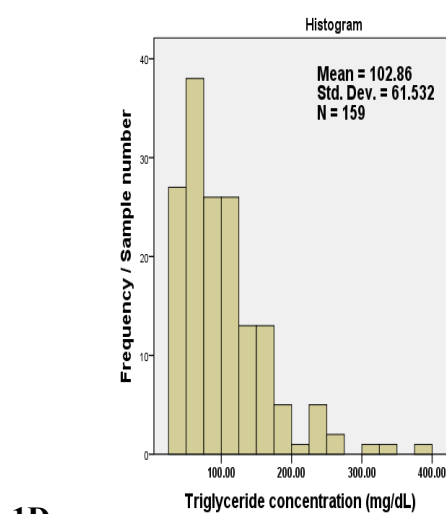
Male <18 years (n= 97)



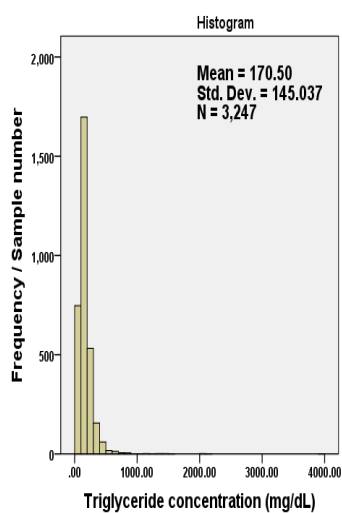
Female <18 years (n = 86)



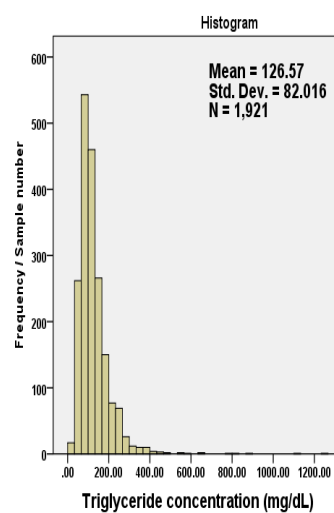
Male 18 – 25 years (n = 210)



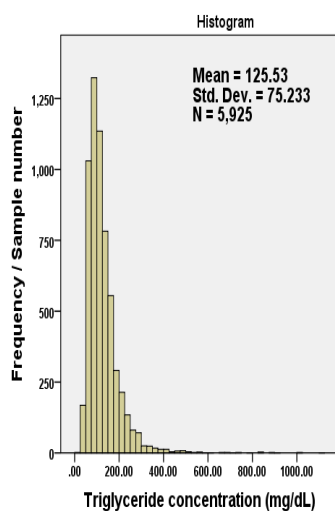
Female 18 – 25 years (n = 159)



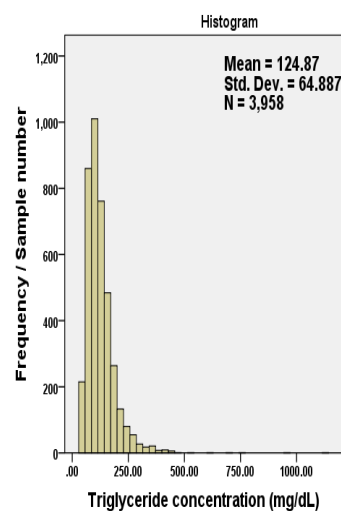
1E. Male 26 – 50 years (n = 3247)



1F. Female 26 – 50 years (n = 1921)



1G. Male >50 years (n = 5925)



1H. Female >50 years (n = 3958)

(Figure 5.1 Continuation)

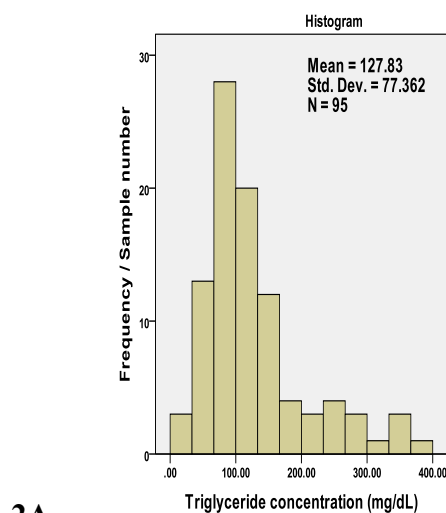
Table 5.2. Sample number and percentage (%) in the histogram regions– Healthy, positively skewed, and Outlier regions of serum triglyceride in various groups.

Age group, Sample number (%) in males (M) and females (F)	Sample number and % in the histogram regions
	a) Healthy TG <150 mg/dL
	b) 150 mg/dL to last positively skewed sample
	c) outlier region
<18 years M, n = 97(100%) F, n = 86(100%)	M: <150 (69, 71.13%) F: <150 (73, 84.88%)
	M: 150 –1093 (27, 27.83%) F: 150 –211 (10, 11.62%)
	M: >1093 –13053 (1, 1.03%) F: >211–299 (3, 3.48%)
18–25 Years M, n = 210 (100%) F, n = 159 (100%)	M: <150 (145, 69.04%) F: <150 (130, 81.76%)
	M: 150 – 494 (64, 30.47%) F: 150 – 260 (26, 16.35%)
	M: >494 – 7749 (1, 0.47%) F: >260 – 388 (3, 1.88%)
26–50 years M, n = 3247(100%) F, n =1921(100%)	M: <150 (1794, 55.25%) F: <150 (1426, 74.23%)
	M: 150 – 924 (1440, 44.34%) F: 150 – 483 (485, 25.24%)
	M: >924 – 3922 (13, 0.40%) F: >483 – 1264 (10, 0.52%)
>50 years M, n = 5925(100%) F, n =3958(100%)	M: <150 (4440, 74.93%) F: <150 (2996, 75.69%)
	M: 150 – 588 (1467, 24.75%) F: 150 – 463 (956, 24.15%)
	M: >588 – 1116 (18, 0.30%) F: >463–1131(6, 0.15%)
Hypertriglyceridemia >18 years M, n = 3003 F, n = 1486	Proportion of patients with hypertriglyceridemia (%) M = 32.00% F = 24.61%

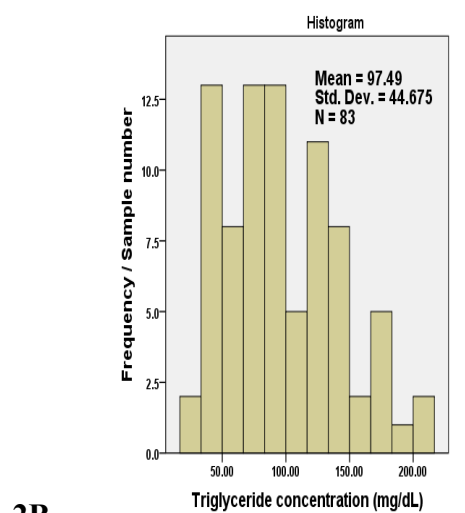
B. Sample number, partitioning, and distribution after removal of outliers**Table 5.3.** Triglyceride (mg/dL) distribution and gender difference after removal of outliers by histogram.

Sample number (n)		Outliers removed by histogram (cycles)	Triglyceride Mean±SD Min –Max	Triglyceride distribution Shapiro – Wilk (Jarque Bera) tests, P		Gender Difference, Mann - Whitney U test (P)
Outlier removal				Log transformation		
Before	After			Before	After	
Samples, age <18 years						
M – 97	M – 95	2 (2)	127.83±77.36 26 – 376	<0.001 (<0.001)	0.562 (0.942)	0.006
F – 86	F – 83	3 (1)	97.49±44.67 28 – 211	<0.001 (0.092)	0.079 (0.211)	
Samples, age 18 – 25 years						
M – 210	M –199	11 (3)	121.28±59.95 27 – 298	0.001 (<0.001)	0.090 (0.196)	<0.001
F – 159	F – 156	3 (1)	98.28±52.10 25 – 260	0.001 (<0.001)	0.149 (0.274)	
Samples, age 26 -50 years						
M –3247	M -3191	56 (4)	157.86±79.98 32 – 486	<0.001 (<0.001)	<0.001 (0.170)	<0.001
F – 1921	F – 1904	17 (3)	121.93±61.85 16 – 411	<0.001 (<0.001)	0.003 (0.575)	
Samples, age > 50 years						
M –5925	M –5880	45 (3)	121.72±59.57 13 – 430	<0.001 (<0.001)	<0.001 (<0.001)	0.015
F – 3958	F – 3933	25 (2)	122.44±55.47 30 – 379	<0.001 (<0.001)	<0.001 (0.001)	

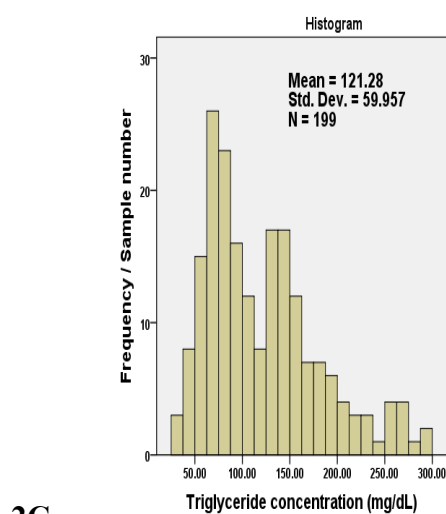
Figure 5.2. Histogram of the sample distribution of serum triglycerides (mg/dL) in various groups after outlier removal. (X axis – Triglyceride concentration (mg/dL), Y axis – Frequency of sample number)



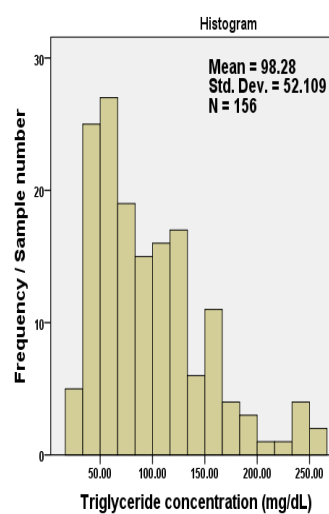
Male <18 years (n = 95)



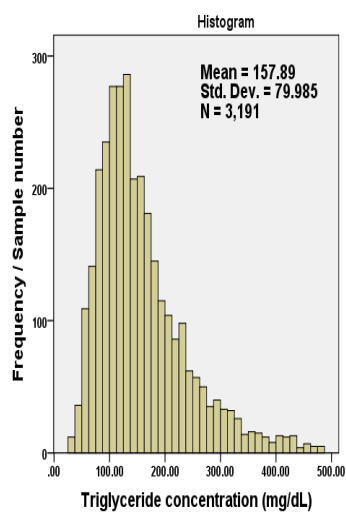
Female <18 years (n = 83)



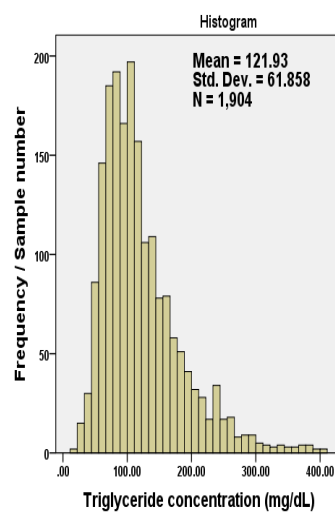
Male 18 – 25 years (n = 199)



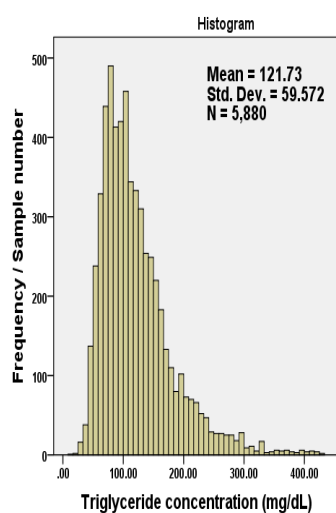
Female 18 – 25 years (n = 156)



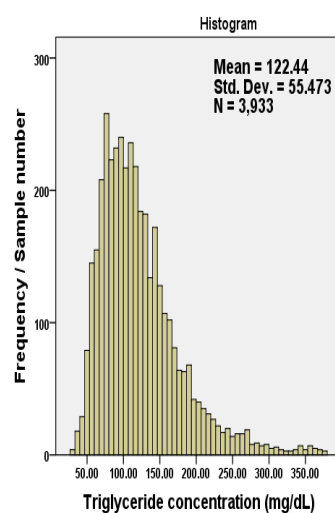
2E. Male 26 – 50 years (n = 3191)



2F. Female 26 – 50 years (n = 1904)



2G. Male >50 years (n = 5880)



2H. Female >50 years (n = 3933)

CHAPTER 6

BOX-WHISKER PLOT USED FOR PARTITIONING SERUM HYPERTRIGLYCERIDEMIA ACCORDING TO QUARTILES AND FENCES TO IDENTIFY GENDER AND AGE-RELATED DIFFERENCES AND SIMILARITIES – A CROSS-SECTIONAL STUDY

6.1. Abstract

Introduction: The Box–Whisker plot is a method of distribution of data series to give a graphical illustration of the dispersion, central tendency, and direction of skewness and the presence of outliers. Earlier we had used histogram (Chapter 5) as a method of sub classifying triglyceride concentration after partitioning the sample according to gender and age. The triglyceride concentration in each of the gender /age group was analyzed using Box-Whisker plot.

Objective: To evaluate the distribution, gender disparities, and the proportion of hypertriglyceridemia in relation to median, quartiles and fences using Box-Whisker plots

Methods: This observational cross-sectional study evaluated serum triglyceride concentrations in individuals with and without dyslipidemia. Samples were collected from the Clinical Biochemistry Laboratory through indirect sampling between September 2018 and March 2022. The large dataset enabled stratification by gender, followed by age groups (<18, 18–25, 26–50, and >50 years), and further division into statistical quartiles and outlier regions using Box-Whisker plots. Continuous multi-week sampling minimized selection bias across gender, age, and patient type (IP/OP). Triglyceride levels were measured using an enzymatic method on a dry chemistry autoanalyzer. Data analysis was performed in SPSS, employing Shapiro-Wilk, Jarque-Bera, Mann-Whitney U tests, and Box-Whisker visualizations.

Results: Box-Whisker plot of serum triglyceride concentration in males and females of all four age groups showed positive skewing and confirmed by Shapiro-Wilk test. The compression of Box-Whisker plot was relieved by removal of outliers in repeated cycles. Number of samples collected rose with increasing patient age. In each distribution plot, the sample number decreased as the concentration of triglyceride increased. The least sample numbers were found in the outlier region. After removal of outliers, samples >18 years still showed positive skewing in all the groups indicating large number of moderate hypertriglyceridemia, especially in the male sample. Males showed higher triglyceride concentrations in the three groups up to 50 years. But in the age group >50 years the gender differences were low due to decrease

in triglycerides in males and increase in females. The triglyceride concentration ranges in different quartiles were higher in males than in females in almost all age groups. The high triglyceride concentration far outlier sample percentage was higher in the male sample of all age groups.

Conclusions: A statistical and clinical method of classification of triglyceride concentration with quartiles, fences and outliers of Box-Whisker plot was proposed. Outliers were sub partitioned into near and far outliers. The concentrations greater than upper whiskers (a_3) are cases of hypertriglyceridemia. Box Whisker Plot of hypertriglyceridemia can be clinically used to differentiate between highly prevalent, multifactorial, moderate hypertriglyceridemia from the rare, severe, monogenic hypertriglyceridemia.

6.2. Introduction

The Box – Whisker plot is a method of distribution of data series to give a graphical illustration of the dispersion, central tendency, direction of skewness and the presence of outliers (Gurumani, 2005). Triglyceride concentrations in the gender and the four age groups were analyzed by histogram in chapter 5 and by Box – Whisker plot in the present chapter. From the distribution analyzed by histogram, it was possible to differentiate the highly prevalent moderate hypertriglyceridemia from the rare severe hypertriglyceridemia. The severe hypertriglyceridemia is considered as outliers of the histogram distribution. While Box-Whisker plot method uses centiles and quartiles of the triglyceride concentration in the ascending order, the histogram uses the concentration of triglyceride plotted against the sample number. The advantage of Box-Whisker plot over histogram was the sub-partitioning of the outliers into near and far outliers. Box-Whisker plot sub-partitioning is a statistical method to further divide the outliers according to clinical severity of increased triglyceride concentration. There are several studies using Box Whisker plot to display distribution of triglyceride levels in patients with hypertriglyceridemia (Goldberg et al., 2011; Haffner et al., 2012; Tonduti et al., 2013). The Box-Whisker plot method is used for verification of the partitioning of hypertriglyceridemia by histogram.

6.2.1. The components of Box-Whisker plot include:

- Box: represent the Interquartile Range (IQR) which contains 50% of data. Top of the box represents the 75th percentile (Q_3), and the bottom represents 25th percentile (Q_1)
- Whiskers: Represent the range of the data, extending from the box to the minimum and maximum values.
- Median: Represented by the line within the box, indicating the middle value of the data
- Outliers: Data points that fall outside the whiskers, indicating extreme values

6.2.2. Interpreting the Box-Whisker plot for triglyceride distribution:

- Central Tendency: The median triglyceride can be identified from the plot.
- Variability: The length of the box and whiskers indicates the spread of triglyceride levels.
- Skewness: if the box is not symmetric, it may indicate skewness in the triglyceride distribution.
- Outliers: the presence of outliers can indicate extreme triglyceride levels.

Box-Whisker plot provides visual representation of triglyceride distribution and thereby making it easier to understand and identify patterns and trends (Williamson et al., 2012) These plots can also be used for comparison of triglyceride levels between different groups (Krzywinski et al., 2013). Box-Whisker plot can be used in identification of outliers, which is important in identifying individuals with extreme triglyceride values (Tukey et al., 1977). After finding outliers in triglyceride distribution using a Box-Whisker plot, it is possible to perform sensitivity analysis to determine how the results change when outliers are included or excluded (Sterne et al., 2011). A study published in the Journal of Clinical Lipidology highlighted the importance of sensitivity analysis in improving model robustness and reducing the

impact of outliers (Goldberg et al., 2011). Researchers in a study published in the journal of statistical Computation and Simulation demonstrated that sensitivity analysis can increase confidence in the results by evaluating the stability of the estimates (Zhang et al. (2012). Sensitivity analysis is important in informing clinical decision-making by providing an accurate understanding of the data (Wood et al., 2013).

6.3. Objectives

To evaluate the distribution, gender disparities, and the proportion of hypertriglyceridemia in relation to median, quartiles and fences using Box-Whisker plots

6.4. Materials and Methods

This observational cross-sectional study on hypertriglyceridemia was conducted at the Clinical Biochemistry Laboratory, Department of Biochemistry, Amala Institute of Medical Sciences. Fasting lipid profile samples were collected from both outpatient (OP) and inpatient (IP) departments between September 2018 and March 2022. The study was carried out following approval from the Institutional Research and Ethics Committees (Ref. AIMSIEC/42/2018, dated 19/10/2018).

Inclusion Criteria:

Participants included both males and females, with or without dyslipidemia and its associated complications. Subjects may have had comorbidities such as diabetes mellitus, obesity, metabolic syndrome, hypertension, alcoholism, thyroid disorders, pancreatitis, smoking habits, unhealthy dietary patterns, sedentary lifestyles, psychological stress, fatty liver disease, or renal dysfunction.

Exclusion criteria:

Subjects were excluded if they had conditions outside those listed in the inclusion criteria. This included individuals with infections, a history of COVID-19, post-operative status, fractures, injuries, malignancies, or those whose samples were collected in emergency or intensive care settings.

6.4.1. Selection of Age Groups

Samples were categorized into four age groups:

- Newborns, children, and adolescents (<18 years)
- Young adults (18–25 years)
- Middle-aged adults (26–50 years)
- Senior adults (>50 years)

An initial validation of this classification was performed after three months of data collection, showing:

- a) Gender-specific differences were evident across all age groups.
- b) A progressive increase in sample size was observed with each ascending age group.

An indirect sampling method was employed instead of direct population-based sampling due to the large sample size needed to detect rare lipid disorders efficiently and cost-effectively (Horowitz, 2012).

6.4.2. Sample size requirement

Sample size was determined using prevalence data from a previous multicentric study on dyslipidemia (Qi et al., 2015).

$$n = \frac{Z^2_{(1-\alpha)}pq}{d^2}$$

Using the following parameters:

- Estimated prevalence (p) = 24%
- Complement (q) = 76%
- Confidence level (α) = 0.05
- Z-score ($Z_{1-\alpha}$) = 1.96

- Desired precision (d) = 20%
- Power = 80%

The initial calculated sample size was 304.12, rounded to 304 participants per group.

However, as the study progressed, it became clear that a larger sample was needed to allow stratification by both gender and age (2 genders $\times$ 4 age groups = 8 groups), and further by lipid profile histogram regions (<150 mg/dL, positively skewed, and outliers), resulting in 24 subgroups (8 $\times$ 3). Thus, the total required sample size was $304 \times 24 = 7,296$.

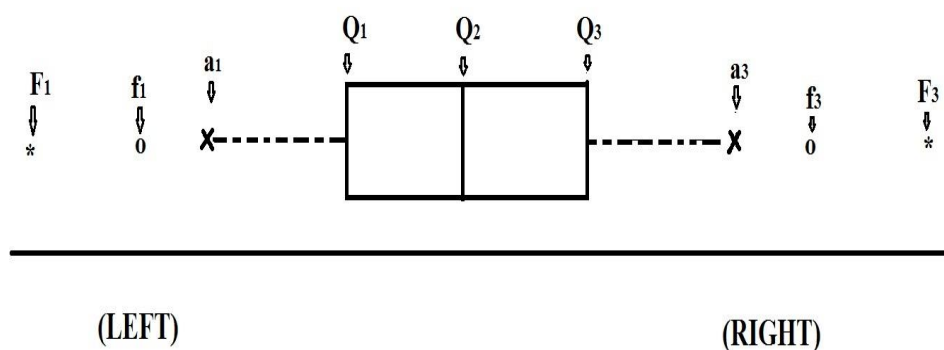
Due to lower sample availability in the <18 age group, the sample size was further doubled, resulting in a total target of 15,603 samples ($304 \times 24 \times 2$). This final sample size met the statistical requirements for analysis across all stratified subgroups.

The same sample set used in the histogram analysis (Chapter 5) was applied in this chapter.

6.4.3. Box-Whisker plot: Diagrammatic representation of triglyceride data by means of Box Whisker plot gives visual information about dispersion, central tendency, outliers, and extent and direction of skewness (Gurumani, 2005). The reference Box-Whisker plot is symmetrically organized (Fig. 6.1).

6.4.3.1. Construction of Box-Whisker plot (Refer General Materials and Methods and Fig. 6.1)

Figure 6.1. Schematic representation of Box Whisker Plot (Horizontally left – right orientation)



6.4.4. Methodology:

- a. Fasting Serum Triglyceride sample data collection from the Clinical Biochemistry Laboratory
- b. Assess distribution using Box-Whisker plots before and after outlier removal in samples, partitioned according to age and gender.
- c. Calculate the cutoff value between moderate and severe hypertriglyceridemia.
- d. Analyze the number and percentage of samples in different Box-Whisker plot regions.

6.4.5. Assay for serum Triglycerides were done with Vitros 5.1 FS autoanalyzer from Ortho Clinical Diagnostics (Refer general materials and methods). The cutoff range for hypertriglyceridemia in this study was ≥ 150 mg/dL (Joshi et al., 2014).

6.4.6. Statistical Methods: SPSS version 19 was used for calculating distribution by Shapiro-Wilk and Jarque-Bera tests, and graphical distribution by Box-Whisker plot before and after removal of outliers. Gender differences in lipid concentrations were analyzed by Mann-Whitney U test (Refer General materials and methods).

6.5. Results

A. Partitioning and Distribution of Serum Triglycerides before removal of outliers

Samples used in this study ($n = 15603$) were the same as that used in the histogram method (Chapter 5). Patient number increased sequentially from the lowest age group (<18 years) to the highest age group (>50 years). The increase in sample number with age indicated increased hospital visits as age increased.

Box-Whisker plot of serum triglycerides distribution of each partitioned gender-age group

Box Whisker plot of the distribution of serum triglyceride concentration in males and females of the four age groups showed positive skewing in the triglyceride values (Fig. 6.2). The Box-Whisker plot was vertically compressed in all the male age groups (Fig. 6.2A, 2C, 2E, and 2G) and in the two female age groups >25 years of age (Fig. 6.2F, 2H). The vertical compression of the Box-Whisker plots was due to the presence of outliers, making it difficult to graphically appreciate the distribution patterns such as positive skewing, position of various fences and quartiles (Fig.6.2). Therefore, the calculated distribution characteristics were used to identify the distribution pattern as given in Table 1 of the previous chapter 5.

As the same sample ($n = 15603$) was used as in the previous chapter 5, the calculated distribution characteristics by Shapiro-Wilk test, Jarque Bera test, minimum-maximum, $\text{mean} \pm \text{SD}$, Mann-Whitney U test are same as that in the earlier related chapter (Table 5.1 of chapter 5).

Box-Whisker plot was used to calculate the number and percentage of samples in the fences and quartiles.

a) Similarities in the distribution of triglycerides in males and females

The lower whisker, $a_1 - Q_1$, $Q_1 - Q_2$ and $Q_2 - Q_3$ regions showed almost similar percentage of samples in all groups. For example, in age group <18 years, the lower whisker, $a_1 - Q_1$, $Q_1 - Q_2$ and $Q_2 - Q_3$ regions showed almost similar percentage in

males (25.7%, 24.7% and 25.7%) and females (25.6%, 25.6% and 24.4%) (Table 6.1). In the male age group <18 years, the total sample number is 97, the number of samples in the lower whisker ($a_1 - Q_1$) was 25 and the percentage was $(25/97 \times 100 = 25.7\%)$ (Table 6.1).

The upper whisker, the upper near outliers and upper far outliers showed a decrease in trend in sample number and percentage of samples of all gender and age groups. The above results indicated a general trend in the sample distribution of all age groups in males and females. There were no samples in lower near outliers and lower far outliers in any of the age groups (Table 6.1).

b) Differences in the distribution of triglycerides in males and females

In the Box-Whisker plot, the ranges of triglyceride concentrations from the lower whisker ($a_1 - Q_1$) up to upper far outliers ($>F_3$) had higher triglyceride ranges in the male samples compared to that of the female sample in all the age groups up to 50 years. As an example, in the male age group <18 years, the triglyceride ranges ($a_1 - Q_1$) = $77 - 26 = 51$; ($Q_1 - Q_2$) = $107 - 77 = 30$; ($Q_2 - Q_3$) $160 - 107 = 53$. The upper near and far outliers' percentage were higher in most of the male samples of all age groups (Table 6.1).

Above 50 years, the upper whisker, upper near outliers and upper far outliers showed almost similar values in both males and females.

These results indicated that there were differences in various partitioning of triglycerides in males and females of different age groups (Table 6.1).

B. Box Whisker plot of Serum Triglycerides after removing the outliers.

When the outliers were removed, the hidden outliers were exposed from the Box Whisker plot. This was done in repeated cycles till there were no more outliers remaining. The total number of outliers, the number of outliers removed, and the number of repeated cycles of outlier removal are given in Table 6.2. As an example, in the male age group <18 years, the sample numbers were 97 before, and 83 after removal of outliers. The total number of outliers removed was 14 in three cycles.

When the outliers were removed the distribution was better appreciated graphically (Fig. 6.3). After removing all the near and far outliers using Box-Whisker plot, the sample distribution showed moderate positive skewing in all age groups (Table 6.2 and Fig. 6.3). The upper limit of minimum–maximum was similar in males and females of <18 years and >50 years. But, in the age groups from 18 – 25 and 26 – 50 years, there were higher upper limit of triglycerides in males than in females of the same age group. The mean triglyceride concentration in the four age groups in males and females were less than 150 mg/dL (Table 6.2). The minimum – maximum extended from the healthy region to the level of mild hypertriglyceridemia. As an example, the minimum – maximum in the males of age group <18 years was 26 to 213 mg/dL. The minimum is within the healthy range of <150 mg/dL and the maximum, 213 mg/dL was in the range of hypertriglyceridemia (Table 6.2).

The median in all age groups of males and females also fall within the healthy range. The Shapiro Wilk test showed significant deviation from normal distribution in males and females of almost all age groups. But after log transformation, the males and females of the age group <18 years only showed p values >0.05. All other three age groups could not be log transformed to normal distribution (Table 6.2) due to positive skewing even after removal of outliers. This may be due to the large percentage of mild and moderate hypertriglyceridemia in the three age groups above 18 years (Table 6.2).

6.6. Discussions

The statistical method of partitioning with quartiles, fences and outliers of Box-Whisker plot distribution was used in this study. Outliers were sub partitioned into near and far outliers. The healthy cutoff value for triglycerides was <150 mg/dL (NCEP, 2001; Mach e al., 2020). The abnormal triglyceride concentrations were grouped by Box-Whisker plot as upper box ($Q_2 - Q_3$), upper whisker ($Q_3 - a_3$), upper near outlier ($f_3 - F_3$), and upper far outlier regions ($>F_3$) (Table 6.1).

As the concentrations of triglycerides increased from the healthy cutoff value, the sample number decreased, with the lowest sample number in the far outlier region (Table 6.1 and Fig.6.2). The outlier region represents high concentration of

triglycerides that are rare and are generally contributed by single gene defects (Mach et al., 2020). These outlier regions are the upper near outliers ($f_3 - F_3$) and upper far outliers ($>F_3$). The concentrations greater than upper whisker (a_3) are rare cases of hypertriglyceridemia. The a_3 concentrations range from 211 mg/dL to 341 mg/dL in the four age groups (Table 6.1). The multifactorial hypertriglyceridemia ranges from 150 mg/dL to <885 mg/dL (Mach et al., 2020). Therefore, the Box-Whisker plot partitions multifactorial moderate hypertriglyceridemia disease states at concentrations >341 mg/dL as multifactorial disease category. The percentage of samples decreased sharply at concentrations >341 mg/dL or $>a_3$. The Box-Whisker plot distribution of triglyceride concentration can be used to calculate the prevalence of hypertriglyceridemia in a partitioned distribution for a particular gender-age group. The proportion of hypertriglyceridemia decreased from the upper whisker to the upper near outlier regions and further decreased in the upper far outlier regions in the four age groups (Table 6.1).

The histogram method may be used to graphically appreciate the relative abundance of moderate hypertriglyceridemia in the positively skewed region from the rare inherited metabolic disorders which may be observed in the outlier region. The Box-Whisker plot of partitioning serum triglycerides was according the median, quartiles and fences, that were used to identify near and far outliers.

It is important to understand positively skewed distribution of triglyceride for accurate clinical interpretation and risk assessment. Accurate interpretation of triglyceride levels is crucial for diagnosing and managing conditions like hypertriglyceridemia (Berglund et al., 2014). Triglyceride levels are a component of cardiovascular risk assessment, and understanding their distribution is essential for accurate risk stratification (Arnett et al., 2019). The methods of partitioning hypertriglyceridemia have important clinical significance that are related to risks of atherosclerosis, xanthoma, xanthelasma, hyperlipoproteinemia, fatty liver, steatohepatitis and cirrhosis liver (Remaley et al., 2012).

The outlier regions were found to vary in males and females of the four age groups. The lower limits of outlier regions increased as age increased. The mean

triglyceride concentration increased in males in the three age groups up to 50 years after removal of outliers, but in males >50 years of age the mean decreased. Clinical interpretation of outliers involves consulting clinical guidelines to determine the clinical significance of outliers (Goldberg et al., 2011). The underlying cause of outliers can be determined by further testing such as genetic testing or imaging studies (Ginsberg et al., 2011)

Factors contributing to positive skewness of triglycerides includes,

a) Biological variation: Triglyceride levels can fluctuate greatly due to factors like physical activity and hormonal changes (Nordestgaard et al., 2010). **b) Metabolic processes:** Triglyceride metabolism involves multiple pathways, leading to a wide range of values (Goldberg et al. (2011). **c) Disease prevalence:** Conditions like hypertriglyceridemia, diabetes, and obesity can contribute to elevated triglyceride levels, skewing the distribution (Miller et al., 2011).

6.7. Conclusions.

A statistical and clinical method of classification of triglyceride concentration with quartiles, fences and outliers of Box-Whisker plot was proposed. Outliers were sub partitioned into near and far outliers. The concentrations greater than upper whisker (a_3) are cases of moderate hypertriglyceridemia. Box-Whisker Plot of hypertriglyceridemia can be clinically used to differentiate between highly prevalent, multifactorial, moderate hypertriglyceridemia from the rare, severe, monogenic hypertriglyceridemia. Genetic and DNA sequence data analysis of the multifactorial and single gene defects are not done, and are topics for future studies. Hormonal influence of triglyceride concentrations resulting in gender differences in individuals between 18 and 50 years of age is another area for future study.

A. Partitioning and Distribution of Serum Triglycerides before removal of outliers

Table 6.1. Sample number and percentage (%) from Box Whisker plot in the skewed and outlier region of serum triglycerides (mg/dL) distribution data in samples of different age

Partitioned regions of Box Whisker plot	Triglyceride concentration (mg/dL)		Sample number in the partitioned regions	
	M	F	M	F
<18 years: Male (n = 97); Female (n = 86): Median: Male – 107; Female – 93				
Lower Near outliers ($F_1 - f_1$)	(-170.5– -47)	(-147 – -42)	0	0
Lower Far outliers ($<F_1$)	$> (-170)$	$> (-147)$	0	0
Lower Whisker ($a_1 - Q_1$)	26– 77 (51)	28– 63 (35)	25 (25.7%)	22 (25.6%)
$Q_1 - Q_2$	77– 107 (30)	63– 93 (30)	24(24.7%)	22 (25.6%)
$Q_2 - Q_3$	107– 160 (53)	93 – 133 (40)	25(25.7%)	21 (24.4%)
Upper Whisker ($Q_3 - a_3$)	160– 273 (113)	133– 211 (78)	15 (15.4%)	18 (20.9%)
Upper Near outliers ($f_3 - F_3$)	284 – 408(124)	238– 343 (105)	6 (6.2%)	3 (3.5%)
Upper Far outliers ($>F_3$)	>408	>343	2 (2.1%)	0

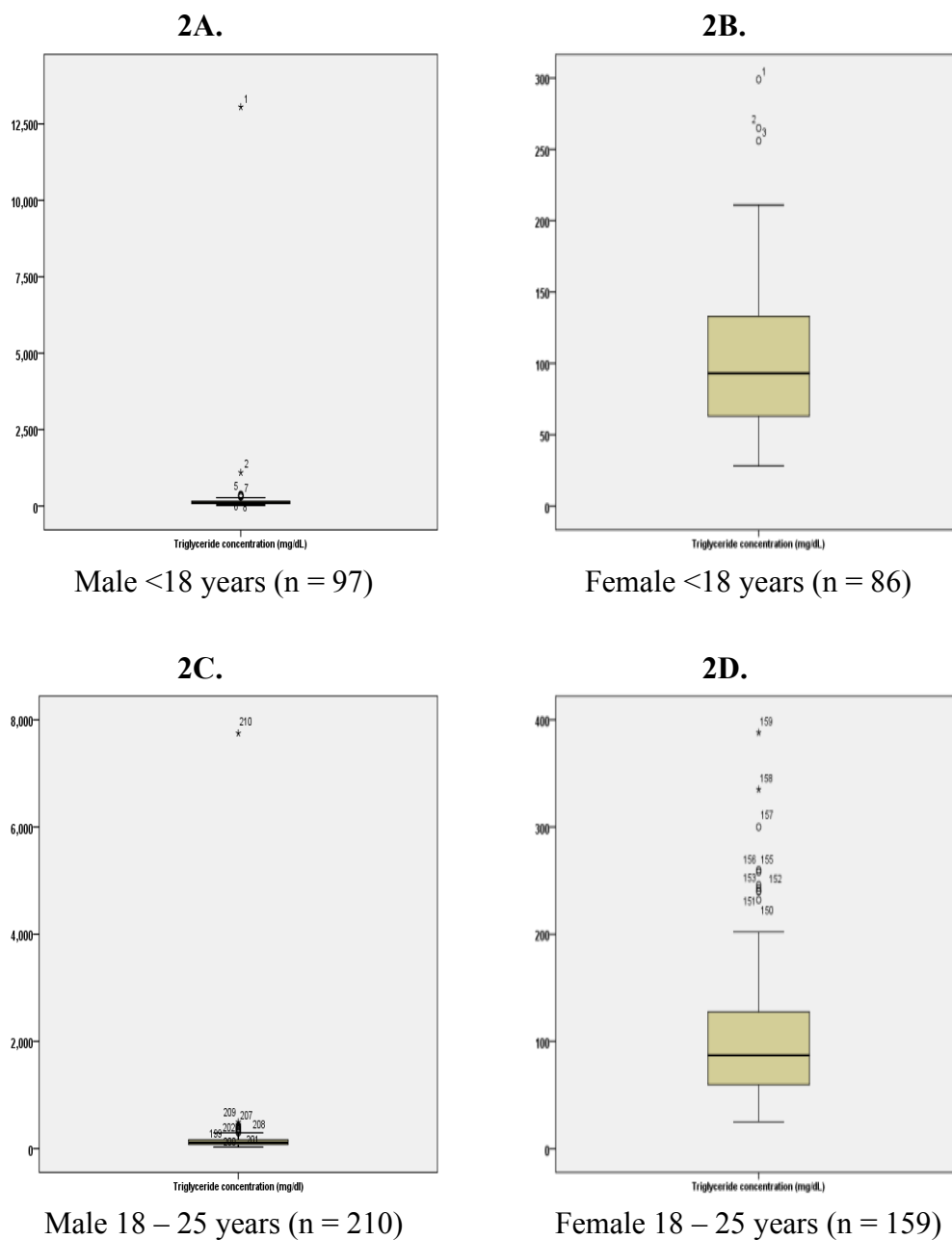
Partitioned regions of Box Whisker plot	Triglyceride concentration (mg/dL)		Sample number in the partitioned regions	
	M	F	M	F
18 – 25 Years: Male (n = 210); Female (n = 159): Median: Male – 114; Female – 87				
Lower Near outliers ($F_1 - f_1$)	(-195 – -60)	(-151 – - 46	0	0
Lower Far outliers ($<F_1$)	(-195)	(-151)	0	0
Lower Whisker ($a_1 - Q_1$)	26– 75 (49)	25– 59 (34)	53 (25.2%)	40 (25.1%)
$Q_1 - Q_2$	75– 144 (59)	59– 87 (28)	53 (25.2%)	40 (25.1%)
$Q_2 - Q_3$	144 – 165 (21)	87 – 129 (42)	53 (25.2%)	40 (25.1%)
Upper Whisker ($Q_3 - a_3$)	165– 298 (133)	129– 232 (103)	40 (19%)	30 (18.9%)
Upper Near outliers ($f_3 - F_3$)	300 – 435 (135)	234– 339 (105)	8 (3.8%)	8 (5.0%)
Upper Far outliers ($>F_3$)	>435	>339	3 (1.4%)	1 (0.6%)
26 – 50 years: Male (n = 3247); Female (n = 1921): Median: Male – 139; Female – 107				
Lower Near outliers ($F_1 - f_1$)	(-186– -42)	(-137 – -29)	0	0
Lower Far outliers ($<F_1$)	(-186)	(-137)	0	0
Lower Whisker ($a_1 - Q_1$)	32– 102 (70)	16– 79 (63)	812 (25%)	494 (25.7%)
$Q_1 - Q_2$	102– 139 (37)	79 – 107 (28)	816 (25.1%)	471 (24.5%)
$Q_2 - Q_3$	139 – 198 (59)	107 – 151 (44)	814 (25.1%)	480 (24.9%)
Upper Whisker ($Q_3 - a_3$)	198– 341 (143)	151 – 256 (105)	812 (25%)	382 (19.8%)
Upper Near outliers ($f_3 - F_3$)	342 – 486 (144)	259 – 367 (108)	119 (3.7%)	66 (3.4%)
Upper Far outliers ($>F_3$)	>486	>367	56 (1.7%)	28 (1.4%)

(Table 6.1 continuation)

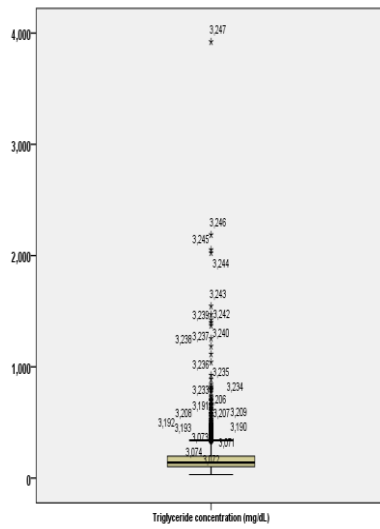
Partitioned regions of Box Whisker plot	Triglyceride concentration (mg/dL)		Sample number in the partitioned regions	
	M	F	M	F
> 50 years: Male (n = 5925); Female (n = 3958): Median: Male – 108; Female – 112				
Lower Near outliers ($F_1 - f_1$)	(-130 – -25)	(-112 – -14.5)	0	0
Lower Far outliers ($<F_1$)	(-130)	(-112)	0	0
Lower Whisker ($a_1 - Q_1$)	13 – 80 (67)	30 – 83 (53)	1514(25.5%)	1008(25.5%)
$Q_1 - Q_2$	80 – 108 (28)	83 – 112 (29)	1467 (24.7%)	1003 (25.3%)
$Q_2 - Q_3$	108 – 150 (42)	112 – 148 (36)	1490 (25.1%)	966 (24.4%)
Upper Whisker ($Q_3 - a_3$)	150 – 254 (104)	148 – 245 (97)	1179 (19.9%)	799 (20.2%)
Upper Near outliers ($f_3 - F_3$)	255 – 360 (105)	245.5 – 343(97.5)	192 (3.2%)	127 (3.2%)
Far outliers on right ($>F_3$)	>360	>343	83(1.4%)	50 (1.3%)

(Table 6.1 continuation)

Figure. 6.2. Box Whisker plot of the sample distribution of serum triglycerides (mg/dL) in various gender age groups before outlier removal. Triglyceride concentration is in Y axis.

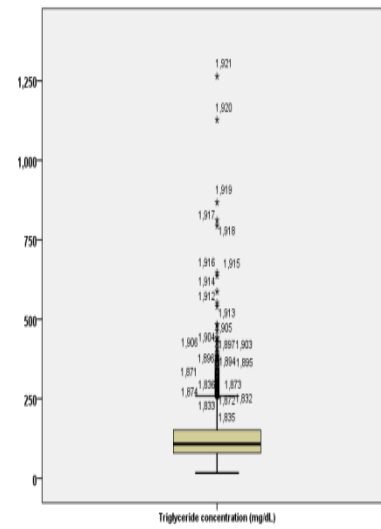


2E.



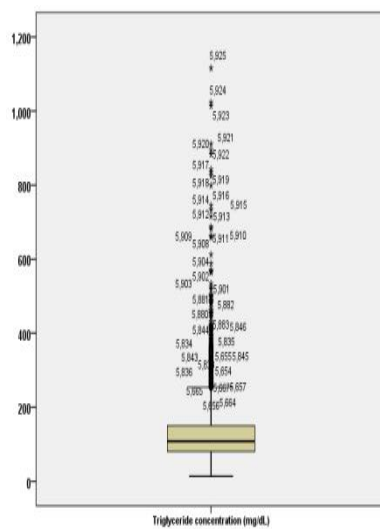
Male 26 – 50 years (n = 3247)

2F.



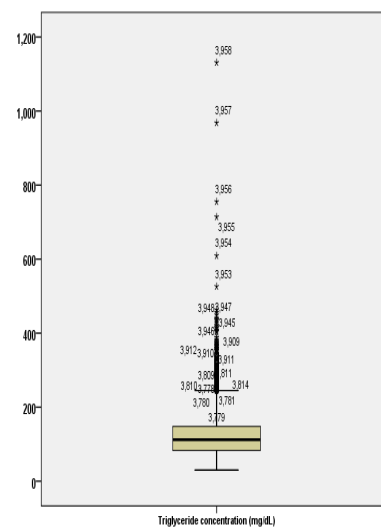
Female 26 – 50 years (n = 1921)

2G.



Male >50 years (n = 5925)

2H.



Female >50 years (n = 3958)

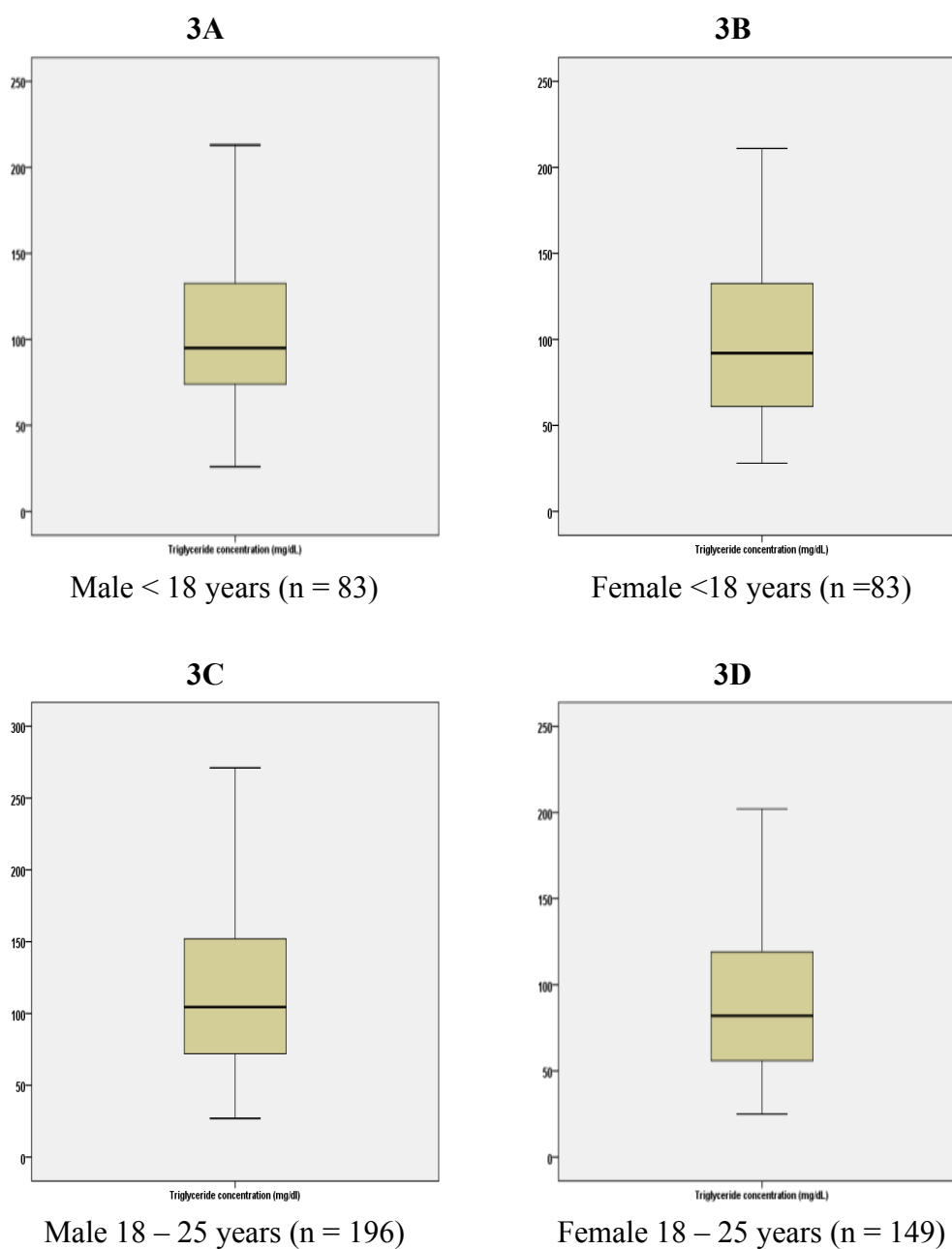
(Figure 6.2 continuation)

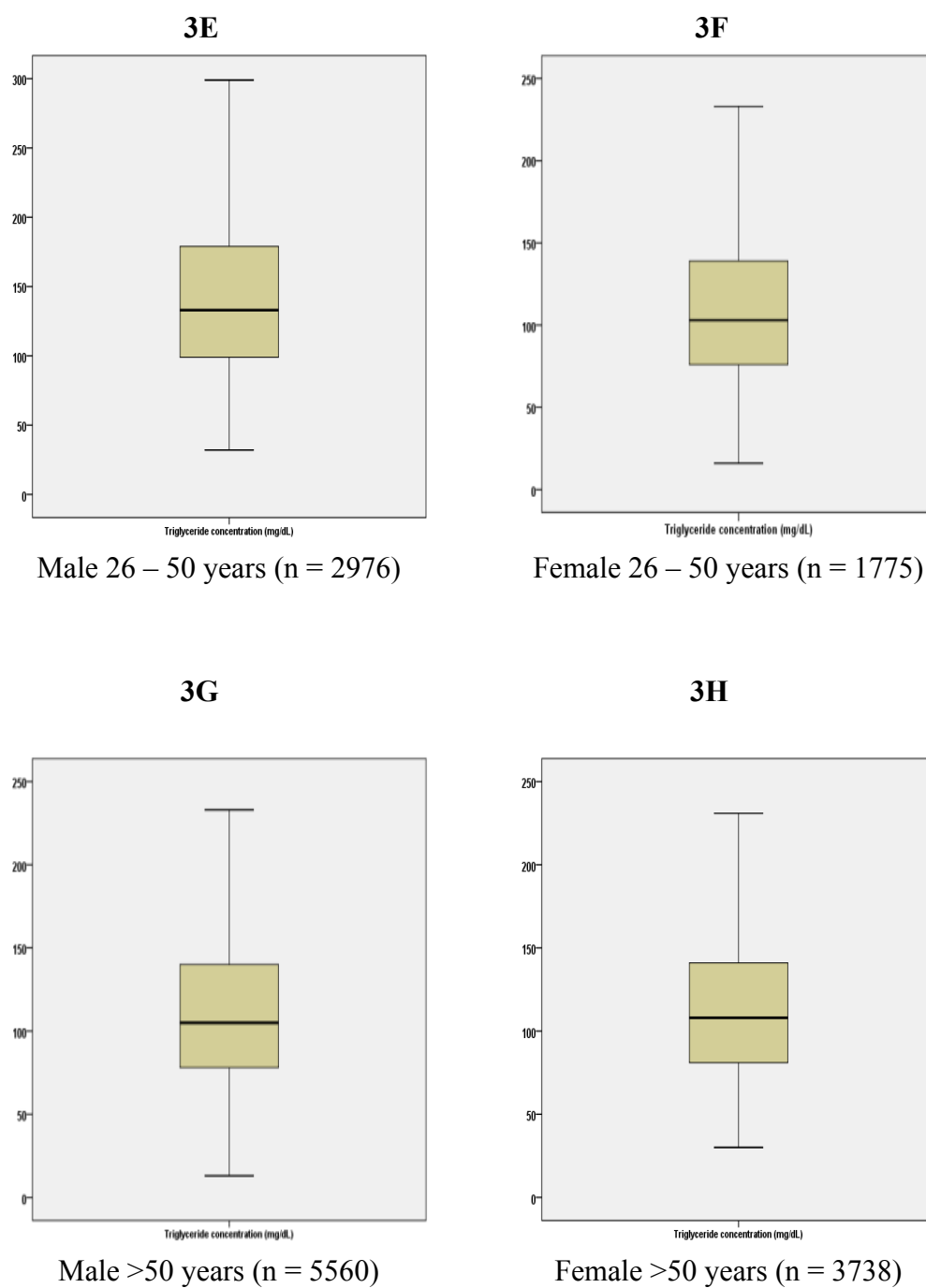
B. Box Whisker plot of Serum Triglycerides after removing the outliers.**Table 6.2.** Distribution of serum triglycerides (mg/dL) after removing outliers by Box-Whisker plot.

Sample number (n)		Total number of outliers removed (cycles)	Mean±SD Min –Max Median	Shapiro – Wilk Jarque Beratest		Mann -Whitney U test
Outlier removal				Log transformation		
Before	After			Before	After	
Samples, age <18 years						
M – 97	M – 83	14 (3)	103.74±43.23 26 – 213 95.00	0.043 (0.121)	0.122 (0.151)	0.272
F – 86	F – 83	3 (1)	97.49±44.67 28 – 211 92.00	0.006 (0.092)	0.079 (0.211)	
Samples, age 18 – 25 years						
M – 210	M – 196	14 (2)	118.69±56.60 27 – 271 104.50	<0.001 (<0.001)	0.047 (0.161)	(<0.001)
F – 159	F – 149	10 (1)	91.36±42.01 25 – 202 82.00	<0.001 (0.004)	0.017 (0.112)	

Sample number (n)		Total number of outliers removed (cycles)	Mean±SD Min –Max Median	Shapiro – Wilk Jarque Beratest		Mann -Whitney U test
Outlier removal				Log transformation		
Before	After			Before	After	
Samples, age 26 – 50 years						
M – 3247	M –2976	271 (4)	143.01±58.20 32 – 299 133.00	<0.001 (<0.001)	<0.001 (<0.001)	(<0.001)
F – 1921	F – 1775	146 (5)	110.31±44.19 16 – 233 103.00	<0.001 (<0.001)	<0.001 (<0.001)	
Samples, age > 50 years						
M – 5925	M –5560	365 (3)	112.07±43.65 13 – 233 105.00	<0.001 (<0.001)	<0.001 (<0.001)	0.002
F – 3958	F – 3738	220 (3)	114.09±41.77 30 -231 108.00	<0.001 (<0.001)	<0.001 (<0.001)	
(Table 6.2 continuation)						

Figure. 6.3. Box-Whisker plot of the sample distribution of serum triglycerides (mg/dL) in various groups after outlier removal. Triglyceride concentration is in Y axis.





(Figure 6.3 continuation)

CHAPTER 7

HISTOGRAM-BASED SUB-CLASSIFICATION OF TOTAL CHOLESTEROL AND PROPORTION OF HYPERCHOLESTEROLEMIA ACROSS GENDER AND AGE GROUPS: A CROSS-SECTIONAL STUDY

7.1. Abstract

Introduction: Family history of heart disease, metabolic syndrome, carotid and femoral plaque, coronary calcium score are some of cause leading to atherosclerotic CVD disease. There is a need for early intervention and management of dyslipidemia in the Indian population. In this study, lipid profile data was collected from Clinical Biochemistry Laboratory and samples were partitioned according to age and gender. In each of the partitioned age and gender group, the Total Cholesterol distribution, percentage and number of samples in healthy, positively skewed and outlier regions of histogram were studied.

Objectives: To investigate the distribution of Total Cholesterol levels based on histogram, determine cutoff level between moderate and severe hypercholesterolemia and analyze the proportion of these categories in different age and gender groups.

Methods: This study, observational and cross-sectional in design, investigated Total cholesterol profiles under normal and dyslipidemic conditions at Amala Institute of Medical Sciences via indirect sampling. Biochemical assays were conducted using the Ortho Clinical Diagnostics Vitros 5.1 FS autoanalyzer. Cholesterol distribution was statistically examined using the Shapiro-Wilk and Jarque-Bera tests and visually assessed using histograms and Normal Q-Q plots. Gender-related differences were studied by means of student's t-test (for Gaussian distributed data) or the Mann-Whitney U test (for non-Gaussian data). All statistical computations were performed using SPSS software.

Results: Male and female samples of Total cholesterol of all age groups were positively skewed as seen in histogram, and deviating from normal distribution as seen by Shapiro-Wilk test. The Normal Q-Q plot and histogram also showed presence of outliers. The sample number increased sharply as age increased in males and females. As concentration of cholesterol increased, the sample number decreased sharply in the histogram from healthy region to positively skewed region and then to outlier region. There was no gender differences in the mean of total cholesterol observed in lower age groups before and after removal of outliers. But there were

gender differences in the older age groups before and after removal of outliers with higher mean total cholesterol in the female sample (177.34 ± 48.27).

Conclusions: Histogram of fasting Total cholesterol samples were found to be positively skewed with the presence of outliers, indicated higher proportion of patients with hypercholesterolemia. Positive skewed region was contributed by moderate hypercholesterolemia which was of common occurrence, and outlier region is contributed by highly increased cholesterolemia which was of rare occurrence and contributed by genetic abnormalities. Outliers were removed in repeated steps to identify and find the total number of outliers, and for graphically appreciating the positively skewed distribution pattern.

7.2. Introduction

The abnormal lipid profile analytes are the causes of common disease conditions that can be identified very early and corrected to significant extend. Lipid profile data from plasma or serum sample includes serum Triglycerides (TG), HDL Cholesterol (HDL-C), Total Cholesterol (TC), and LDL Cholesterol (LDL-C). These measurands are commonly assayed in a Clinical Biochemistry Laboratory. But seldom does clinicians analyse the lipid profile data for further diagnostic and therapeutic purpose, except when there is a gross increase in the value. In this chapter, the gender differences and age-dependent changes in Total Cholesterol analytes were evaluated for diagnosis of common clinical conditions and to understand their further scope.

LDL-C and associated Total Cholesterol are major risk factors for coronary artery disease. Plasma concentrations of LDL-C are positively associated with increased risk of atherosclerotic cardiovascular disease (Master and Rader, 2013). HDL-C negatively associated with risk of atherosclerotic cardiovascular disease.

In 2019, 17.9 million people died from CVDs representing 32% of all deaths worldwide (WHO, 2021). In 2016, India reported 63% of total deaths due to Non-Communicable Diseases, of which 27% were attributed to CVDs (WHO, 2021). In Kerala, according to a survey report in 2022, by the state health department, 25 percent of people above 30 years suffer from lifestyle diseases (Mathrubhumi, 2022).

Indian population is more prone to develop cardiovascular disease and more

than 50% of coronary artery disease – associated death occurs in younger age usually before 50 years (Bansal et al., 2024). LDL lipoprotein contains cholesteryl ester as the predominant core hydrophobic lipid. The outer shell consists of phospholipids and single molecule of the large protein apolipoprotein B-100 (apo B). LDL carries about 60% to 70% of serum cholesterol and it transports cholesterol from the liver to peripheral tissues (Talbert, 2005). Small dense LDL particles have much less cholesterol than large, buoyant LDL particles which are more atherogenic (Berneis and Krauss, 2002). VLDL-C is synthesized in the liver. Almost all LDL-C is derived from VLDL-C (Mahley and Bersot, 2006). HDL-C is produced in the intestine and liver. It is composed of Apo A lipoproteins, phospholipids and cholesterol. It is known to be anti-atherogenic as it is involved in the reverse cholesterol transport pathway, thereby maintaining body cholesterol homeostasis. HDL-C particles carry approximately 30% of the serum cholesterol (Remaley et al, 2012).

In lipoproteins, free cholesterol occupies the surface layer and cholesterol ester is a core component. The ratio of the surface to core component is inversely proportional to the size of the lipoprotein particle. Surface cholesterol and core cholesterol ester are found in all the different types of lipoproteins which is presented in Table 7.1 and Table 7.2 (Remaley et al., 2012)

Table 7.1 Chemical Composition (%) of Normal Human Plasma Lipoproteins

	Surface components			Core lipids	
Lipoprotein	Cholesterol	Phospho-lipid	Apo-lipoproteins	Triglyceride	Cholesteryl Esters
Chylomicrons	2	7	2	86	3
VLDL	7	18	8	55	12
IDL	9	19	19	26	29
LDL	8	22	22	6	42
HDL2	5	33	40	5	17
HDL3	4	25	55	3	13

Ratio of surface cholesterol to core cholesterol ester components were given in Table 7.2

Table 7.2 Ratio of surface cholesterol to core cholesterol ester components in Lipoproteins

Lipoprotein	Cholesterol/ Cholesteryl Esters	Ratio of surface to core components
Chylomicrons	2/3	1/1.5
VLDL	7/12	1/1.71
IDL	9/29	1/3.22
LDL	8/42	1/5.25
HDL2	5/17	1/3.4
HDL3	4/13	1/3.25

The ratio was converted to one as numerator, to identify the relative amount cholesterol ester as the core component with reference to free cholesterol. The denominator is low for chylomicron and VLDL due to larger triglyceride core component. The core cholesterol is intermediate for IDL and HDL. LDL also has intermediate size and has high concentration of cholesterol ester and very low concentration of triglyceride as the core component. ICMR-INDIA B study-2014 and NCEP-2001 guidelines recommended a healthy cutoff of Total Cholesterol as <200 mg/dL (Joshi et al., 2014; NCEP, 2001)

In this chapter, the histogram characteristics, gender and age-related differences in Total Cholesterol were studied. The gender differences have biochemical significances. The gender differences in cholesterol may be altered with age and after menopause in women (ormazabal et al., 2018). The distribution pattern of Total Cholesterol, Gaussian or skewed, and presence of outliers can give significant information regarding the state of the analytes in the population.

7.3. Objectives

- a) Collection of fasting Total Cholesterol sample data from the Clinical Biochemistry Laboratory
- b) Evaluation of the Total Cholesterol distribution by histogram before and after outlier removal in samples, partitioned according to age and gender
- c) Calculating the cut-off value between moderate and severe hypercholesterolemia, and analyzing the number and percentage of samples in different histogram regions.

7.4. Materials and methods

7.4.1. Research Design

An observational cross-sectional study of hypercholesterolemia was conducted at the Clinical Biochemistry Laboratory, Department of Biochemistry, Amala Institute of Medical Sciences using indirect sampling. This method was favoured over direct sampling from the general population because it allowed for the

rapid collection of a large sample size necessary to detect rare lipid profile abnormalities at lower cost (Horowitz et al., 2012). Fasting lipid profile samples were continuously obtained from both outpatient and inpatient departments between September 2018 and March 2022 to ensure randomized data. The study protocol was approved by the Institutional Research and Ethics Committees (Ref. AIMSIEC/42/2018, dated 19/10/2018) for both the Department of Biochemistry and the Clinical Biochemistry Laboratory.

7.4.2. Inclusion Criteria and Exclusion criteria: (Refer General Materials and Methods)

7.4.3. Selection of Age Groups for the study

To facilitate age-related analysis, the samples were grouped into adolescents (below 18 years), young adults (18 to 25 years), middle-aged individuals (26 to 50 years), and senior adults (over 50 years).

7.4.4. Sample size requirement

The sample size was determined by a statistician based on prevalence data from a multicentric dyslipidemia study (Qi et al., 2015). Using parameters of 20% prevalence (d), a significance level (α) of 0.05, prevalence rate (p) of 24%, $q = 1 - p$, Z value of 1.96 at 95% confidence, and 80% power, the calculated sample size was 304.12, which was rounded to 304 for each group. The final total sample size of 15,603 met the required number for all subgroups (see General Materials and Methods). The cutoff for healthy total cholesterol in this study was set at <200 mg/dL, in accordance with the ICMR-INDIA B study (2014) and NCEP-2001 guidelines.

7.4.5. Methodology:

- a) Collection of Fasting Total Cholesterol sample data from the Clinical Biochemistry Laboratory
- b) Evaluation of the Total Cholesterol distribution by histogram before and after outlier removal in samples, partitioned according to age and gender

- c) Calculating the cutoff value between moderate and severe hypercholesterolemia, and analyzing the number and percentage of samples in different histogram regions.

7.4.6. Assay of Total Cholesterol (Refer General materials and methods)

7.4.7. Statistical Methods

SPSS version 19 was utilized to assess data distribution using the Shapiro-Wilk and Jarque-Bera tests, as well as to generate histograms depicting distribution before and after outlier removal. Graphical representation of the data was performed using histograms and Normal Q-Q plots. Gender differences in lipid levels were evaluated using either the student's t-test or the Mann-Whitney U test (Altman, 1991; Gao and Smith, 2003).

7.5. Results

A. Distribution Characteristics of Serum Total Cholesterol after partitioning according to gender and age.

The male samples ($n = 97$) of age group <18 years had higher mean Total Cholesterol (176.81 ± 82.19) values than the female samples ($n = 86$) of the same age group. The minimum to maximum values of Total Cholesterol in male samples <18 years of age showed very large range ($83 - 881 \text{ mg/dL}$) as compared to the female sample ($84 - 316 \text{ mg/dL}$). There was no gender difference in Total Cholesterol values in samples <18 years of age (Mann Whitney U test $P = 0.560$) (Table 7.3).

In the $18 - 25$ years of age group, male samples ($n = 210$) had higher mean Total Cholesterol values (184.34 ± 80.58) than the female samples ($n = 159$). But there are no gender differences in Total cholesterol as the P value for Mann-Whitney U test was 0.190 (Table 7.3). Standard deviation range for males in all four age groups were from 45.60 to 82.19 and for female were from 39.40 to 48.27 (Table 7.3).

The mean values for Total Cholesterol were higher in female samples ($n = 1921$; mean 190.84 ± 41.68) than that in the male samples ($n = 3247$; mean: 188.43 ± 46.25) of age group between $26 - 50$ years. The minimum to maximum range

for Total Cholesterol values in male samples (26 – 50 years of age) were from 50 – 378 mg/dL which was slightly less than in the female sample from 55 – 401 mg/dL. There was no gender differences in Total Cholesterol values in the sample of age between 26 – 50 years (Mann Whitney U test $P = 0.082$) (Table.7.3).

The mean values for Total Cholesterol were higher in female samples ($n = 3958$; mean: 177.34 ± 48.27) than that in the male samples ($n = 5925$; mean: 158.47 ± 45.60) of age group >50 years, with significant gender difference with Mann Whitney U test value ($P = <0.001$) (Table.7.3).

In samples, <18 years and samples of age group 18 – 25 years, the Normal Q-Q plot showed very large deviation of the sample distribution away from the straight-line normal distribution plot in male samples than in total female samples (Fig. 7.1).

Histogram of Total Cholesterol distribution in male and female samples of age group <18 years showed positive skewing (Fig. 7.2). Histograms of Total Cholesterol distribution in male and female samples of all the age groups showed that there is positive skewing in Total Cholesterol values in which Shapiro-Wilk test showing deviation from normal distribution.

The number and percentage of samples in healthy, positively skewed and outlier region of histogram.

The three different regions of histogram (healthy Total Cholesterol <200 mg/dL, positively skewed region and outlier region) were analyzed in each of the gender and four age groups for number and percentage of samples in each of the three regions. In <18 years age group, there were higher percentages of female samples (82.55%) in the healthy region (<200 mg/dL) compared to that of the male samples (80.41%). There were 17.52% of male samples and 15.11% of female sample in positively skewed region (Table 7.4).

In 18 – 25 years of age group, the percentage of samples in positively skewed region were higher for male samples (23.80%) compared to female samples (19.49%). The result indicated that there were more males having increased Total Cholesterol in age group below 25 years.

In 26 – 50 years, there was nearly equal percentage of samples in healthy, positively skewed regions in male and female sample. In >50 years, females had higher percentage of samples in positively skewed region (29.88%) compared to male samples (18.34%) (Table 7.4).

In < 18 years group there were three outliers in male samples, but female samples had two outliers removed from the histogram. The number of outliers was high in males (9) of age group 18 – 25 years compared to those of females (5). In the age group 26 – 50 years, there were 17 outliers (removed in 2 cycles) and 11 outliers (removed in 2 cycles) in male and female samples, respectively. Male samples > 50 years of age, had large number of outliers compared to those of females (Table 7.5)

In younger age group below 25 years, there were more males with hypercholesterolemia. But, as age increases (26 – 50 years) the Total Cholesterol concentrations are nearly equal in male and females. But after 50 years, hypercholesterolemia is higher in females. This transition of cholesterol levels with age may be related to changes in hormonal levels, diet, and physical activity.

The numbers of outliers are generally few in all age group in males and females (Table 7.4). These samples are ones which are more likely to be inherited metabolic disorders associated with Total Cholesterol. The positively skewed regions with hypercholesterolemia, which are generally multifactorial, inherited and acquired metabolic disorders.

B. Distribution Characteristics of Total Cholesterol after outlier removal

The mean values are decreased after removal of outliers only in males in age group 18 -25 years. In females of 18 – 25 years, the mean cholesterol values are similar before and after removal of outliers. These results indicate higher mean cholesterol level in males in lower age group after removal of outliers. But above 25 years, after removal of outliers, the mean Total Cholesterol levels are equal in males and females (Table 7.5).

Total Cholesterol outliers are rare occurrences compared to positively skewed region. Outliers are removed in repeated steps as there are several hidden outliers.

Even after removal of outliers and the hidden outliers, the large number of samples still remain in positively skewed region in age groups >25 years in both male and females. Even after log transformation the distribution remain positively skewed in age groups >25 years as Shapiro Wilk value is P is <0.05 (Table 7.5). histogram showed, after removal of outliers few age and gender groups have Gaussian distribution (Fig 7.3)

There is no gender difference in Total Cholesterol values in samples <18 years of age (Mann Whitney U test $P = 0.450$), and in age group 18 – 25 years (Mann Whitney U test $P = 0.587$) (Table 7.5).

The mean Total Cholesterol were higher in female samples of both age groups 26 – 50 years and >50 years with 190.81 ± 40.23 and 176.60 ± 46.83 , respectively. There were gender difference in Total Cholesterol values in age group 26 – 50 years (Mann Whitney U test $P = 0.051$), and in age group >50 years (Mann Whitney U test $P = <0.001$) (Table 7.5).

Before removal of outliers there appears to be negative correlation between Total Cholesterol and age ($r = -0.334$, $P = <0.001$ in males and $r = -0.159$, $P = <0.001$ in females). Outliers in X-Y Scatter plots of Total Cholesterol above 400 mg/dL were removed for graphical appreciation of the relationship between Total Cholesterol with age. There was negative correlation between Total Cholesterol and age with correlation coefficient $r = -0.333$; $P = <0.001$ and, $r = -0.158$, $P = <0.001$). The results indicated that Total Cholesterol decreases with age (Fig.7.4 and Fig. 7.5).

7.6. Discussions

Increase in Total Cholesterol sample number as age increased represented increased hospital visits. The maximum sample number was seen in males and females above fifty years of age. There were no gender differences in the two lower age groups. But, as age advanced, gender differences increased, the age group above 50 years showed maximum gender difference. The male sample number was higher than the female sample number for all age groups. The negative correlation of Total Cholesterol with age was an unexpected result. As atherosclerosis and increase in

heart diseases increase with age, it was assumed that Total Cholesterol also would increase with age. But it was not so.

The sample distribution of total cholesterol was positively skewed, especially in the higher age groups, indicating large number of samples with increased Total Cholesterol (Table 7.3, Fig 7.1).

Histograms are used for graphical interpretation of distribution patterns such as positive or negative skewing, and presence of positive or negative outliers.

The findings that males below 25 years develop hypercholesterolemia than females of same age group have implications for cardiovascular disease prevention and management in adults.

The higher concentration of Total Cholesterol is of rare occurrence; therefore, they present as outliers. Such rare cases of hyperlipidemia are typically seen in inherited metabolic disorders of hypercholesterolemia. But outliers can also be “hidden outliers”. These hidden outliers are exposed by removing the graphically visible outliers in the histogram. These hidden outliers may also be removed for complete removal of outliers. There may be about 1 to 3 hidden sets of outliers in a single sample set. For complete removal of outliers, repeated plotting of histogram of serum Total Cholesterol is required. Cycles of outliers removed are given in the Table 7.5.

Suspected double peak is seen in Fig 7.3A. Platykurtic is seen in Fig.7.3C, and positive skewing is clearly seen in most of the distribution pattern except Fig 7.3E.

The clinical need for outlier removal is

1. To understand total number of outliers, including hidden outliers.
2. The positively skewed region after removal of outliers indicates the moderate hypercholesterolemia.
3. The total number of outliers indicates the prevalence of rare cases of high concentration hypercholesterolemia.

4. The number of moderate hypercholesterolemia is calculated from the positively skewed concentration $\geq 200\text{mg/dL}$.
5. The presence of multiple peaks in the distribution pattern was graphically visualized after removal of outliers. Examples in Fig7.3A and 7.4D.
6. The presence of platykurtic (Fig 7.3C) and leptokurtic (Fig 7.3A) are observed after removal of outliers.

The relationship between Total Cholesterol and triglycerides with age should be understood in more. Outliers were removed to graphically appreciate and relate the X-Y Scatter plot. The negative correlation of Total Cholesterol with age was an unexpected result. Although heart disease risk increases with age, this study found that Total Cholesterol levels did not follow the same trend.

7.7. Conclusions

Graphical analysis by histogram and Normal Q-Q plot showed presence of positive skewing which increased in the higher age groups and was confirmed by Shapiro-Wilk and Jarque Bera test. In the higher age groups even log transformation did not convert the distribution to Gaussian. But after removal of outliers, log transformation of the non-Gaussian distributed groups was transformed to normal distribution in the lower age groups. Log transformation could not transform the distribution to Gaussian even after removal of outliers in higher age groups. This is due to increased proportion of hypercholesterolemia in the higher age groups. the sample numbers were higher in the males than females of all age group. In each of the gender age groups maximum sample number was seen in the healthy region and it decreased in the positively skewed region and further decreased in the outlier region. It was important to note that mean total cholesterol values were higher in female sample and there was negative correlation of total cholesterol with age in males and females. The presence of multiple peaks in the distribution pattern was graphically visualized after removal of outliers.

A. Distribution Characteristics of Serum Total Cholesterol after partitioning according to gender and age.**Table 7.3.** Distribution of Total cholesterol (mg/dL) in samples of age group below 18, 18 – 25, 26 – 50, and >50 years

Samples	Sample number (n)	Mean±SD 95%CI for mean Range:Min –Max	Median Skewness kurtosis	Shapiro–Wilk Jarque Bera (Log transformation)		Mann-Whitney U Test (P)
				Before	After	
Samples: Age <18 years						
Male	97	176.81±82.19 160.24 – 193.38 83 – 881	167.00 6.742 56.931	(<0.001) (<0.001)	(<0.001) (<0.001)	0.560
Female	86	166.14±40.34 157.49 – 174.78 84 – 316	162.00 0.849 2.154	0.007 (<0.001)	0.539 0.449	
Samples: Age group 18 – 25 years						
Male	210	184.34±80.58 173.38 – 195.29 87 – 912	173.50 5.305 39.89	(<0.001) (<0.001)	(<0.001) (<0.001)	0.190
Female	159	170.93±39.40 164.76 – 177.10 70 – 355	165.00 0.923 2.709	(<0.001) (<0.001)	0.011 (<0.001)	
Samples: Age group 26 – 50 years						
Male	3247	188.43±46.25 186.84 – 190.02 50 – 378	188.00 0.174 0.098	(<0.001) (<0.001)	(<0.001) (<0.001)	0.082
Female	1921	190.84±41.68 188.97 – 192.70 55 – 401	189.00 0.284 0.575	(<0.001) (<0.001)	(<0.001) (<0.001)	

Samples	Sample number (n)	Mean±SD 95%CI for mean Range:Min –Max	Median Skewness kurtosis	Shapiro–Wilk Jarque Bera (Log transformation)		Mann-Whitney U Test (P)
				Before	After	
Samples: Age group > 50 years						
Male	5925	158.47±45.60 157.31 – 159.63 50 – 413	154.00 0.564 0.612	(<0.001) (<0.001)	(<0.001) (<0.001)	(<0.001)
Female	3958	177.34±48.27 175.83 – 178.84 50 – 457	174.00 0.521 0.730	(<0.001) (<0.001)	(<0.001) (<0.001)	
Table 7.3. continuation						

Figure. 7. 1 Comparison of sample distribution of Total Cholesterol (mg/dL) in different gender- age groups visualized by Normal Q-Q plot

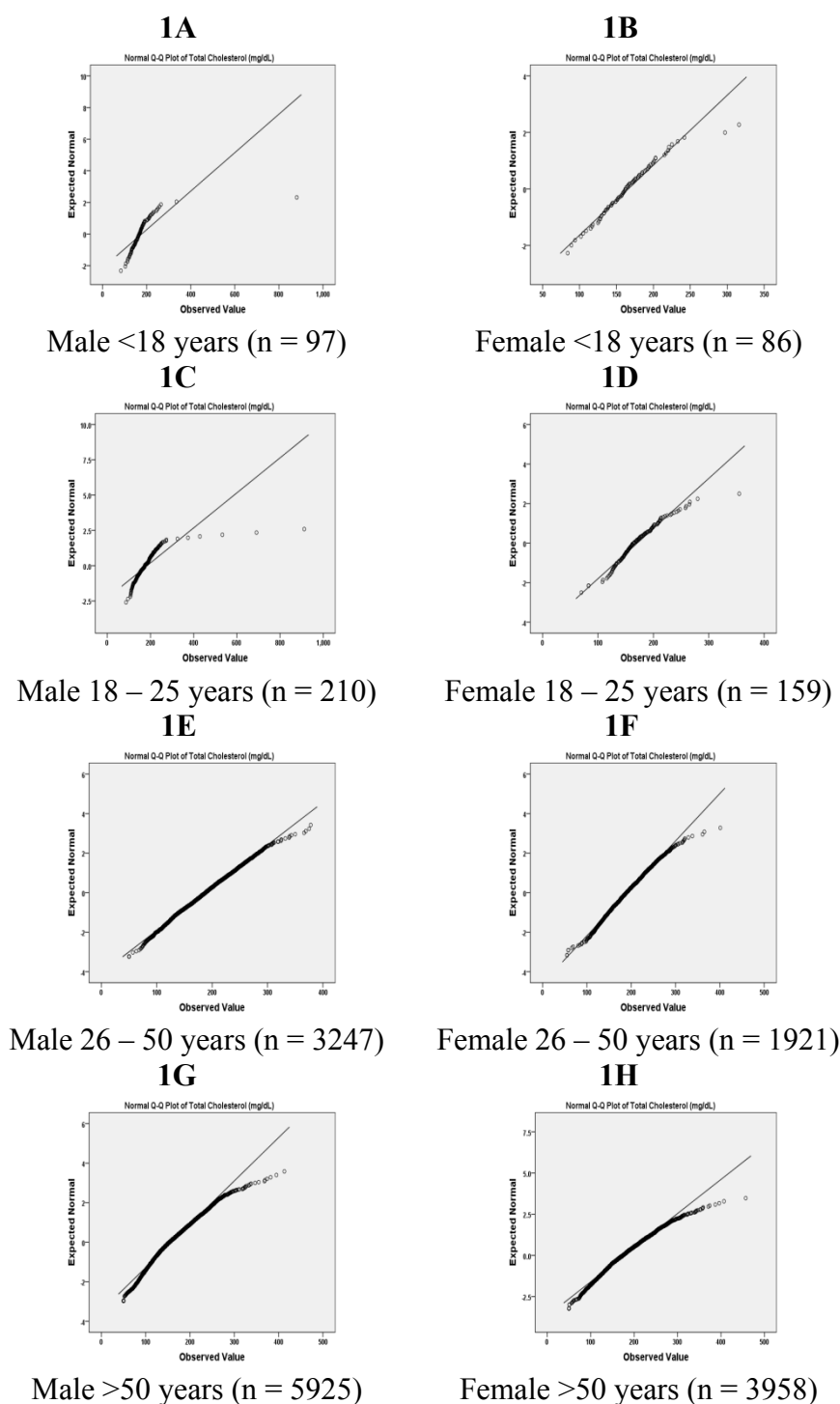
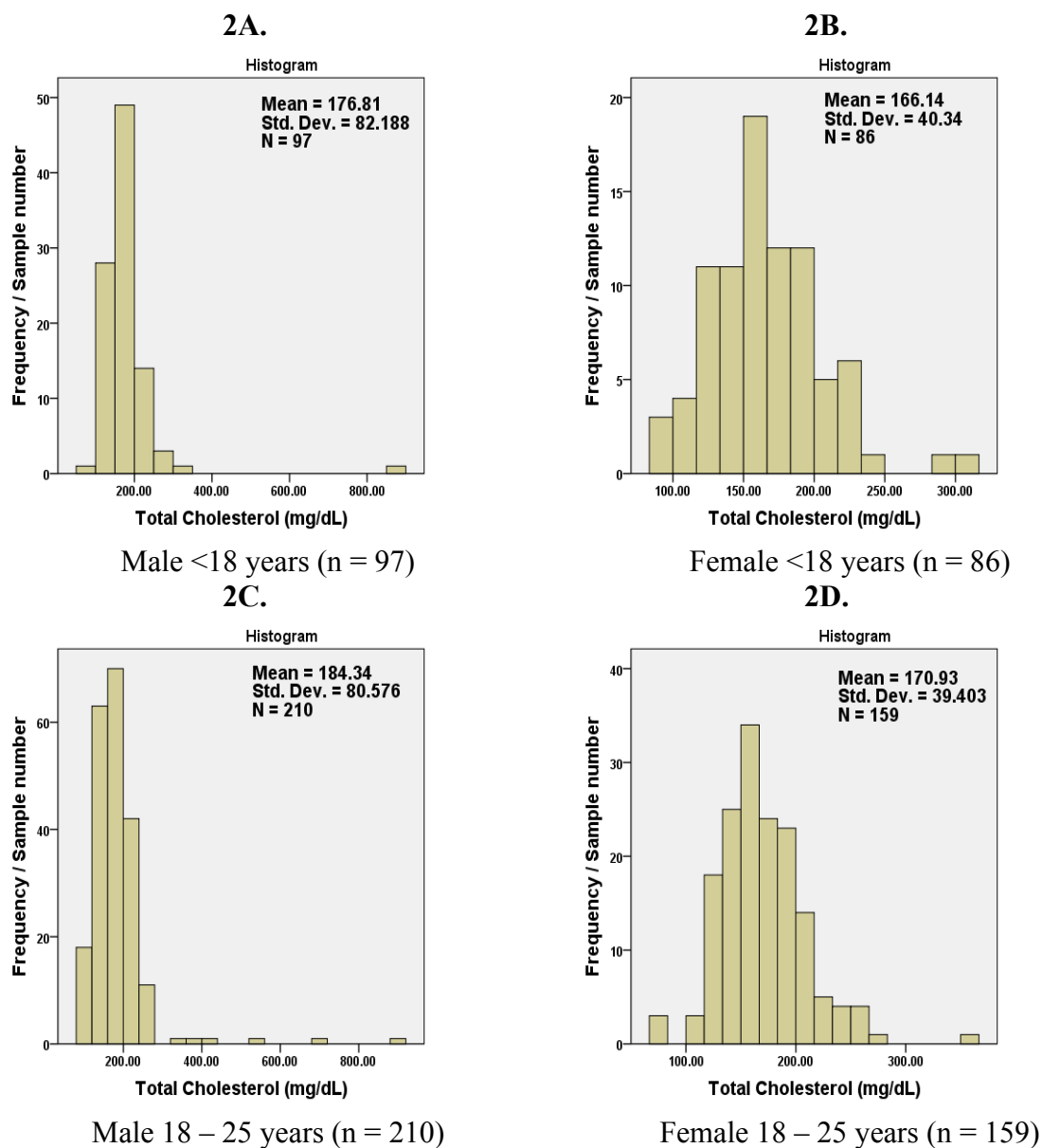
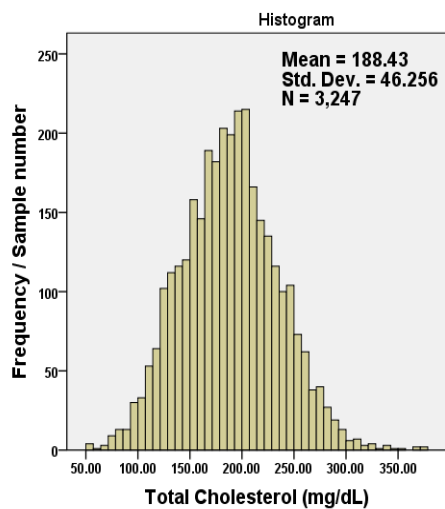


Figure. 7. 2. Comparison of sample distribution of Total Cholesterol (mg/dL) before outlier removal in different gender-age groups visualized by histogram (X axis – Total Cholesterol (mg/dL), Y axis – Frequency of sample number)

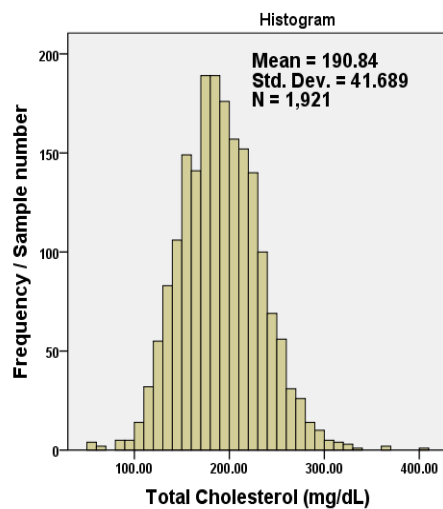


2E.



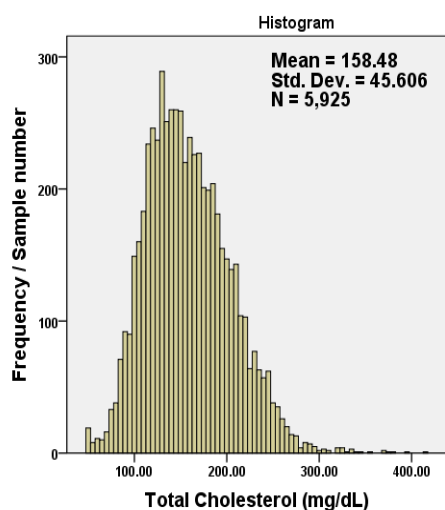
Male 26 – 50 years (n = 3247)

2F.



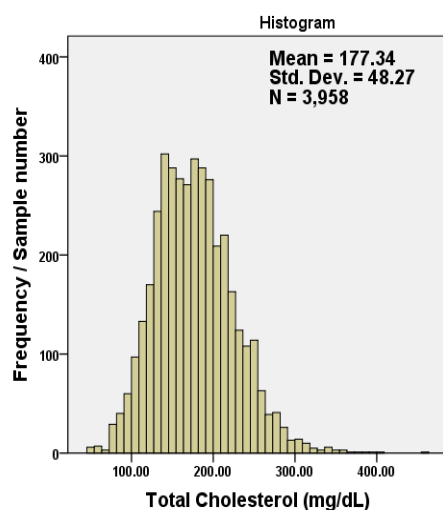
Female 26 – 50 years (n = 1921)

2G.



Male >50 years (n = 5925)

2H.



Female >50 years (n = 3958)

(Figure 7.2 continuation)

Table 7.4. The sample number and its percentage (%) in the histogram, distributed in the region with Total Cholesterol <200 mg/dL (**Healthy region**), Total Cholesterol $\geq$ 200 mg/dL to last positively skewed sample (**Skewed region**), and last positively skewed to last outlier region (**Outlier region**) of Total Cholesterol (mg/dL) in different age and gender groups. F and M are males and females.

Age group	Total Cholesterol <200	Sample number and %	
Sample number and %	200 to last positively skewed sample in M & F		
	Outlier region in M & F	Male	Female
<18 years M, n = 97 (100%) F, n = 86(100%)	<200	78 (80.41%)	71 (82.55%)
	M =200 – 265 F = 200 – 242	17 (17.52%)	13 (15.11%)
	M – >265 – 881 F – >242 – 316	2 (2.06%)	2 (2.32%)
18 – 25 Years M, n = 210(100%) F, n = 159(100%)	<200	151 (71.90%)	126 (79.24%)
	M = 200 – 257 F = 200 –266	50 (23.80%)	31(19.49%)
	M – >257 – 912 F – >266 – 355 F - <108 (Negative outlier)	9 (4.28%)	2 (1.25%) 3 (1.88%)
26 – 50 years M, n = 3247(100%) F, n =1921(100%)	<200	1964 (60.48%)	1150 (59.86%)
	M = 200 – 324 F = 200 – 321	1270 (39.11%)	766 (39.87%)
	M – >324 – 378 M - <63(Negative outlier) F – >321 – 401 F - <80 (Negative outlier)	13 (0.40%) 4 (0.09%)	5 (0.26%) 6 (0.31%)
>50 years M, n = 5925(100%) F, n =3958(100%)	<200	4817 (81.29%)	2760 (69.73%)
	M = 200 – 311 F = 200 – 341	1087 (18.34%)	1183 (29.88%)
	M – >311 – 413 F – >341–457	21 (0.35%)	15 (0.37%)

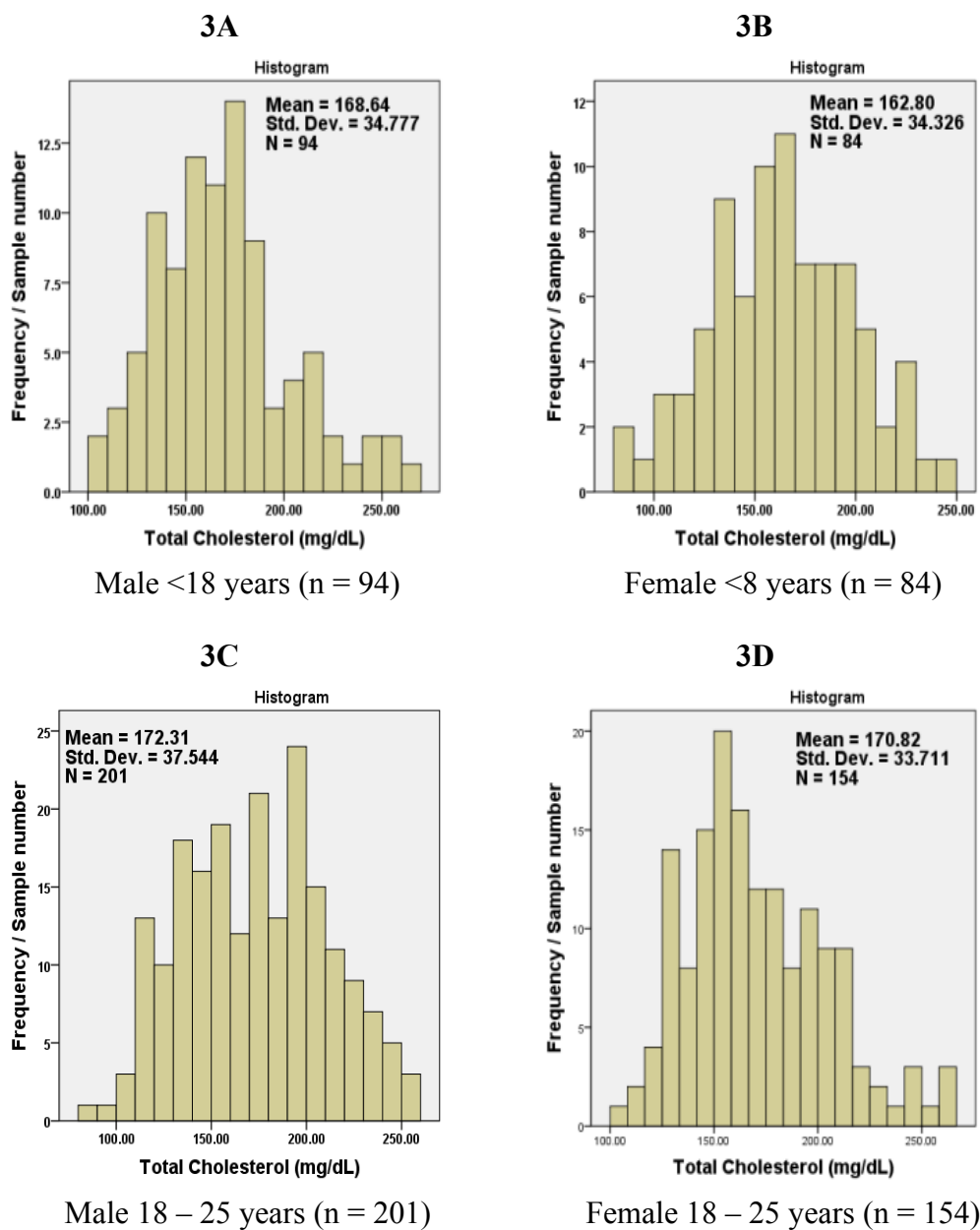
B. Distribution Characteristics of Total Cholesterol after outlier removal**Table 7.5.** Distribution characteristics and gender differences in Total Cholesterol (mg/dL) after removal of outliers using histogram. F and M are males and females

Sample number (n)		Outliers removed (cycles)	Total Cholesterol Mean±SD 95%CI for mean Min –Max Median Skewness/kurtosis	Total Cholesterol Shapiro – Wilk, P (Jarque Bera)		Gender Difference, Student t test/Mann - Whitney U test (P)
Outlier removal				Log transformation		
Before	After			Before	After	
Samples, age <18 years						
M – 97	M – 94	3 (2)	168.63±34.77 161.51 – 175.76 103 – 265 166.00 0.633/0.315	0.018 (0.043)	0.749 (0.193)	0.450
F – 86	F – 84	2 (1)	162.79±34.32 155.34 – 170.24 84 – 242 162.00 0.001/-0.315	0.960 (0.790)	0.097 (0.090)	
Samples, age 18 – 25 years						
M –210	M –201	9 (1)	172.30±37.54 167.08 – 177.53 87 – 257 173.00 0.099/-0.733	0.030 (0.083)	0.005 (0.041)	0.587
F – 159	F – 154	5 (2)	170.82±33.71 165.45 – 176.19 108 – 266 165.00 0.643/0.116	0.001 (0.005)	0.413 (0.390)	

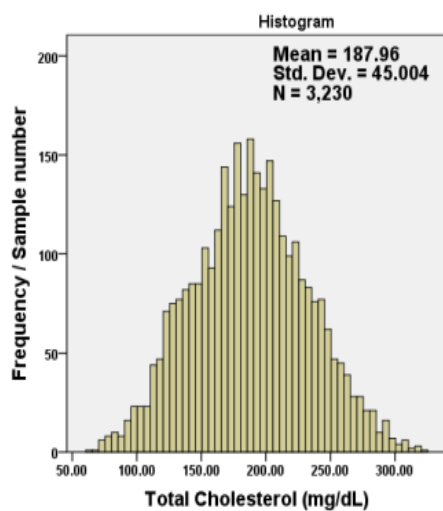
Table 7.5 (continuation)

Sample number (n)		Outliers removed (cycles)	Total Cholesterol Mean±SD 95%CI for mean Min –Max Median Skewness/kurtosis	Total Cholesterol Shapiro – Wilk, P (Jarque Bera)		Gender Difference, Student t test/Mann - Whitney U test (P)
Outlier removal				Log transformation		
Before	After			Before	After	
Samples, age 26 -50 years						
M – 3247	M – 3230	17 (2)	187.96±45.00 186.40 – 189.51 63 – 324 45.00 0.073/-0.296	<0.001 (<0.001)	<0.001 (<0.001)	0.051
F – 1921	F – 1910	11 (2)	190.81±40.23 189.00 – 192.61 80 – 321 189.00 0.224/-0.119	<0.001 (<0.001)	<0.001 (<0.001)	
Samples, age > 50 years						
M – 5925	M – 5904	21 (2)	157.81±44.27 156.68 – 158.94 50 – 311 153.00 0.379/-0.183	<0.001 (<0.001)	<0.001 (<0.001)	<0.001
F – 3958	F – 3943	15 (2)	176.60±46.83 175.45 – 178.06 50 – 341 174.00 0.334/-0.034	<0.001 (<0.001)	<0.001 (<0.001)	

Figure. 7. 3. Histogram of the sample distribution of Total Cholesterol (mg/dL) in males and females of various age groups after outlier removal. (X axis – Total Cholesterol (mg/dL), Y axis – Frequency of sample number)

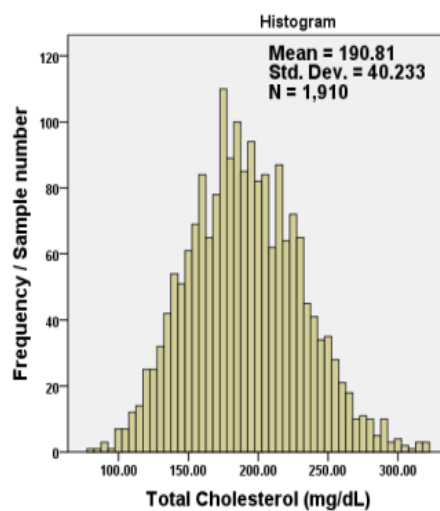


3E



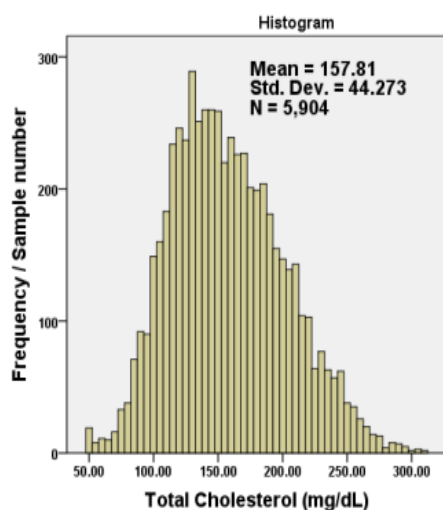
Male 26 – 50 years (n = 3230)

3F



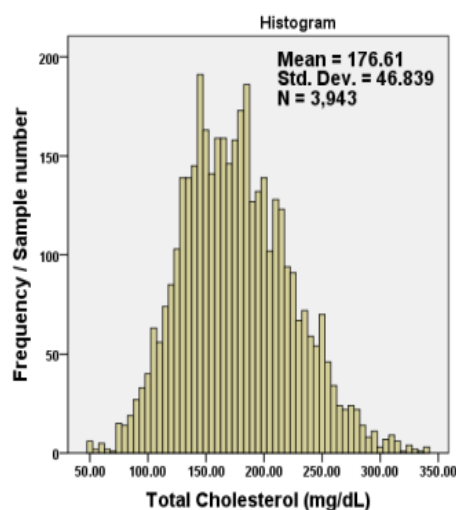
Female 26 – 50 years (n = 1910)

3G



Male >50 years (n = 5904)

3H



Female >50 years (n = 3943)

Figure. 7. 3 (continuation)

Figure.7.4. Correlation between Total Cholesterol and Age in males and females

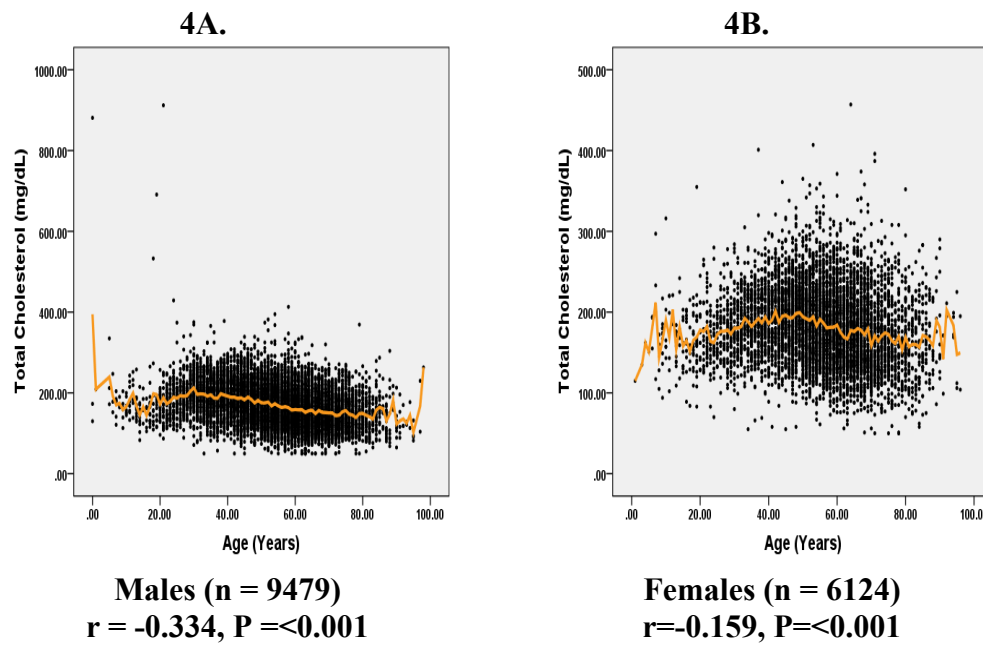
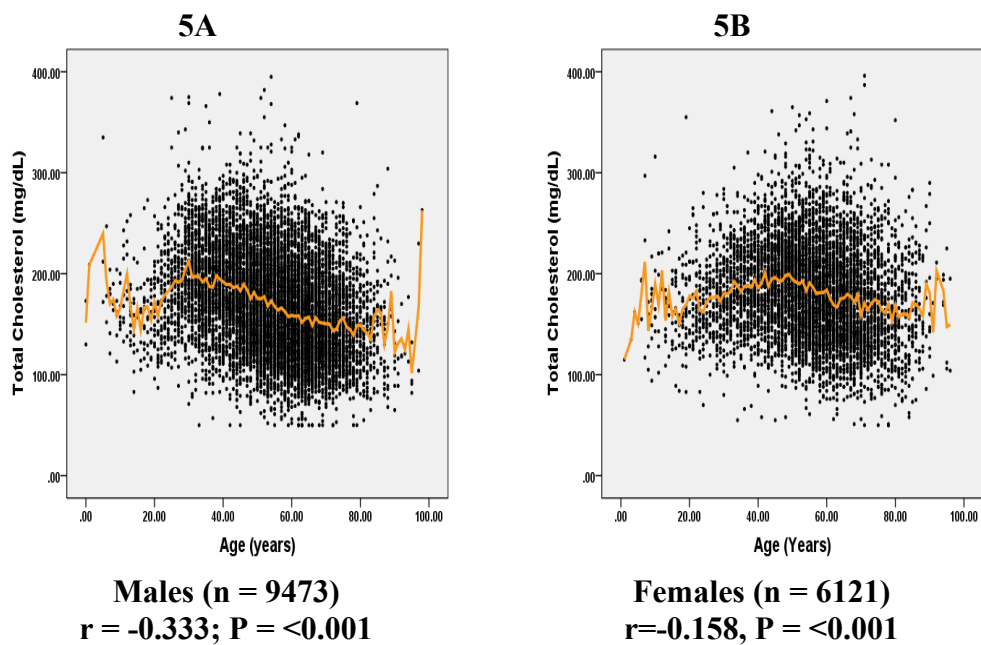


Figure.7.5. Correlation between Total Cholesterol and Age in males and females after removal of samples with Total Cholesterol above 400 mg/dL



CHAPTER 8

**BOX-WHISKER PLOT USED FOR PARTITIONING SERUM
HYPERCHOLESTEROLEMIA ACCORDING TO QUARTILES
AND FENCES TO IDENTIFY GENDER AND AGE-RELATED
DIFFERENCES AND SIMILARITIES – A CROSS-SECTIONAL
STUDY**

8.1. Abstract

Introduction: Total Cholesterol is an important biomarker for assessing cardiovascular risk. Understanding the distribution of Total Cholesterol levels in a population is essential for developing effective prevention and treatment strategies. In this study, Lipid profile data was collected from Clinical Biochemistry Laboratory and samples were partitioned according to age and gender. In each of the partitioned age and gender group, the Total Cholesterol distribution, percentage and number of samples in different regions of Box-Whisker plot were studied.

Objective: To evaluate the distribution, gender disparities, and the proportion of patients with hypercholesterolemia in relation to median, quartiles and fences using Box-Whisker plots

Methods: This cross-sectional observational study, based on indirect sampling, evaluated total cholesterol levels in both normal and dyslipidemic states at Amala Institute of Medical Sciences. The analyses were performed using the Vitros 5.1 FS autoanalyzer from Ortho Clinical Diagnostics. Distribution of cholesterol values was examined using Box-Whisker plots. Gender differences in lipid concentrations were tested by Student t-test or Mann-Whitney U test. Statistical analysis was conducted with SPSS software.

Results: Male and female samples of Total cholesterol of all age groups were positively skewed and vertically compressed as seen in Box-Whisker plot. The Box-Whisker plot also showed presence of near and far outliers. In each of the eight gender age groups, there were 0 to 1 sample in the Lower Near outliers ($F_1 - f_1$) and the Lower Far outliers ($<F_1$) region in the two lower age groups and in the fourth group above 50 years of age in both males and females. In the age group 26 – 50 years these two regions had sample numbers between 0 and 6. The sample number increased sharply as age increased in males and females. There were no gender differences in the mean of Total cholesterol observed in age groups below 50 years before and after removal of outliers.

Conclusions: Box-Whisker plot of fasting total cholesterol samples were found to be

positively skewed with the presence of outliers. A statistical and clinical method of classification of Total Cholesterol concentration with quartiles, fences and outliers of Box-Whisker plot was proposed. Outliers were sub partitioned into near and far outliers. The concentrations greater than upper whisker (a_3) are cases of hypercholesterolemia. The a_3 concentrations range was from 242 mg/dL to 311 mg/dL in the four age groups. Box Whisker Plot of hypercholesterolemia can be used to differentiate between prevalent, multifactorial states from the rare, monogenic forms of the condition.

8.2. Introduction

High serum Cholesterol concentration is one of the major risk factors for atherosclerosis and myocardial infarction. Several methods such as histogram, Box-Whisker plot, Normal Q-Q plot, Shapiro Wilk test and Jarque Bera are used to study the distribution pattern of Total Cholesterol measurand. The histogram method, Normal Q-Q plot and Shapiro Wilk test were used in the chapter 7 to study the distribution of Total Cholesterol. In this chapter the Box Whisker plot, Shapiro-Wilk test, and Jarque Bera test used to study distribution of Total Cholesterol before and after removal of outliers. Outliers were identified using Box-Whisker plot and may be divided in to near and far outlier according to concentration of Total Cholesterol.

The advantage of Box-Whisker plot over histogram was that there is a procedure for partitioning the distribution pattern in the Box-Whisker plot and it is possible to subdivide the outliers as near and far outliers. Box Whisker plot can also be used to identify graphically the Gaussian distribution and presence of positive skewing of Total Cholesterol Concentration.

A study on “Lipid profiles and cardiovascular risk factors in a rural Indian population” used Box plots to display the distribution of Total cholesterol levels in rural Indian adults. The study reported that there is a significant difference in Total cholesterol levels between men and women (Gupta, R et al., 2017).

Box plots were utilized to illustrate the distribution of Total cholesterol level in Chinese adults and observed significant correlations between Total cholesterol

levels and cardiovascular risk factors. (Wang, Y et al., 2019). Box plots were employed to display the distribution of total cholesterol level in Iranian adults and observed significant differences in total cholesterol levels between different age groups. (Sadeghi, M et al., 2018)

A study published in the journal of Clinical Lipidology used Box-Whisker plots to display the distribution of Total cholesterol, LDL Cholesterol in patients with familial hypercholesterolemia (Goldberg et al., 2011). Box-Whisker plots were used to compare the lipid profiles between different ethnic groups in a study published in journal of Lipid research. (Haffner et al., 2012) A study published in the European Journal of Preventive Cardiology used box-whisker plots to display changes in lipid profiles after lifestyle interventions (Wood et al., 2013).

8.3. Objectives

- a) Collection of Fasting Total Cholesterol data from the Clinical Biochemistry Laboratory
- b) Evaluation of the Total Cholesterol distribution by Box-Whisker plot before and after outlier removal in samples, partitioned according to age and gender
- c) Calculating the cut-off value between moderate and severe hypercholesterolemia, and analyzing the number and percentage of samples in different Box-Whisker plot regions.

8.4. Materials and Methods

8.4.1. Research Design

This observational cross-sectional study on hypercholesterolemia was conducted at the Clinical Biochemistry Laboratory, Department of Biochemistry, Amala Institute of Medical Sciences, utilizing an indirect sampling method. Indirect sampling was chosen over direct population sampling due to the need for a large sample size to detect rare lipid profile abnormalities efficiently, within a shorter timeframe and at reduced cost (Horowitz et al., 2012). Fasting lipid profile samples were continuously collected from both outpatient and inpatient departments between

September 2018 and March 2022 to ensure randomization of data. The study received ethical approval from the Institutional Research and Ethics Committees (Ref. AIMSIEC/42/2018, dated 19/10/2018) for data collection under the Department of Biochemistry.

8.4.2. Inclusion Criteria and Exclusion criteria: (Refer General Materials and Methods)

8.4.3. Selection of Age Groups for the study

Samples were divided into age-based categories: <18 years (adolescents), 18–25 years (young adults), 26–50 years (middle-aged adults), and >50 years (senior adults).

8.4.4. Sample size requirement

The sample size was determined by a statistician based on a previously published multicentric study on dyslipidemia prevalence (Qi et al., 2015). Parameters used included: $d = 20\%$ prevalence, $\alpha = 0.05$, $p = 24\%$, $q = 1 - p$, $Z(1-\alpha) = 1.96$, and a power of 80%, resulting in a calculated sample size of 304.12, which was rounded to 304 per group. The final sample size of 15,603 was adequate to meet the requirements for all defined subgroups (refer to General Materials and Methods). A total cholesterol level of <200 mg/dL was considered the healthy cutoff, following ICMR-INDIA B Study (2014) and NCEP-2001 guidelines. The same sample set used in the histogram analysis (Chapter 7) was applied in this chapter.

8.4.5. Methodology:

- a) Collect fasting Total Cholesterol sample data from the Clinical Biochemistry Laboratory
- b) Assess distribution using Box-Whisker plots before and after outlier removal in samples, partitioned according to age and gender.
- c) Calculate the cutoff value between moderate and severe hypercholesterolemia.
- d) Analyze the number and percentage of samples in different Box-Whisker plot regions

8.4.6. Assay of Total Cholesterol

Assay for Total Cholesterol was done with Vitros 5.1 FS autoanalyzer from Ortho Clinical Diagnostics (Refer General Materials and Methods)

8.4.7. Statistical Methods

Data distribution was analysed using SPSS version 19 through the Shapiro-Wilk and Jarque-Bera tests, with Box-Whisker plots used for graphical representation before and after excluding outliers (Gurumani, 2005). Differences in lipid levels between genders were tested using either the student t-test or Mann-Whitney U test, as appropriate (Altman, 1991; Gao and Smith, 2003).

8.5. Results

A. Distribution Characteristics of Total Cholesterol before outlier removal

In the presence of outliers, the Box-Whisker plot was found to be vertically compressed in all the groups. (Fig. 8.1). The vertical compression was severe in the male sample of the younger age groups below 18 years and 18 – 25 years. (Fig. 8.1A and 1C). The positive skewing can be appreciated in some the female samples of the lower age group <18 years and 18 – 25 years (Fig 8.1B and 1D).

The number of samples was calculated in each region of the Box-Whisker plot. In each of the eight gender age groups, there were 0 to 1 sample in the Lower Near outliers ($F_1 - f_1$) and the Lower Far outliers ($<F_1$) region in the two lower age groups and in the fourth group above 50 years of age in both males and females. In the age group 26 – 50 years these two regions had sample numbers between 0 and 6 (Table 8.1).

The percentage of samples significantly increased in the next four Box-Whisker plot regions. These regions were from the Lower Whisker ($a_1 - Q_1$) to the Upper Whisker ($Q_3 - a_3$). These four regions in males and females of age group <18 years had sample number between 17.5% and 26.7%. The maximum percentages of sample number were observed in these four regions of Box-Whisker plot in male and female samples of all age groups (Table 8.1). The upper near outlier and upper far outlier region had percentage samples between 0 and 4.1% in the eight gender-age groups (Table 8.1).

B. Distribution Characteristics of Total Cholesterol after outlier removal

The distribution of Total cholesterol after removal of outliers using Box-Whisker plot in repeated cycles of 1 to 2 resulted in complete removal of outliers. The number of outliers removed ranged from 2 to 47. After removal of outliers the male and female gender groups between 18 years showed Gaussian distribution. The higher age groups had positively skewed distribution. But after log transformation the higher age group samples showed a tendency towards non-Gaussian distribution. Shapiro – Wilk test showed increased tendency for positive skewing with increase in age. This was observed before and after removal of outliers (Table 8.2).

Visual observation of Box-Whisker plot after removal of outliers showed a near normal distribution in most of the eight gender groups except Fig. 8.2A, 3D, 3G and 3H.

After removal of outliers the tendency for positive skewing could not be easily appreciated. Therefore, the main advantage of Box-Whisker plot was to identify the near and far outliers and remove the outliers.

8.6. Discussions

The advantage of Box-Whisker plot over histogram is the following. This is

- a) Method for sub grouping outliers as near and far outliers.
- b) The percentile method is used for partitioning the sample, such as interquartile range, median and the upper and lower whisker region.
- c) In Box Whisker plot, the percentiles of each sample are used as the distribution criteria and not the concentration of the measurand which is Total Cholesterol.

These groupings of the percentile sequence of Total Cholesterol resulted in eight subgroups as follows.

- a) Lower Near outliers ($F_1 - f_1$)
- b) Lower Far outliers ($<F_1$)
- c) Lower Whisker ($a_1 - Q_1$)

- d) $Q_1 - Q_2$
- e) $Q_2 - Q_3$
- f) Upper Whisker ($Q_3 - a_3$)
- g) Upper Near outliers ($f_3 - F_3$)
- h) Upper Far outliers ($>F_3$)

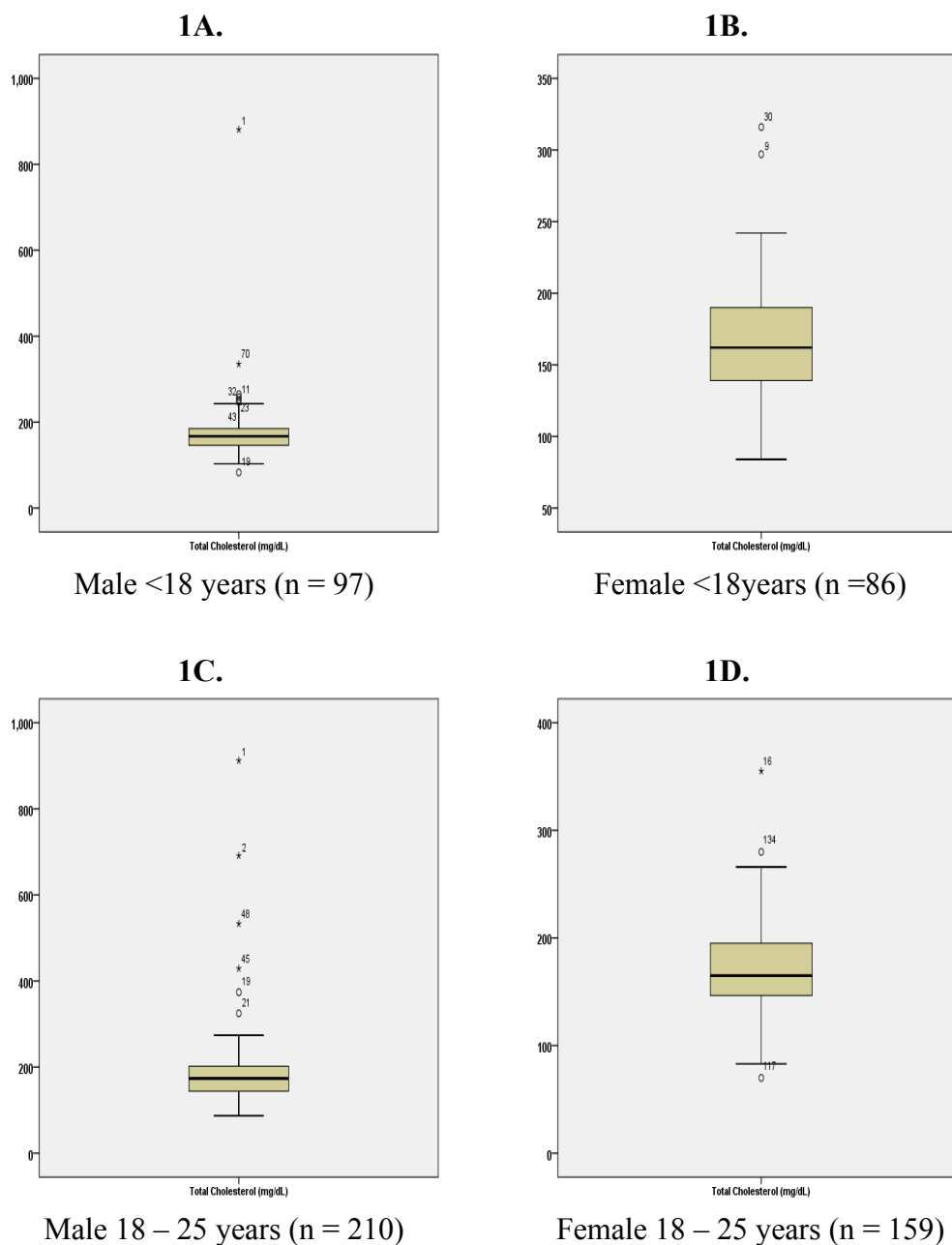
These eight partition regions are used to partition the two gender groups of the four age groups. Therefore, the total number of partition groups is equal to 2 (gender) x 4 (age groups) x eight (Box-Whisker plot region) = 64 groups are there in total. The sample number increased as age increased in both males and females (Table 8.1). The reason for this is due to increase in number of hospital visits as patients increases with advancing their age. Outlier removal will help in better graphical appreciation of the Box-Whisker plot regions before removal of outliers. The Box-Whisker plot was compressed due to the presence of high concentration of Total Cholesterol outliers. The concentrations greater than upper whisker (a_3) are cases of hypercholesterolemia. The a_3 concentrations range from 242 mg/dL to 311 mg/dL in the four age groups. Box Whisker Plot of hypercholesterolemia can be used to differentiate between prevalent, multifactorial, and rare, monogenic forms of the condition. Factors contributing to positive skewness of Total Cholesterol distribution were Genetic Predisposition, Diet and Life style, Age and sex, Obesity and physical inactivity, Metabolic syndrome and insulin resistance.

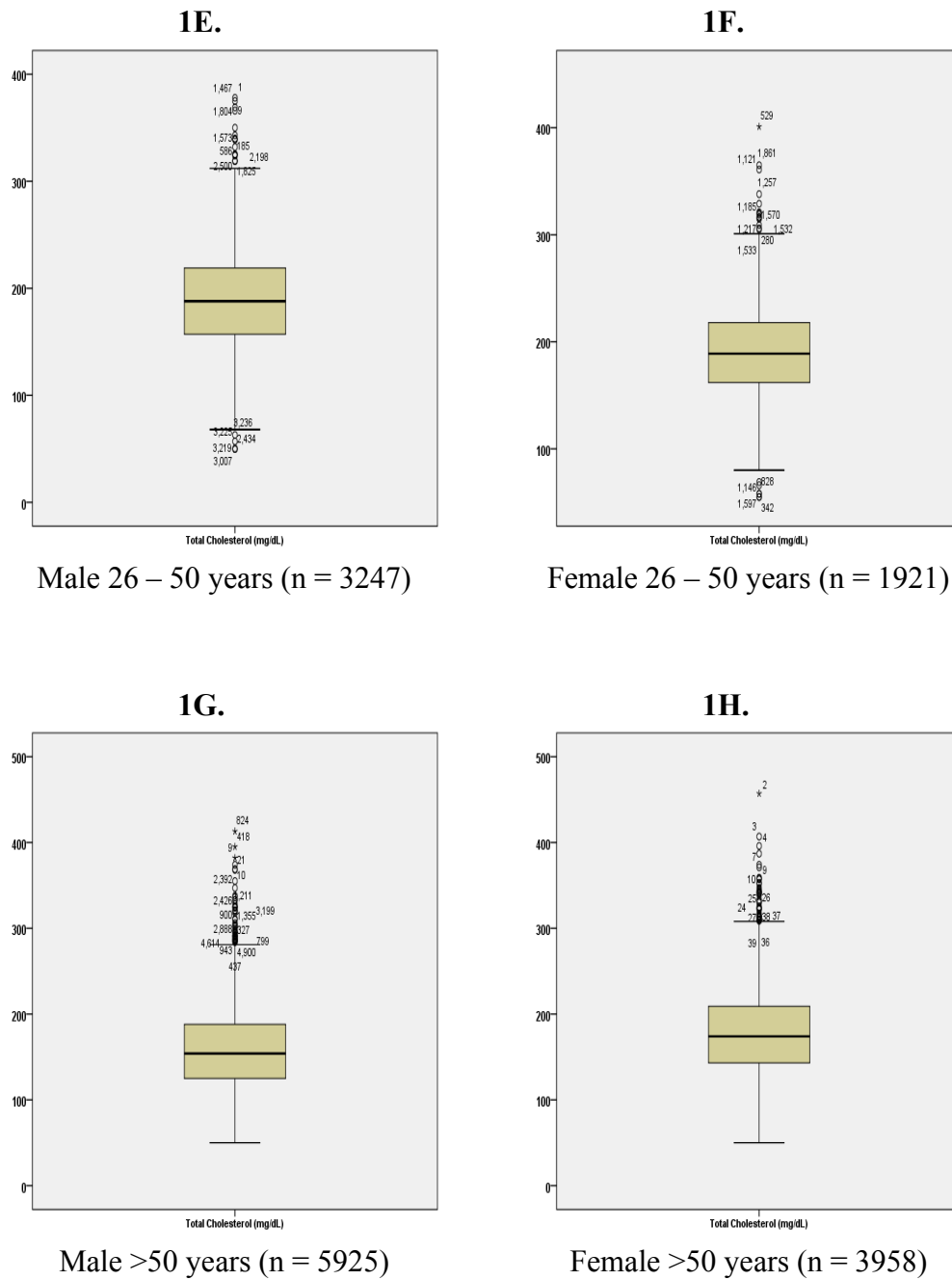
8.7. Conclusions

A statistical and clinical method of classification of Total Cholesterol concentration with quartiles, fences and outliers of Box-Whisker plot was proposed. Outliers were sub partitioned into near and far outliers. Genetic and DNA sequence data analysis of the multifactorial and single gene defects are not done, and are topics for future studies. Hormonal influence of Total Cholesterol concentrations resulting in gender differences in individuals between 18 and 50 years of age is another area for future study.

A. Distribution Characteristics of Total Cholesterol before outlier removal

Figure. 8.1. Box Whisker plot of the sample distribution of serum Total Cholesterol (mg/dL) in males and females of various age groups before outlier removal.





(Figure 8.1 continuation)

Table 8.1. The number of samples calculated from Box Whisker plot in the skewed and outlier region of Total Cholesterol (mg/dL) distribution data in samples of different age groups.

Partitioned regions of Box Whisker plot	Total Cholesterol concentration (mg/dL)		Sample number in the partitioned regions	
	M	F	M	F
<18 years: Male (n = 97); Female (n = 86): Median: Male – 167; Female – 162				
Lower Near outliers ($F_1 - f_1$)	(6 – 86)	(-14 – 63)	1 (1.03%)	0
Lower Far outliers ($<F_1$)	< (6)	< (-14)	0	0
Lower Whisker ($a_1 - Q_1$)	103 – 146	84 – 139	24 (24.7%)	23 (26.7%)
$Q_1 - Q_2$	146 – 167	139 – 162	25 (25.7%)	21 (24.4%)
$Q_2 - Q_3$	167 – 186	162 – 190	24 (24.7%)	21 (24.4%)
Upper Whisker ($Q_3 - a_3$)	186 – 243	190 – 242	17 (17.5%)	19 (22.0%)
Upper Near outliers ($f_3 - F_3$)	246 – 306	267 – 343	4 (4.1%)	2 (2.3%)
Upper Far outliers ($>F_3$)	>306	>343	2 (2.0%)	0
18 – 25 Years: Male (n = 210); Female (n = 159): Median: Male – 174; Female – 165				
Lower Near outliers ($F_1 - f_1$)	(0 – 87)	(-1 – 73)	1 (0.47%)	1 (0.62%)
Lower Far outliers ($<F_1$)	(0)	(-1)	0	0
Lower Whisker ($a_1 - Q_1$)	96 – 144	83 – 146	53 (25.2%)	39 (24.5%)
$Q_1 - Q_2$	144 – 174	146 – 165	55 (26.1%)	42 (26.4%)
$Q_2 - Q_3$	174 – 202	165 – 195	50 (23.8%)	38 (23.8%)
Upper Whisker ($Q_3 - a_3$)	202 – 274	195 – 266	45 (21.4%)	37 (23.2%)
Upper Near outliers ($f_3 - F_3$)	289 – 376	268 – 342	2 (0.9%)	1 (0.6%)
Upper Far outliers ($>F_3$)	>376	>342	4 (1.9%)	1 (0.6%)

Table 8.1 continued

Partitioned regions of Box Whisker plot	Total Cholesterol concentration (mg/dL)		Sample number in the partitioned regions	
	M	F	M	F
26 – 50 years: Male (n = 3247); Female (n = 1921): Median: Male – 188; Female – 189				
Lower Near outliers ($F_1 - f_1$)	(-29 – 64)	(-6 – 78)	5 (0.15%)	6 (0.31%)
Lower Far outliers ($<F_1$)	(-29)	(-6)	0	0
Lower Whisker ($a_1 - Q_1$)	68 – 157	69 – 162	826 (25.4%)	489 (25.4%)
$Q_1 - Q_2$	157 – 188	162 – 189	806 (24.8%)	479 (24.9%)
$Q_2 - Q_3$	188 – 219	189 – 218	809 (24.9%)	475 (24.7%)
Upper Whisker ($Q_3 - a_3$)	219 – 311	218 – 301	783 (24.1%)	458 (23.8%)
Upper Near outliers ($f_3 - F_3$)	312 – 405	302 – 386	18 (0.5%)	13 (0.6%)
Upper Far outliers ($>F_3$)	>405	>386	0	1 (0.05%)
> 50 years: Male (n = 5925); Female (n = 3958): Median: Male – 154; Female – 174				
Lower Near outliers ($F_1 - f_1$)	(-64 – 31)	(-55 – 44)	0	0
Lower Far outliers ($<F_1$)	(-64)	(-55)	0	0
Lower Whisker ($a_1 - Q_1$)	50 – 125	50 – 143	1496 (25.2%)	1008 (25.4%)
$Q_1 - Q_2$	125 – 154	143 – 174	1520 (25.6%)	983 (24.8%)
$Q_2 - Q_3$	154 – 188	174 – 209	1450 (24.4%)	1006 (25.4%)
Upper Whisker ($Q_3 - a_3$)	188 – 281	209 – 307	1411 (23.8%)	920 (23.2%)
Upper Near outliers ($f_3 - F_3$)	283 – 377	308 – 407	45 (0.7%)	40 (1.0%)
Far outliers on right ($>F_3$)	>377	>407	3 (0.05%)	1 (0.02%)

B. Distribution Characteristics of Total Cholesterol after outlier removal.**Table 8.2.** Distribution of Total cholesterol (mg/dL) after removing outliers using Box Whisker plot in samples of age<18, 18 – 25 years, 26 - 50 years and >50 years (M – Male, F – Female)

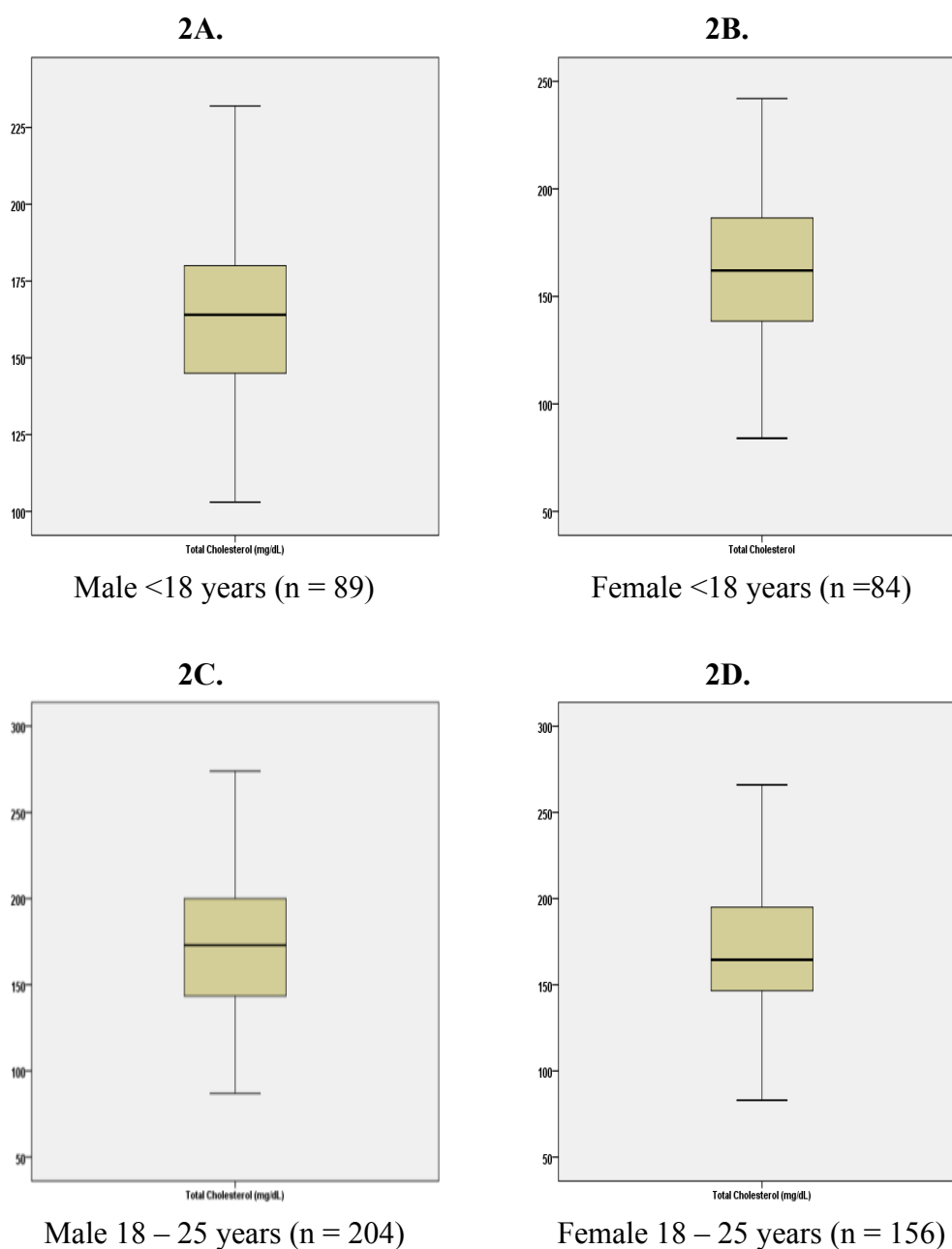
Sample number (n)		Number of outliers removed (cycles)	Mean±SD 95%CI for mean Min –Max Median Skewness kurtosis	Shapiro – Wilk (Jarque Bera) P value		Gender Difference, Mann-Whitney U test
Outlier removal				Log transformation		
Before	After			Before	After	
Samples, age <18 years						
M – 97	M –89	8 (2)	163.89±29.11 157.76 – 170.03 103 – 232 164.00 0.194 -0.285	0.525 0.619	0.488 0.544	0.880
F – 86	F – 84	2 (1)	162.79±34.32 155.34 – 170.24 84 – 242 162.00 0.001 -0.315	0.960 0.790	0.097 0.090	
Samples, age 18 – 25 years						
M – 210	M –204	6 (1)	173.75±39.12 168.35 – 179.16 87 - 274 173.00	0.051 0.128	0.035 0.092	

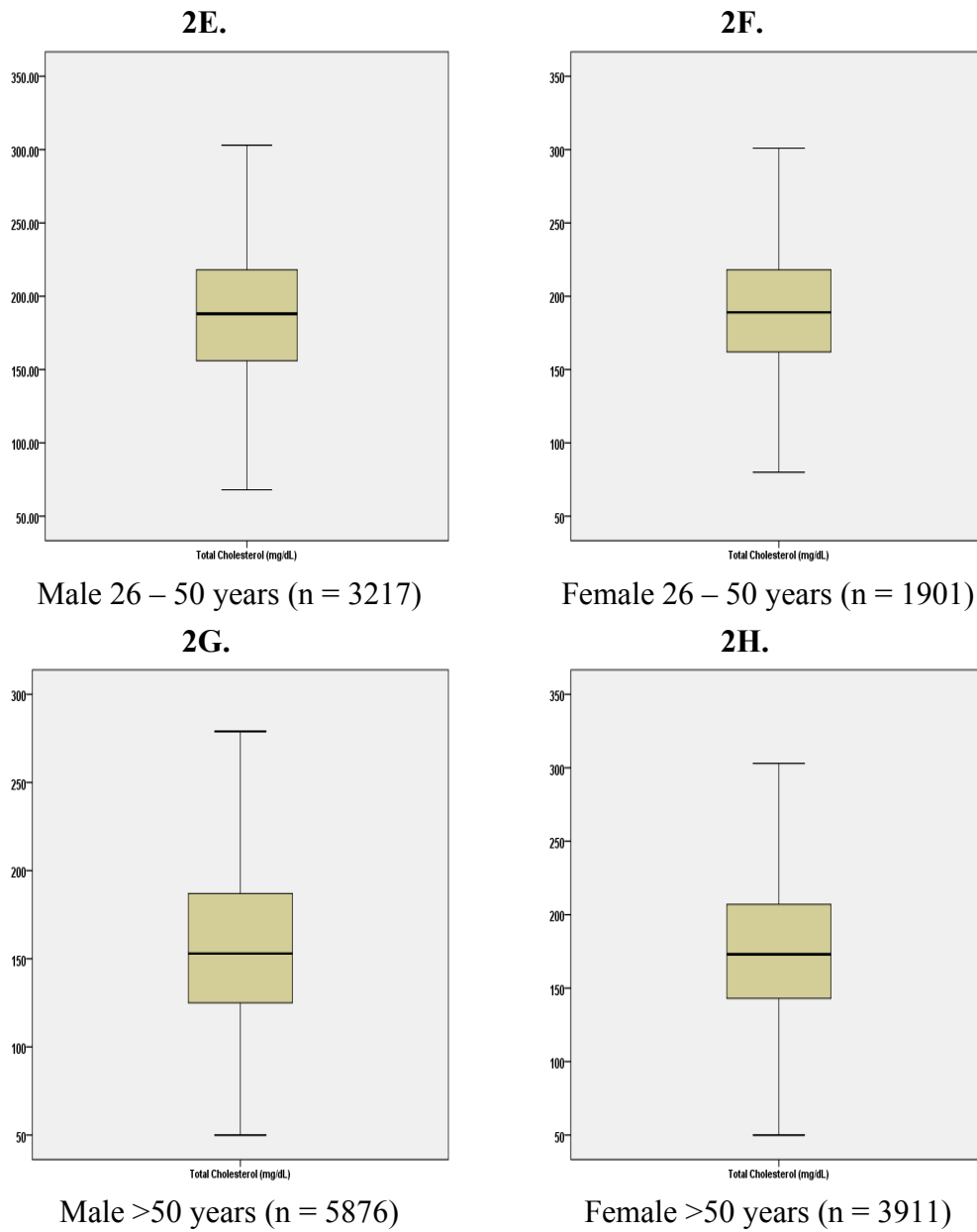
Sample number (n)		Number of outliers removed (cycles)	Mean±SD 95%CI for mean Min –Max Median Skewness kurtosis	Shapiro – Wilk (Jarque Bera) P value		Gender Difference, Mann-Whitney U test
Outlier removal				Log transformation		
Before	After			Before	After	
			0.214 -0.534			0.329
F – 159	F – 156	3 (1)	169.69±34.92 164.17 – 175.22 83 - 266 164.50 0.461 0.250	0.017 0.058	0.123 0.172	
Samples, age 26 – 50 years						
M – 3247	M – 3217	30 (2)	187.53±44.38 185.99 – 189.06 68 – 303 188.00 0.029 -0.387	<0.001 <0.001	<0.001 <0.001	0.059
F – 1921	F – 1901	20 (2)	190.22±39.41 188.45 – 191.99 80 – 301 189.00 0.137 -0.313	<0.001 <0.001	<0.001 <0.001	

Table 8.2 continuation

Sample number (n)		Number of outliers removed (cycles)	Mean±SD 95%CI for mean Min –Max Median Skewness kurtosis	Shapiro – Wilk (Jarque Bera) P Value		Gender Difference, Mann -Whitney U test
Outlier removal				Log transformation		
Before	After			Before	After	
Samples, age > 50 years						
M – 5925	M – 5876	49 (2)	157.16±43.37 156.05 – 158.27 50 - 279 153.00 0.303 -0.374	<0.001 <0.001	<0.001 <0.001	<0.001
F – 3958	F – 3911	47 (2)	175.45±45.26 174.04 – 176.87 50 – 303 173.00 0.202 -0.336	<0.001 <0.001	<0.001 <0.001	

Figure. 8.2. Box Whisker plot after outlier removal of Total Cholesterol (mg/dL) in males and females of various age groups after outlier removal.





(Figure. 8.2 continuation)

CHAPTER 9

HISTOGRAM-BASED SUB-CLASSIFICATION OF LDL CHOLESTEROL AND PROPORTION OF HYPERCHOLESTEROLEMIA ACROSS GENDER AND AGE GROUPS: A CROSS-SECTIONAL STUDY

9.1. Abstract

Introduction: Low-Density Lipoprotein (LDL-C) has been a significant focus of research due to its important role in determining cardiovascular disease burden. The available data suggests that LDL cholesterol prevalence is a public health concern in the Indian population. In this study, Lipid profile data was collected from Clinical Biochemistry Laboratory and samples were partitioned according to age and gender. In each of the partitioned age and gender group, the LDL Cholesterol distribution, percentage and number of samples in healthy, positively skewed and outlier regions of histogram were studied.

Objective: To investigate the distribution of LDL Cholesterol based on histogram, determine cutoff level between moderate and severe hypercholesterolemia and analyze the proportion of these categories in different age and gender groups.

Methods: This study, conducted at the Amala Institute of Medical Sciences, used an observational cross-sectional design and indirect sampling to investigate LDL cholesterol in normal and dyslipidemic individuals. Laboratory assessments were completed with the Ortho Clinical Diagnostics Vitros 5.1 FS analyzer. Statistical distribution was examined using the Shapiro-Wilk and Jarque-Bera tests and visualized with histograms and Q-Q plots. Differences between male and female lipid levels were tested by student's t-test or the Mann-Whitney U test. Spearman's method was used for correlation analysis, and SPSS software facilitated all statistical computations.

Results: In both males and females, sample number increased with advancing age. In each of the gender age groups, maximum sample number was seen in the healthy region and it decreased in the positively skewed region and further decreased in the outlier region. The mean LDL value increased in both males and females up to 50 years of age. Above 50 years of age LDL-C value appears to decrease. There were no gender differences in mean LDL-C value before 18 years and in the age group between 18 – 25 years. But in the age group 26 – 50 and >50 years, showed strong gender differences with higher cholesterol in the female sample. The positive skewing is due to moderate increase in LDL-C and outliers are due to very high increase in LDL

Cholesterol.

Conclusions: A statistical and clinical method of classification of LDL Cholesterol concentration using histogram was proposed. Graphical analysis by histogram and Normal Q-Q plot showed presence of positive skewing and was confirmed by Shapiro-Wilk and Jarque Bera test. The mean LDL Cholesterol values were higher in female sample. Log transformation could not transform the distribution to Gaussian even after removal of outliers in higher age groups. This is due to increased prevalence of hypercholesterolemia in the higher age groups. Hormonal influence of LDL Cholesterol concentrations resulting in gender differences in individuals between 18 and 50 years of age is another area for future study.

9.2. Introduction

Low-density lipoprotein (LDL) cholesterol plays important role in the development and progression of atherosclerotic cardiovascular disease (ASCVD) (Grundy et al., 2019; Ference et al., 2017). High levels of LDL cholesterol in blood, results in accumulation of cholesterol in arterial wall, which further lead to initiation of an inflammatory response and promote plaque formation (Libby et al., 2019). The plaque formation can further lead to cardiovascular events such as myocardial infarction and stroke.

The Framingham Heart Study demonstrated a strong positive correlation between LDL cholesterol levels and cardiovascular disease risk (Kannel et al., 1964).

Recent studies have explored various aspects of LDL cholesterol, such as its estimation, (Ephraim et al., 2023) effects of diet on LDL cholesterol (Schoeneck and Iggman, 2021), and management through novel therapeutic approaches (Pariyani, et al., 2025)

9.3. Objectives

- a) Collection of fasting LDL Cholesterol data from the Clinical Biochemistry Laboratory
- b) Evaluation of the LDL Cholesterol distribution by histogram before and after outlier removal in samples, partitioned according to age and gender
- c) Calculating the cutoff value between moderate and severe hypercholesterolemia, and analyzing the number and percentage of samples in different histogram regions.

9.4. Materials and Methods

9.4.1. Research Design

The study, designed as an observational cross-sectional investigation of hypercholesterolemia, was conducted at the Clinical Biochemistry Laboratory, Department of Biochemistry, Amala Institute of Medical Sciences. An indirect sampling approach was utilized, favoring it over direct population sampling to allow for the efficient study of rare lipid abnormalities, minimizing time and financial constraints associated with larger sample sizes (Horowitz et al., 2012). Fasting lipid profile data were continuously collected from outpatient and inpatient departments between September 2018 and March 2022 to ensure randomized representation. The Institutional Research and Ethics Committees approved the study (Ref. AIMSIEC/42/2018, dated 19/10/2018).

9.4.2. Inclusion Criteria and Exclusion criteria: (Refer General Materials and Methods)

9.4.3. Selection of Age Groups for the study

Participants were stratified by age into the following groups: adolescents (under 18 years), young adults (18 to 25 years), middle-aged adults (26 to 50 years), and older adults (above 50 years).

9.4.4. Sample size requirement

Sample size calculation was performed by a statistician using data from a previous multicenter study on dyslipidemia prevalence (Qi et al., 2015). The sample size was calculated to be 304.12. This was rounded to 304 for each subgroup. The final sample size of 15,603 was sufficient to meet the requirements across all defined subgroups (see General Materials and Methods). LDL cholesterol values below 130 mg/dL were considered normal, based on guidelines from the ICMR-INDIA B study (2014) and NCEP (2001).

9.4.5. Methodology:

- a) Collect fasting LDL Cholesterol sample data from the Clinical Biochemistry Laboratory
- b) Evaluate the LDL Cholesterol distribution by histogram before and after outlier removal in samples partitioned according to age and gender
- c) Calculate the cutoff value between moderate and severe hypercholesterolemia.
- d) Analyze the number and percentage of samples in different histogram regions.

9.4.6. Assay for LDL Cholesterol

When the LDL cholesterol was below 400 mg/dL, **LDL cholesterol** was assayed by indirect method which is done using Friedwald equation. $TC = LDL-C + HDL-C + (Total\ TG/5)$. LDL cholesterol was directly assayed when LDL cholesterol was above 400 mg/dL. In lipemic samples cloudiness is cleared by cooling to 4°C overnight and centrifuging at 4°C at 10,000 rpm for 20 minutes (Remaley et al., 2012)

9.4.7. Statistical Methods

Using SPSS version 19, the normality of the lipid data distribution was evaluated through the Shapiro-Wilk and Jarque-Bera tests, conducted both before and after eliminating outliers. Histograms and Normal Q-Q plots were employed for visual inspection of distribution characteristics. To examine gender-based variations in lipid concentrations, the student t-test was applied for parametric data and the Mann-

Whitney U test for non-parametric data, following the methodologies of Altman (1991) and Gao and Smith (2003).

9.5. Results

A. Distribution of LDL Cholesterol distribution after partitioning according to gender and age.

In both males and females, sample number increased with advancing age. The mean LDL value increased in both male and females up to 50 years of age. Above 50 years of age LDL-C value appears to decrease. Similarly, the median value was also found to increase in both males and females up to 50 years of age and, above 50 years, the median decreases (Table 9.1).

The distribution of LDL Cholesterol was found to be positively skewed in almost all samples before log transformation. The distribution as evaluated by Shapiro-Wilk or Jarque Bera test, after log transformation did not convert the positively skewed distribution to Gaussian distribution.

There is no gender difference in LDL Cholesterol values in samples <18 years of age (Mann Whitney U test $P = 0.824$).

In 18 – 25 years of age group, male ($n = 209$) samples had higher mean LDL Cholesterol values than the female ($n = 159$) samples (Table 1). But there was no gender difference in LDL Cholesterol values in samples 18 – 25 years of age (Mann Whitney U test $P = 0.336$) (Table 9.1).

The minimum to maximum range for LDL Cholesterol values in male samples (26 – 50 years of age) were from 2 to 299 mg/dL and in female samples were from 18 – 345 mg/dL. There was gender difference in LDL Cholesterol values in the sample of age between 26 – 50 years with mean values higher (121.88 ± 36.87 mg/dL) in female sample. The Mann Whitney U Test P value was <0.001 in samples of age between 26 – 50 years of age (Table 9.1).

There was significant gender difference in the LDL cholesterol levels of clinically apparent healthy individuals of >50 years of age with Mann Whitney U test value ($P = <0.001$). The mean values for LDL cholesterol were higher in female ($n =$

3952; Mean $\pm$ SD = 107.71 $\pm$ 43.39 mg/dL) samples than the male (n = 5908) samples of age group >50 years. (Table 9.1)

There were no gender differences in mean LDL-C value before 18 years and in the age group between 18 – 25 years. But in the age group 26 – 50 and >50 years, showed strong gender differences with higher cholesterol in the female sample. Below 25 years of age, male samples had higher mean LDL-C levels. However, the increased value was not statistically significant (Table 9.1)

Graphical Distribution Characteristics of LDL cholesterol in groups after partitioning according to gender and age

Except in samples of age group 26 -50 years and samples of age group >50 years, the normal Q-Q plot of LDL Cholesterol distribution showed large deviation of the sample distribution away from the straight-line normal distribution in male samples than in female sample. The normal Q-Q plot shows the presence of outliers and the presence of positive skewing (Fig.9.1).

The histogram of LDL-C showed the presence of outliers in all the samples, except in males below 18 years (Fig 9.2). Histograms of LDL cholesterol distribution in male (n = 209) sample were highly positively skewed than the female (n = 159) samples of age group between 18 – 25 years (Fig 9.2). Histogram of LDL cholesterol distribution in male (n = 3229) and female (n = 1916) samples of age group 26 – 50 years of age also showed slight positive skewing. There was moderate positive skewing in samples of age group >50 years (Fig 9.2).

The number and percentage of samples in healthy, positively skewed and outlier region of histogram

The number and percentage of samples in three different regions of histogram were analyzed for each of the gender and age groups.

Both males and females exhibited the highest percentage of samples within the healthy region of histogram. The number of samples decreased as LDL Cholesterol concentration increased in the positively skewed region and continued to

decline as LDL Cholesterol levels rose further in to the outlier region. This decrease is seen in both males and females. The percentage of sample in healthy region decreased in male and female up to 50 years of age. Above 50 years of age there were more percentage of samples in the healthy regions in both males and females (Table 9.2).

In < 18 years age group, there were 22.10% of male samples and 17.44% of female sample in positively skewed region. In 18 – 25 years of age group, the percentage of samples in positively skewed region and in outlier region were higher for male samples compared to female samples. In 26 – 50 years and >50 years, females had higher percentage of samples in positively skewed region with 40.34% and 28.51%, respectively compared to male samples (Table 9.2).

Results also indicated that the percentage of patients with high LDL-C increased up to 50 years of age in males and females (Table 9.2).

B. Distribution Characteristics of LDL Cholesterol after outlier removal

In < 18 years group there were no outliers in male samples, but female samples had 7 outliers removed in two repeated cycles from the histogram. The number of outliers was high in males (18) of age group 18 – 25 years compared to those of females (6) in the same age group. In the age group 26 – 50 years, there were 5 outliers (removed in 1 cycle) and 10 outliers (removed in 3 cycles) in male and female samples, respectively. Male samples > 50 years of age, had large number of outliers compared to those of females (Table 9.3)

When outliers were removed in repeated steps, there is no gender difference in LDL Cholesterol values in samples <18 years of age (Mann Whitney U test $P = 0.523$), and in age group 18 – 25 years (Mann Whitney U test $P = 0.790$),

The mean LDL cholesterol was higher in female samples of both age groups 26 – 50 years and >50 years with 121.14 ± 35.46 and 106.98 ± 42.01 , respectively. There was gender difference in LDL Cholesterol values in age group 26 – 50 years (Mann Whitney U test $P = <0.001$), and in age group >50 years (Mann Whitney U test $P = <0.001$) (Table 9.3)

Log transformation did not convert the positively skewed sample to normal distribution. This may be due to large number of high concentrations of positively skewed LDL Cholesterol sample. Shapiro-Wilk and Jarque – Bera test observations were graphically observed in histogram of LDL-C after removal of outliers. Males and females in the two lower age groups were graphically found to be normally distributed in the two higher age groups. The histogram was found to be positively skewed. These graphical observations were in agreement with Shapiro-Wilk and Jarque Bera test.

9.6. Discussions

Similar pattern of increase in mean LDL Cholesterol and its median was seen in males and females of three groups up to 50 years of age. The mean and the median LDL Cholesterol concentration decreased in the group above 50 years of age in males and females. In the two groups above 25 years of age, the mean LDL Cholesterol was higher in female samples when compared to that in males. These changes may be related to decrease physical activity above 25 years.

LDL-Cholesterol tend to be increased in females 26 – 50 years and >50 years than in males. In females, pre-menopausal period is 25 – 45 years. During this period, women's estrogen levels begin to decline. Estrogen is the hormone that helps to regulate LDL Cholesterol levels. As estrogen levels decreases, LDL Cholesterol levels may increase (Mathews et al., 2009). Women tend to gain weight and body fat during pre-menopausal period, particularly around the abdominal area. This can lead to increased LDL Cholesterol production (Krotkiewski, 2003).

Menopausal period in females is considered to be from 45 – 55 years of age. The decline in estrogen levels during this period can further contribute to increased LDL-C levels (Walsh and Schiff, 1997). Menopause is associated with changes in lipid metabolism, leading to increased LDL cholesterol and decreased HDL Cholesterol (Jensen et al., 2010).

Females above 55 years are considered to be in their post-menopausal period. The loss of estrogen hormones protective effects on lipid metabolism can lead to increased LDL Cholesterol levels (Mendelsohn and Karas, 2005). Post-menopausal

women often experience increased inflammation which can contribute to higher LDL-C levels. (Puder et al., 2005).

The lipid association of India recommends LDL- C goals to prevent atherosclerotic cardiovascular disease (Bansal et al., 2023). To relieve the horizontal compression of histogram caused by the outliers and for better graphical observation of distribution pattern, outliers of LDL-C were removed (Fig 9.3)

The positive skewing is due to moderate increase in LDL-C and outliers are due to very high increase in LDL Cholesterol. As the numbers of outliers are few in number compared to sample number in each group, there is not much influence in the mean cholesterol value. When log transformation is done to convert a positively skewed sample to Gaussian distribution, the distribution does not become Gaussian. This is because of the relatively larger number of samples with higher levels of moderately increased LDL-C (Fig 9.2).

9.6. Conclusions

A statistical and clinical method of classification of LDL Cholesterol concentration was proposed. Graphical analysis by histogram and Normal Q-Q plot showed presence of positive skewing and was confirmed by Shapiro-Wilk and Jarque Bera test. In each of the gender age groups maximum sample number was seen in the healthy region and it decreased in the positively skewed region and further decreased in the outlier region. The mean LDL Cholesterol values were higher in female sample. Log transformation could not transform the distribution to Gaussian even after removal of outliers in higher age groups. This is due to increased proportion of hypercholesterolemia in the higher age groups. Hormonal influence of LDL Cholesterol concentrations resulting in gender differences in individuals between 18 and 50 years of age is another area for future study.

A. Distribution of LDL Cholesterol distribution after partitioning according to gender and age.**Table.9.1.** LDL Cholesterol distribution and the Gender difference in gender – age groups before removal of outliers

Samples number, n (Total n = 15603)	Mean±SD 95%CI for mean Min –Max	Median Skewness kurtosis	Shapiro – Wilk, P (Jarque Bera)		Gender Difference, Mann-Whitney U Test (P)
			Log transformation		
			Before	After	
Samples, Age group < 18 years					
Male 95	103.93±34.92 96.81 – 111.03 21 – 215	99.00 0.525 0.510	0.112 (0.085)	0.003 (<0.001)	0.824
Female 86	103.22±37.38 95.20 – 111.23 24 – 251	100.00 1.260 3.688	(<0.001) (<0.001)	0.012 (<0.001)	
Samples, Age group 18 - 25 years					
Male 209	115.36±54.70 107.89 – 122.81 26 – 531	109.00 3.862 23.675	(<0.001) (<0.001)	(<0.001) (<0.001)	0.336
Female 159	107.64±32.96 102.48 – 112.81 30 – 275	104.00 1.071 3.858	(<0.001) (<0.001)	(<0.001) (<0.001)	
Samples, age group 26 – 50 years					

Samples number, n (Total n = 15603)	Mean±SD 95%CI for mean Min –Max	Median Skewness kurtosis	Shapiro – Wilk, P (Jarque Bera)		Gender Difference, Mann-Whitney U Test (P)
			Log transformation		
			Before	After	
Male 3229	115.54±40.95 114.13 – 116.95 2 – 299	115.00 0.256 0.118	(<0.001) (<0.001)	(<0.001) (<0.001)	(<0.001)
Female 1916	121.88±36.87 120.23 – 123.53 18 – 345	121.00 0.381 0.847	(<0.001) (<0.001)	(<0.001) (<0.001)	
Samples, age group> 50 years					
Male 5908	93.66±39.15 92.65 – 94.65 5 – 314	88.00 0.642 0.365	(<0.001) (<0.001)	(<0.001) (<0.001)	(<0.001)
Female 3952	107.71±43.39 106.36 – 109.06 2 – 351	102.00 0.641 0.534	(<0.001) (<0.001)	(<0.001) (<0.001)	
Table 9.1 continuation					

Figure. 9.1 Comparison of sample distribution of LDL Cholesterol (mg/dL) in various age and gender groups visualized by Normal Q-Q plot

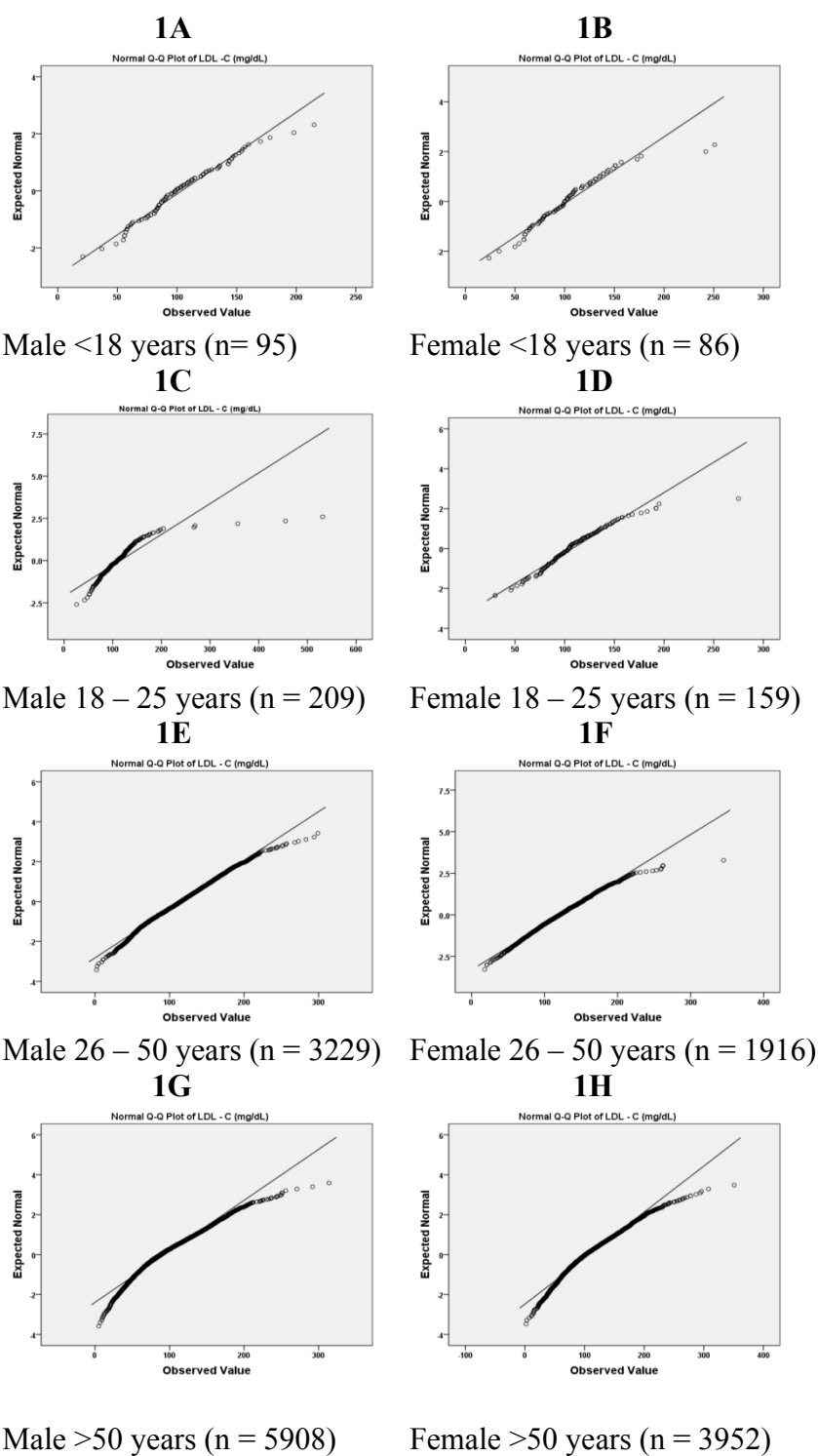
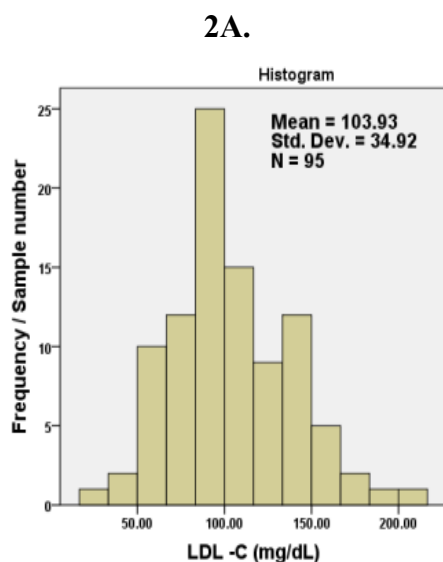
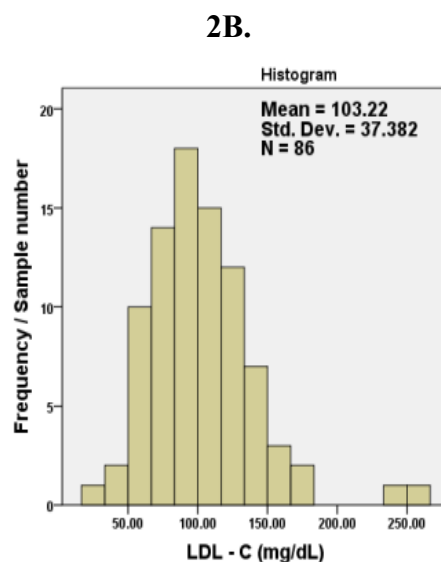


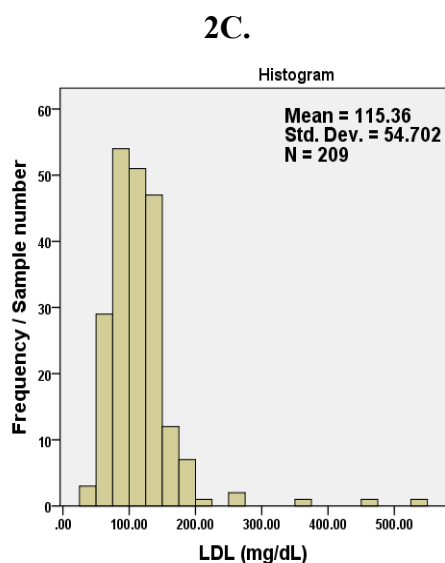
Figure. 9.2. Histogram of the sample distribution of LDL Cholesterol (mg/dL) in males and females of various age groups before outlier removal. (X axis – LDL Cholesterol (mg/dL), Y axis – Frequency of sample number)



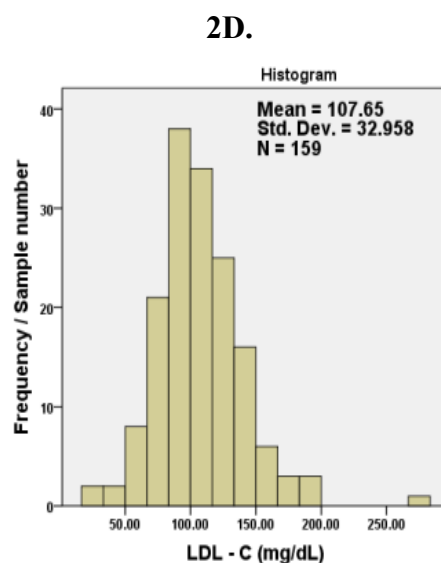
Male <18 years (n= 95)



Female <18 years (n = 86)

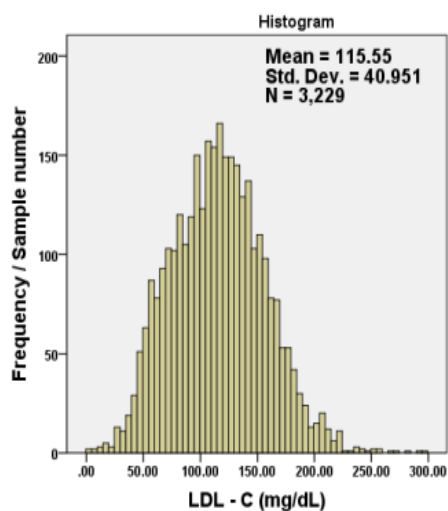


Male 18 – 25 years (n = 209)



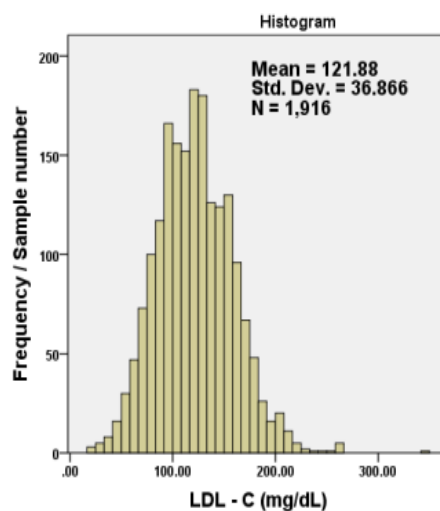
Female 18 – 25 years (n =159)

2E.



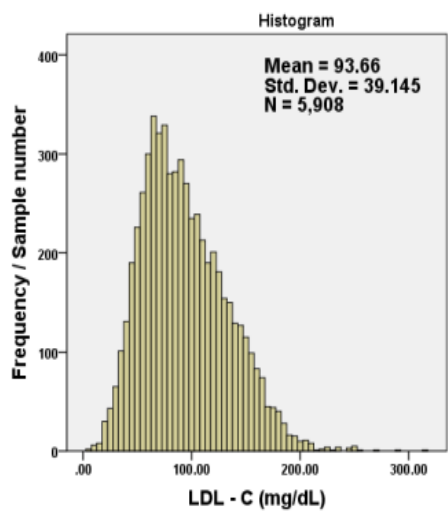
Male 26 – 50 years (n = 3229)

2F.



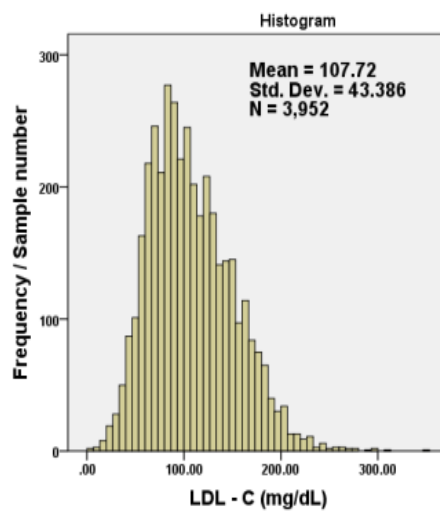
Female 26 – 50 years (n = 1916)

2G.



Male >50 years (n = 5908)

2H.



Female >50 years (n = 3952)

(Figure 9.2 continuation)

Table 9.2. The sample number and its percentage (%) in the histogram region of LDL Cholesterol <130mg/dL (**Healthy region**), LDL Cholesterol $\geq$ 130 mg/dL to last positively skewed sample (**Skewed region**), and last positively skewed to last outlier region (**Outlier region**) of LDL Cholesterol (mg/dL) in different age and gender groups. F and M are males and females

Age group	LDL Cholesterol <130	Sample number and %	
Sample number and %	130 to last positively skewed sample in M & F		
	Outlier region in M & F	Male	Female
<18 years M, n = 95 (100%) F, n = 86(100%)	<130	74(77.89%)	69(80.23%)
	M =130 – 215 F = 130 – 177	21(22.10%)	15(17.44%)
	M – >215 F – >177 – 251	0 (0%)	2 (2.32%)
18 – 25 Years M, n = 209(100%) F, n = 159(100%)	<130	152(72.72%)	124(77.98%)
	M = 130 – 204 F = 130 – 195	52(24.88%)	34(21.38%)
	M – >204 – 531 F – >195 – 275	5 (2.39%)	1(0.62%)
26 – 50 years M, n = 3229(100%) F, n =1916(100%)	<130	2056 (63.67%)	1142(59.60%)
	M =130 – 224 F = 130 – 262	1156 (35.80%)	773(40.34%)
	M – >224 – 299 F – >262 – 345	17 (0.52%)	1(0.05%)
>50 years M, n = 5908(100%) F, n =3952(100%)	<130	4799 (81.22%)	2818(71.30%)
	M = 130 – 209 F = 130 – 272	1081 (18.29%)	1127 (28.51%)
	M – >209 – 314 F – >272 – 351	28(0.47%)	7(0.17%)

B. Distribution Characteristics of LDL Cholesterol after outlier removal**Table.9.3.** Distribution characteristics and gender differences of LDL Cholesterol (mg/dL) after outlier removal by histogram. F and M are males and females

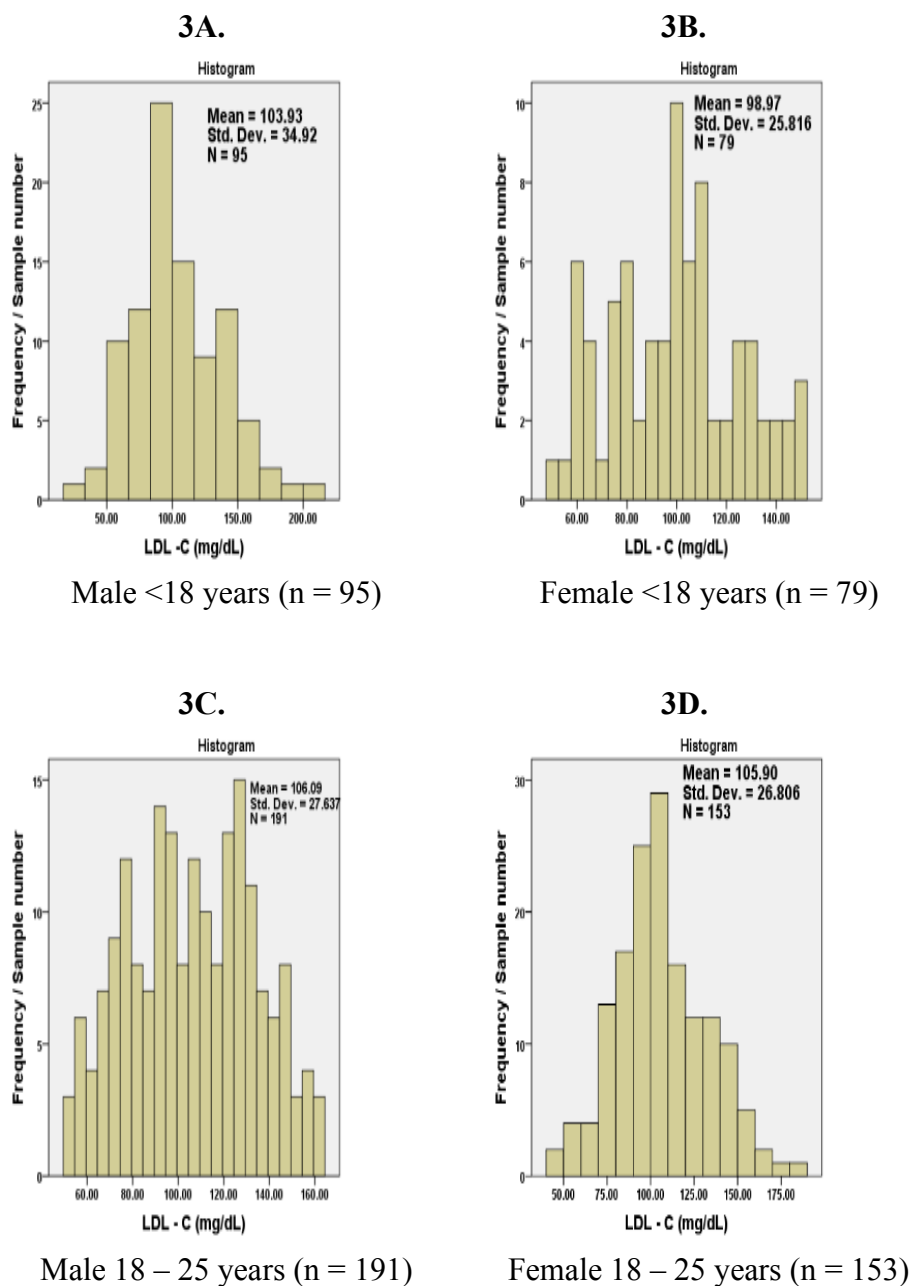
Sample number (n)		Outliers removed (cycles)	Mean±SD 95%CI for mean Min –Max Median Skewness kurtosis	Shapiro – Wilk, P (Jarque Bera)		Gender Difference, Mann -Whitney U test (P)
Outlier removal				Log transformation		
Before	After			Before	After	
Samples, age <18 years						
M – 95	M – 95	0	103.93±34.92 96.81 – 111.03 21 – 215 99.00 0.525 0.510	0.112 (0.085)	0.003 (<0.001)	0.523
F – 86	F – 79	7 (2)	98.97±25.81 93.19 – 104.75 50– 151 100.00 0.125 -0.735	0.135 (0.346)	0.042 (0.025)	
Samples, age 18 – 25 years						
M – 209	M – 191	18 (4)	106.09±27.63 102.14 - 110.38 52 – 164	0.008 (0.045)	<0.001 (0.006)	

Sample number (n)		Outliers removed (cycles)	Mean±SD 95%CI for mean Min –Max Median Skewness kurtosis	Shapiro – Wilk, P (Jarque Bera)		Gender Difference, Mann -Whitney U test (P)
Outlier removal				Log transformation		
Before	After			Before	After	
			108.00 -0.012 -0.871			0.790
F – 159	F – 153	6 (2)	105.89±26.80 106.61 – 110.71 46 – 183 104.00 0.326 -0.021	0.249 (0.262)	0.067 (0.061)	
Samples, age 26 -50 years						
M – 3229	M – 3224	5 (1)	115.28±40.44 113.88 – 116.68 2 – 257 115.00 0.172 -0.188	<0.001 (<0.001)	<0.001 (<0.001)	<0.001
F – 1916	F – 1906	10 (3)	121.14±35.46 119.55 – 122.74 18 – 225 120.50 0.119 -0.218	<0.001 (0.015)	0.007 (<0.001)	

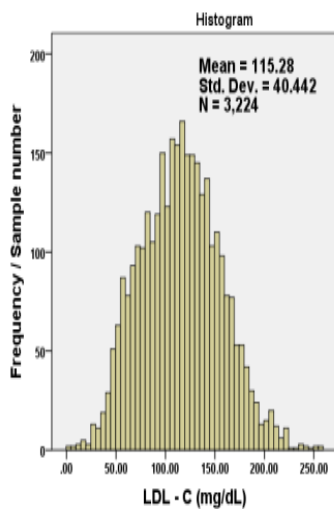
Table.9.3. (Continuation)

Sample number (n)		Outliers removed (cycles)	Mean±SD 95%CI for mean Min –Max Median Skewness kurtosis	Shapiro – Wilk, P (Jarque Bera)		Gender Difference, Mann -Whitney U test (P)
Outlier removal				Log transformation		
Before	After			Before	After	
Samples, age > 50 years						
M – 5908	M – 5884	24 (6)	93.04±38.04 92.07 -94.01 5 – 212 88.00 0.484 -0.312	<0.001 (<0.001)	<0.001 (<0.001)	<0.001
F – 3952	F – 3935	17 (2)	106.98±42.01 105.67 – 108.30 2 – 244 101.00 0.463 -0.216	<0.001 (<0.001)	<0.001 (<0.001)	

Figure.9.3. Histogram of the sample distribution of LDL Cholesterol (mg/dL) in males and females of various age groups after outlier removal. (X axis – LDL Cholesterol (mg/dL), Y axis – Frequency of sample number)

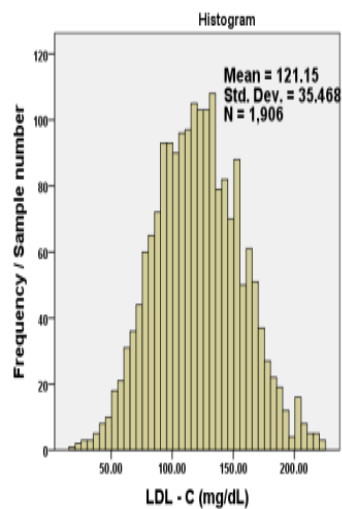


3E.



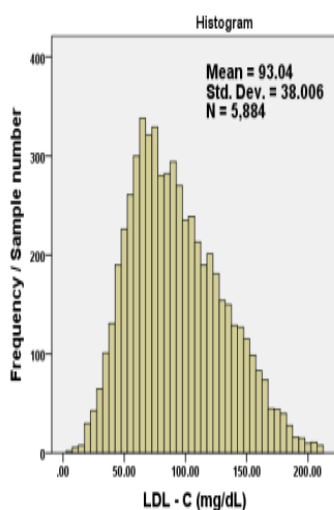
Male 26 – 50 years (n = 3224)

3F.



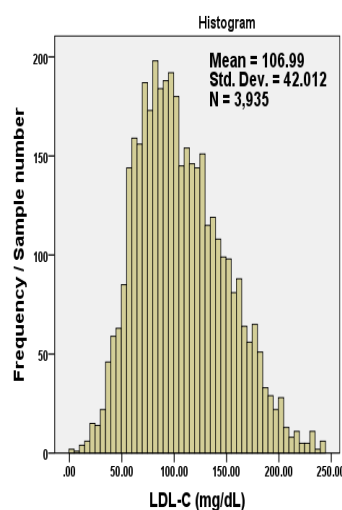
Female 26 – 50 years (n = 1906)

3G.



Male >50 years (n = 5884)

3H.



Female >50 years (n = 3935)

(Figure 9.3 continuation)

CHAPTER 10

**BOX-WHISKER PLOT USED FOR PARTITIONING LDL
CHOLESTEROL ACCORDING TO QUARTILES AND FENCES TO
IDENTIFY GENDER AND AGE-RELATED DIFFERENCES AND
SIMILARITIES – A CROSS-SECTIONAL STUDY**

10.1. Abstract

Introduction: Increase in LDL Cholesterol is a significant risk factor for CVD in Indians. In this study, fasting LDL Cholesterol samples was collected from Clinical Biochemistry Laboratory and samples were partitioned according to age and gender. In each of the partitioned age and gender group, the LDL Cholesterol distribution, percentage and number of samples in different regions of Box-Whisker plot were studied. Visualizing the distribution of LDL Cholesterol levels can provide insights in to the characteristics of data, such as central tendency, variability and outliers.

Objectives: To evaluate the LDL Cholesterol distribution, gender disparities, and the proportion of hypercholesterolemia in relation to median, quartiles and fences using Box-Whisker plots

Methods: This cross-sectional observational study focused on normal and dyslipidemic LDL cholesterol levels, using indirect sampling, and was carried out at Amala Institute of Medical Sciences. Lipid profiles were assayed using the Vitros 5.1 FS analyzer. Data analysis was conducted with SPSS software, and distribution characteristics were visualized through Box-Whisker plots. Statistical comparisons of lipid concentrations between genders were done by student t-test or Mann-Whitney U test.

Results: Box-Whisker plot showed Positive skewing and presence of outliers. Box-Whisker plot of LDL cholesterol revealed an increasing trend in outliers with advancing age in both males and females. Samples in the lower near outlier regions may be due to abetalipoproteinemia below 25 years of age after removal of outliers; LDL-C was normally distributed. No gender differences were observed after outlier removal in < 18 years. In 18 – 25 years, 26 – 50 years and >50 years, there were strong gender differences with mean LDL Cholesterol value higher in females. The gender differences in samples >25 years of age was contributed by high mean LDL-C in female samples.

Conclusions: A statistical and clinical method of classification of LDL Cholesterol concentration with quartiles, fences and outliers of Box-Whisker plot was proposed.

Outliers were sub partitioned into near and far outliers. The concentrations greater than upper whisker (a_3) are cases of hypercholesterolemia. The a_3 concentrations range from 177mg/dL to 319 mg/dL in the four age groups. Box Whisker Plot of hypercholesterolemia can help clinicians distinguish between common, multifactorial, moderate hypercholesterolemia and rare, severe, monogenic forms.

10.2. Introduction

The liver produces Very Low-Density Lipoprotein, which is often converted in to LDL Cholesterol through the action of lipoprotein lipase (Remaley et al., 2012). Several factors like genetics, diet, obesity, physical inactivity and smoking can influence LDL Cholesterol level. Elevated levels of LDL can contribute to the development of cardiovascular disease (CVD) through several mechanisms like atherogenesis, inflammation and endothelial dysfunction. Understanding the physiology, factors influencing levels, role in cardiovascular disease, measurement, classification, and management of LDL cholesterol is essential for prevention and treatment of CVD.

Understanding the distribution of LDL Cholesterol levels is essential for identifying high-risk populations and developing effective prevention strategies. Box Whisker can provide clear and concise visual representation of data, making it easier to identify patterns and trends. (Tuft, 2001). Shape of the distribution, including skewness and outliers can be studied by using Box-Whisker plots.

In this chapter the Box Whisker plot, Shapiro-Wilk test and Jarque Bera test were used to study distribution of LDL Cholesterol before and after removal of outliers. Outliers were identified using Box-Whisker plot and may be divided in to near and far outlier according to concentration of LDL Cholesterol.

10.3. Objectives

- a) Collection of Fasting LDL Cholesterol sample data from the Clinical Biochemistry Laboratory
- b) Evaluation of the LDL Cholesterol distribution by Box-Whisker plot before and after outlier removal in samples, partitioned according to age and gender
- c) Calculating the cut-off value between moderate and severe

hypercholesterolemia, and analyzing the number and percentage of samples in different Box-Whisker plot regions.

10.4. Materials and Methods

10.4.1. Research Design

This observational cross-sectional study on hypercholesterolemia was conducted in the Clinical Biochemistry Laboratory, Department of Biochemistry, Amala Institute of Medical Sciences, using an indirect sampling method. This approach was selected over direct population sampling due to its efficiency in handling large sample sizes, essential for identifying rare lipid profile disorders within a shorter timeframe and with lower costs (Horowitz et al., 2012). Fasting lipid profile samples were continuously collected from both outpatient and inpatient departments between September 2018 and March 2022, ensuring randomization of lipid profile data. The study protocol received approval from the Institutional Research and Ethics Committees (Ref. AIMSIEC/42/2018, dated 19/10/2018) for the Department of Biochemistry and data usage from the Clinical Biochemistry Laboratory.

10.4.2. Inclusion Criteria and Exclusion criteria: (Refer General Materials and Methods)

10.4.3. Selection of Age Groups for the study

The collected samples were categorized into four age groups: adolescents (<18 years), young adults (18–25 years), middle-aged adults (26–50 years), and senior adults (>50 years).

10.4.4. Sample size requirement

Sample size estimation was performed by a statistician using prevalence data from a multicentric study on dyslipidaemia (Qi et al., 2015). The required sample size was calculated as 304.12 and rounded to 304 per group. The final dataset included 15,603 samples, satisfying subgroup requirements (see General Material and Methods). The LDL cholesterol cutoff for healthy individuals was <130 mg/dL, as per ICMR-INDIA B (2014) and NCEP (2001) guidelines. The sample set used in chapter 9 was applied for this study

10.4.5. Methodology:

- a) Collect fasting LDL Cholesterol sample data from the Clinical Biochemistry Laboratory
- b) Evaluate the LDL Cholesterol distribution by Box-Whisker plot before and after outlier removal in samples partitioned according to age and gender
- c) Calculate the cut-off value between moderate and severe hypercholesterolemia.
- d) Analyze the number and percentage of samples in different Box-Whisker plot regions.

10.4.6. Assay for LDL Cholesterol

When the LDL cholesterol was below 400 mg/dL, **LDL cholesterol** was assayed by indirect method which is done using Friedwald equation. $TC = LDL + HDL + C + (Total\ TG/5)$ (12), LDL cholesterol was directly assayed when LDL cholesterol was above 400 mg/dL. In lipemic samples cloudiness is cleared by cooling to 4°C overnight and centrifuging at 4°C at 10,000 rpm for 20 minutes (Remaley et al., 2012)

10.4.7. Statistical Methods

Data distribution was assessed in SPSS version 19 using Shapiro-Wilk and Jarque-Bera tests, and visualized via Box-Whisker plots before and after excluding outliers (Gurumani, 2005). Gender differences in lipid levels were analysed using either the student t-test or the Mann-Whitney U test, depending on data normality (Altman, 1991; Gao & Smith, 2003).

10.5. Results**A. Box -Whisker plot of LDL Cholesterol before removal of outliers.**

Box-Whisker plot of LDL cholesterol showed increase in the number of outliers as age increased in males and females (Fig.10.1). The positive skewing cannot be clearly appreciated before removal of outliers in some groups. The positive skewing is represented predominantly by Box region above the median. The Box

region is increased in Fig 10.1A, 1D, 1G and 1H. In these groups the presence of outliers was vertically compressing the Box-Whisker plot.

Outliers were very few in the age group below 18 years. The percentage of positive outliers was ranging from 0 to 2.5% in males and females between 18 – 25 years of age. But in the age group 26 – 50 years, the percentage of outliers ranged from 0 to 0.6%. The percentage of outliers in the age group >50 years was 0 – 0.9% (Table 10.1).

There were near equal percentage of samples in the lower whisker ($a_1 - Q_1$), ($Q_1 - Q_2$), ($Q_2 - Q_3$) and upper whisker ($Q_3 - a_3$). In these four age groups, the percentages of samples were ranging between 21.3 – 26.7%. There were no samples in almost all lower outlier regions.

B. Box -Whisker plot of LDL Cholesterol after removal of outliers.

One of the reasons for removal of outliers was to relief the vertical compression of Box-Whisker plot. This was done for appreciating the distribution pattern. Even after log transformation the positively skewed samples in the two groups above 25 years of age in males and females were found to be having non-Gaussian distribution due to positive skewing. Below 25 years of age after removal of outliers, LDL-C was normally distributed and the normal distribution was converted in to non-Gaussian distribution after log transformation (Table 10.2).

There was no gender differences observed after outlier removal in the age group below 18 years (Table 10.2). In the three age groups >18 years, there were strong gender differences. In the age groups above 25 years, the female samples had higher LDL-C. The positive skewing could not be easily appreciated graphically using Box Whisker plot (Fig. 10.2).

10.6. Discussions

The advantage of Box-Whisker plot was to identify near and far outliers. But the major disadvantage is to graphically identify minor positive skewing and inability to identify double peak formation. Double peak formation may be due to

heterogeneity in LDL-C distribution.

Near and far outliers arise when there are high concentrations of LDL-C. The positive skewing is better appreciated after removal of outliers, when vertical compression is relieved.

Log-transformation was done to convert a non-Gaussian positively skewed distribution. After removal of outliers, log transformation can be more clearly transform a non-Gaussian positively skewed distribution to a Gaussian one. In some cases, even after removal of outliers in samples from age group >25 years, the log transformation does not bring distribution to normal. This was due to presence of larger number of samples with high LDL-C. Such changes in distribution can also indicate differences in the contribution of genetic component and the environmental component in samples with increased LDL-C.

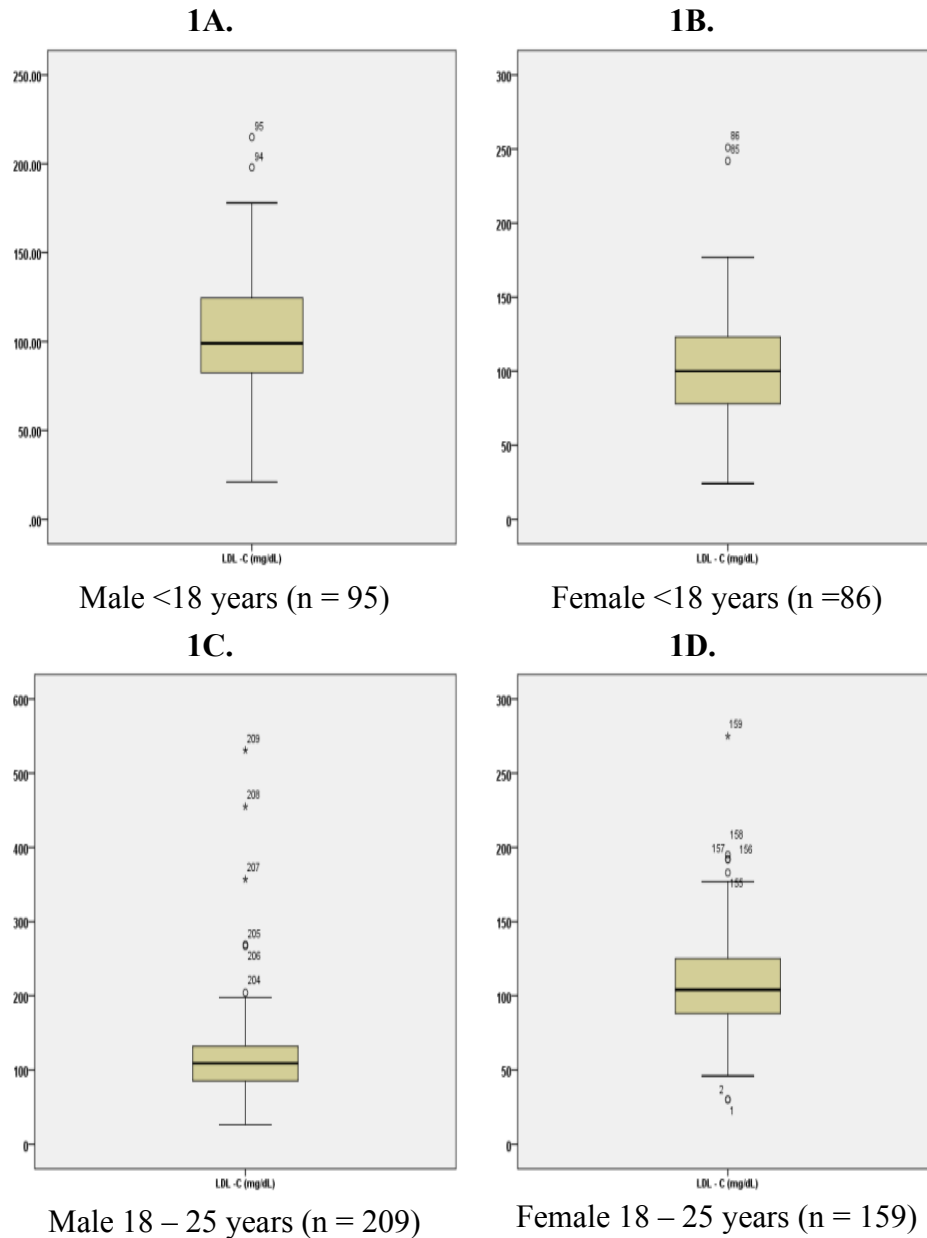
The gender differences in samples >25 years of age was contributed by high mean LDL-C in female samples. The LDL-C, as expected, were lower in age group <18, 18 -25 years after outlier removal. These results indicated the importance of distribution analysis for clinical interpretation of increased LDL-C in the population.

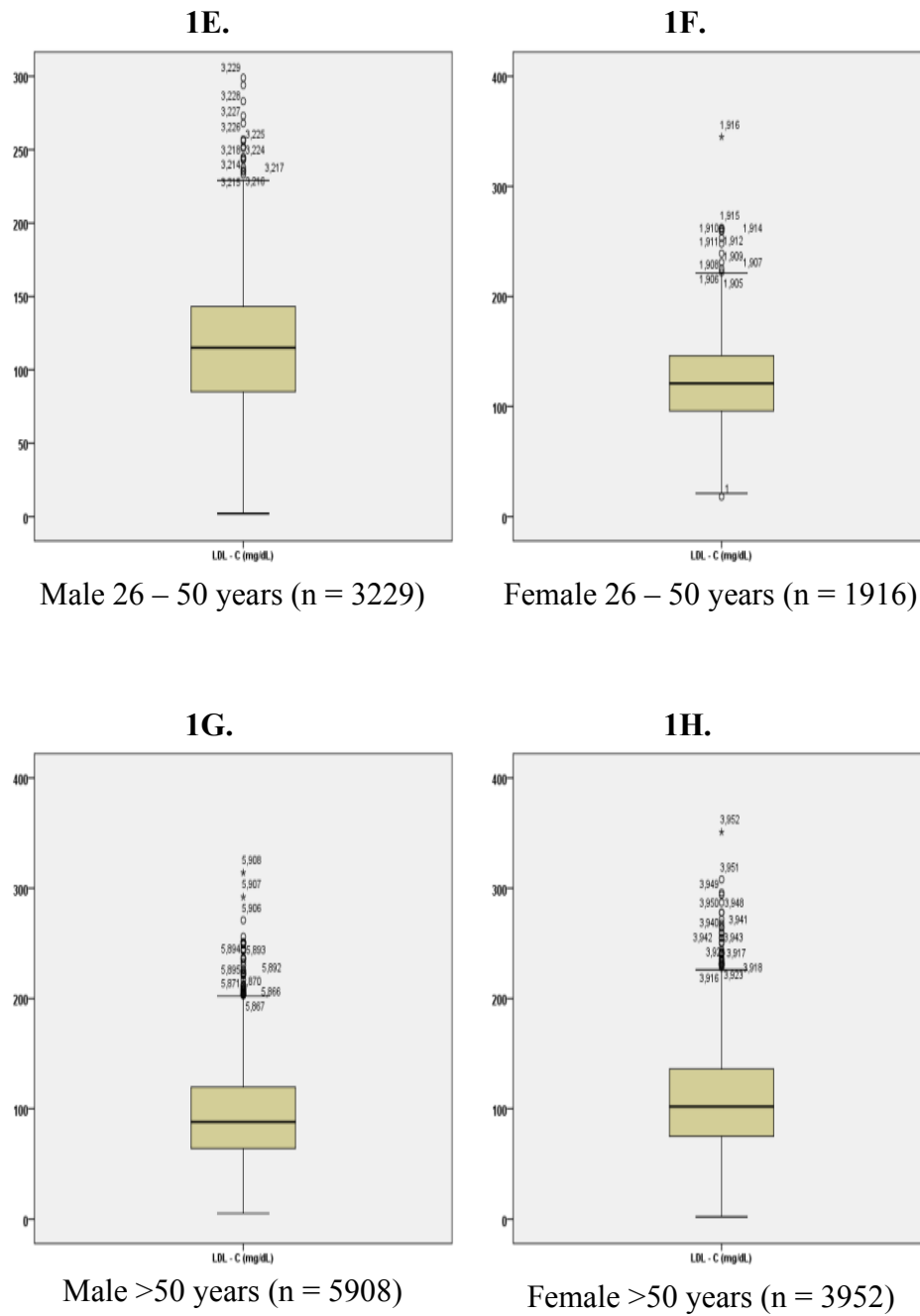
10.7. Conclusions

Graphical analysis and calculation of distribution character of LDL-C were done using Box-Whisker plot and Shapiro-Wilk or Jarque Bera test. As expected LDL-C increases with age but increase is more in female sample, which is seen from the gender differences of LDL-C. A statistical and clinical method of classification of LDL Cholesterol concentration with quartiles, fences and outliers of Box-Whisker plot was proposed. The concentrations greater than upper whisker (a_3) are cases of hypercholesterolemia. The a_3 concentrations range from 177mg/dL to 319 mg/dL in the four age groups. Box Whisker Plot of hypercholesterolemia can help clinicians distinguish between common, multifactorial, moderate hypercholesterolemia and rare, severe, monogenic forms.

A. Box -Whisker plot of LDL Cholesterol before removal of outliers.

Figure. 10.1. Box Whisker plot of the sample distribution of LDL Cholesterol (mg/dL) in males and females of various age groups before outlier removal.





(Figure 10.1 continuation)

Table 10.1. The number of samples calculated from Box Whisker plot in the skewed and outlier region of LDL Cholesterol (mg/dL) distribution data in samples of different age groups.

Partitioned regions of Box Whisker plot	LDL Cholesterol concentration (mg/dL)		Sample number in the partitioned regions	
	M	F	M	F
<18 years: Male (n = 95); Female (n = 86): Median: Male – 99; Female – 100				
Lower Near outliers ($F_1 - f_1$)	(-47 – 18)	(-57 – 11)	0	0
Lower Far outliers ($<F_1$)	$< (-47)$	$< (-57)$	0	0
Lower Whisker ($a_1 - Q_1$)	21 – 82	24 – 78	24 (25.2%)	22 (25.5%)
$Q_1 - Q_2$	82 – 99	78 – 100	25 (26.3%)	23 (26.7%)
$Q_2 - Q_3$	99 – 125	100 – 123	23 (24.2%)	20 (23.3%)
Upper Whisker ($Q_3 - a_3$)	125 – 178	123 – 177	21 (22.1%)	19 (22.0%)
Upper Near outliers ($f_3 - F_3$)	190 – 254	191 – 263	2 (2.1%)	2 (2.3%)
Upper Far outliers ($>F_3$)	>254	>263	0	0
18 – 25 Years: Male (n = 209); Female (n = 159): Median: Male – 109; Female – 104				
Lower Near outliers ($F_1 - f_1$)	(-56 – 15)	(-26 – 31)	0	2 (1.25%)
Lower Far outliers ($<F_1$)	(-56)	(-26)	0	0
Lower Whisker ($a_1 - Q_1$)	26 – 85	40 – 88	51 (24.4%)	40 (25.1%)
$Q_1 - Q_2$	85 – 109	88 – 104	51 (24.4%)	38 (23.8%)
$Q_2 - Q_3$	109 – 132	104 – 126	52 (24.8%)	40 (25.1%)
Upper Whisker ($Q_3 - a_3$)	132 – 198	126 – 177	45 (21.5%)	34 (21.3%)
Upper Near outliers ($f_3 - F_3$)	203 – 273	183 – 240	3 (1.4%)	4 (2.5%)
Upper Far outliers ($>F_3$)	>273	>240	3 (1.4%)	1 (0.6%)

Table 10.1. (continuation)

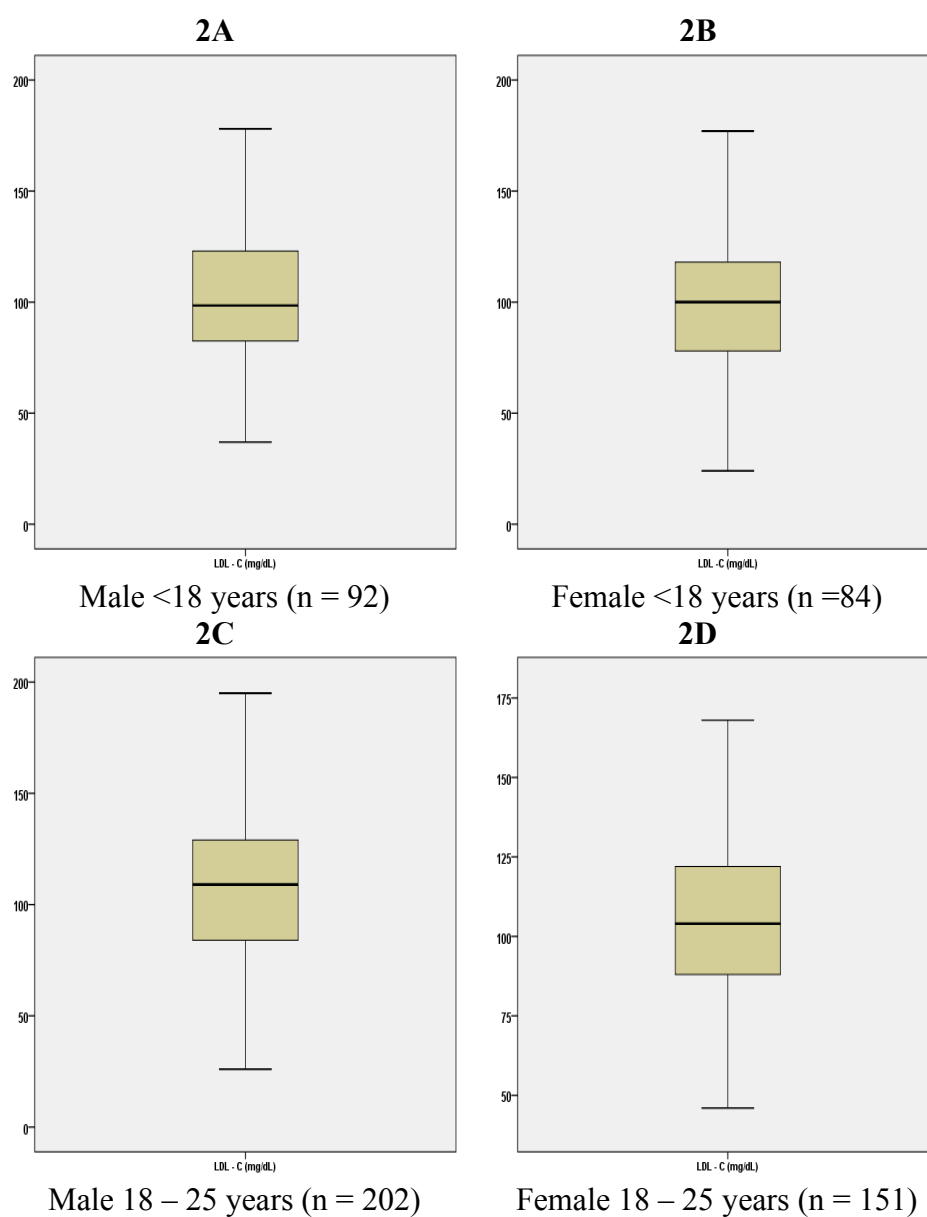
Partitioned regions of Box Whisker plot	LDL Cholesterol concentration (mg/dL)		Sample number in the partitioned regions	
	M	F	M	F
26 – 50 years: Male (n = 3229); Female (n = 1916): Median: Male – 115; Female – 121				
Lower Near outliers ($F_1 - f_1$)	(-3 – -89)	(-54 – 21)	0	3 (0.15%)
Lower Far outliers ($<F_1$)	(-3)	(-54)	0	0
Lower Whisker ($a_1 - Q_1$)	2 – 85	26 – 96	809 (25.0%)	487 (25.4%)
$Q_1 - Q_2$	85 – 115	96 – 121	815 (25.2%)	486 (25.3%)
$Q_2 - Q_3$	115 – 143	121 – 146	809 (25.0%)	463 (24.1%)
Upper Whisker ($Q_3 - a_3$)	143 – 229	146 – 220	780 (24.1%)	464 (24.2%)
Upper Near outliers ($f_3 - F_3$)	230 – 317	221 – 292	16 (0.5%)	12(0.6%)
Upper Far outliers ($>F_3$)	>317	>292	0	1 (0.05%)
> 50 years: Male (n = 5908); Female (n = 3952): Median: Male – 88; Female – 102				
Lower Near outliers ($F_1 - f_1$)	(-104 – 20)	(-108 – -17)	0	0
Lower Far outliers ($<F_1$)	(-104)	(-108)	0	0
Lower Whisker ($a_1 - Q_1$)	5 – 64	2 – 75	1495 (25.3%)	992 (25.1%)
$Q_1 - Q_2$	64 – 88	75 – 102	1482 (25.0%)	1016 (25.7%)
$Q_2 - Q_3$	88– 120	102 – 136	1492 (25.2%)	972 (24.5%)
Upper Whisker ($Q_3 - a_3$)	120 – 203	136 – 226	1398 (23.6%)	935(23.6%)
Upper Near outliers ($f_3 - F_3$)	204 – 288	228– 319	39 (0.7%)	36 (0.9%)
Far outliers on right ($>F_3$)	>288	>319	2 (0.03%)	1 (0.02%)

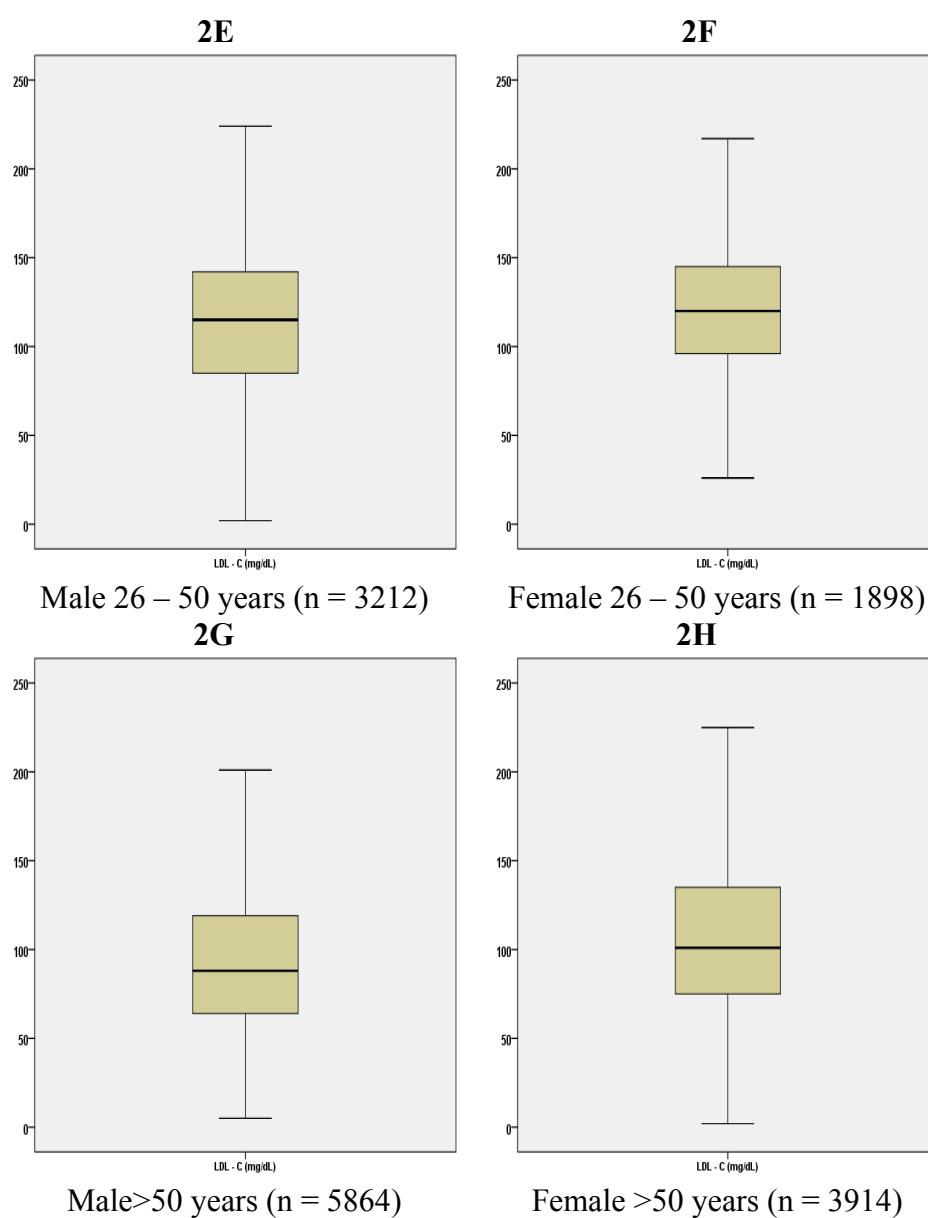
B. Box -Whisker plot of LDL Cholesterol after removal of outliers.**Table 10.2.** Distribution of LDL cholesterol (mg/dL) after removing outliers using Box Whisker plot in samples of age<18, 18 – 25 years, 26 - 50 years and >50 years (M – male, F – female)

Sample number (n)		Number of outliers removed (cycles)	Mean±SD 95%CI for mean Min –Max Median Skewness kurtosis	Shapiro – Wilk P (Jarque Bera)		Mann - Whitney U test P
Outlier removal				Log transformation		
Before	After			Before	After	
Samples, age <18 years						
M – 95	M –92	3 (2)	102.59±30.81 96.21 – 108.97 37 - 178 98.50 0.295/-0.517	0.141 (0.291)	0.094 (0.245)	0.603
F – 86	F – 84	2 (1)	99.80±30.39 93.21 – 106.40 24 – 177 100.00 0.154/-0.036	0.856 (0.835)	0.001 (<0.001)	
Samples, age 18 – 25 years						
M – 209	M – 202	7 (2)	108.06±31.84 103.64 – 112.48 26 - 195 109.00 0.200/-0.243	0.349 (0.381)	<0.001 (<0.001)	0.002
F – 159	F – 151	8 (2)	104.91±25.57 100.80 – 109.02 46 – 168 104.00 0.167/-0.311	0.374 (0.492)	0.012 (0.019)	

Sample number (n)		Number of outliers removed (cycles)	Mean±SD 95%CI for mean Min –Max Median Skewness/ kurtosis	Shapiro – Wilk P (Jarque Bera)		Mann - Whitney U test P
Outlier removal				Log transformation		
Before	After			Before	After	
Samples, age 26 – 50 years						
M – 3229	M – 3212	17 (2)	114.80±39.74 113.43 – 116.18 2 - 224 115.00 0.092/-0.386	<0.001 (<0.001)	<0.001 (<0.001)	<0.001
F – 1916	F – 1898	18 (3)	121.04±34.93 119.47 – 122.61 25 - 217 120.00 0.110/-0.323	0.001 (0.002)	<0.001 (<0.001)	
Samples, age >50 years						
M – 5908	M – 5864	44 (2)	92.65±37.48 91.69 – 93.61 5 - 201 88.00 0.442/-0.423	<0.001 (<0.001)	<0.001 (<0.001)	<0.001
F – 3952	F – 3914	38 (2)	106.30±41.07 105.01 – 107.59 2 - 225 101.00 0.388/-0.413	<0.001 (<0.001)	<0.001 (<0.001)	

Figure.10.2. Box Whisker plot of LDL Cholesterol (mg/dL) after outlier removal in males and females of various age groups.





(Figure 10.2. continuation)

CHAPTER 11

HISTOGRAM-BASED SUB-CLASSIFICATION OF HDL CHOLESTEROL AND PROPORTION OF HYPER/HYPOALPHALIPOPROTEINEMIA ACROSS GENDER AND AGE GROUPS: A CROSS-SECTIONAL STUDY

11.1. Abstract

Introduction: HDL cholesterol has important function in maintaining cardiovascular health, by extracting excess cholesterol from the circulation and carrying it to the liver for excretion. In this study, Lipid profile data was collected from Clinical Biochemistry Laboratory and samples were partitioned according to age and gender. In each of the partitioned age and gender group, the HDL Cholesterol distribution, percentage and number of samples in healthy, positively skewed and outlier regions of histogram were studied.

Objective: To investigate the distribution of HDL Cholesterol based on histogram, and analyze the proportion of low, normal, and elevated HDL Cholesterol levels across different age and gender groups.

Methods: An observational cross-sectional study on HDL cholesterol in normal and dyslipidemic conditions was conducted at Amala Institute of Medical Sciences using indirect sampling. HDL levels were assayed using autoanalyzer. Distribution was assessed using Shapiro-Wilk and Jarque-Bera tests, and graphically represented with histograms and Normal Q-Q plots. Gender differences were analyzed by student t-test or Mann-Whitney U test based on data distribution. All statistical analysis were done by SPSS software.

Results: There was significant gender difference in all the four age groups with mean HDL Cholesterol values higher in females than in males. Graphical analysis by histogram and Normal Q-Q plot showed presence of positive skewing and was confirmed by Shapiro-Wilk and Jarque Bera test. In each of the gender age groups maximum sample number was seen in the low HDL Cholesterol region and minimum number in the outlier region (High HDL Cholesterol region). In all partitioned groups (except 18 – 25 years), the Normal Q-Q plot of HDL Cholesterol distribution showed, and very large deviation. There was Increase in positive skewing in males and females above 25 years of age. There was horizontal compression in the samples due to positive skewing and presence of outliers. In samples of age group between 18 – 25 years, 26 – 50 years and samples of age >50 years there were gender differences with Mann Whitney U test $P = <0.001$ after removal of outliers.

Conclusions: A statistical and clinical method of classification of HDL Cholesterol concentration using histogram was proposed. The mean HDL Cholesterol values were higher in female sample. The higher mean cholesterol and higher 95% confidence interval of HDL-C in females is a prominent gender difference in HDL-C indicating the possibility of lower risk for atherosclerosis and myocardial infarction in females.

11.2. Introduction

High Density lipoprotein is an important measurand of the serum lipid profile panel, playing a vital role in maintaining cardiovascular health. HDL cholesterol is synthesized in liver and intestine, and its levels are influenced by various factors, including genetics, diet, exercise and smoking (Rosenson et al., 2012). HDL cholesterol is composed of several subfractions, including HDL2 and HDL3, which have distinct functions and properties. (Barter et al., 2004). HDL Cholesterol facilitates removal of excess cholesterol from peripheral tissues and thereby play important role in Reverse cholesterol transport. HDL Cholesterol has anti-inflammatory properties, which can help mitigate the development of cardiovascular diseases. (Barter et al., 2001; Bandiali et al., 2012). Endothelial function can be improved by HDL cholesterol, enhancing the ability of blood vessels to relax and improving blood flow.

HDL cholesterol Cutoff value used in this study is low HDL Cholesterol: <40 mg/dL in males and <43 mg/dL in females. Normal HDL Cholesterol: 40 – 60 mg/dL in males and 43 – 60 mg/dL in females. High HDL values: >60 mg/dL (NCEP guidelines, Procedure manual -Vitros 5.1FS). Analyzing the distribution of HDL cholesterol levels by histogram is important for visualizing peaks, troughs, skewness, kurtosis of distribution (Rosenson et al., 2012; Barter et al., 2004) Factors like age, sex, and ethnicity can influence the distribution of HDL cholesterol. (Grundy et al., 2019; NLA, 2014; Harris et al, 2017).

11.3. Objectives

- a) Collection of Fasting HDL – C sample data from the Clinical Biochemistry Laboratory
- b) Evaluation of the HDL Cholesterol distribution by histogram before and after outlier removal in samples, partitioned according to age and gender
- c) Calculating the cut-off value between moderate and severe hyperalphalipoproteinemia, and analyzing the number and percentage of samples in different histogram regions.

11.4. Materials and Methods

11.4.1. Research Design

This study on hyperalphalipoproteinemia was designed as an observational cross-sectional analysis, conducted at the Clinical Biochemistry Laboratory, Department of Biochemistry, Amala Institute of Medical Sciences. An indirect sampling method was employed instead of direct population sampling, as it allowed for the efficient collection of a large number of samples necessary for identifying rare lipid disorders, while reducing time and cost (Horowitz et al., 2012). Fasting lipid profile samples from both outpatient and inpatient departments were continuously gathered between September 2018 and March 2022 to support data randomization. The study protocol was approved by the Institutional Research and Ethics Committees (Ref. AIMSIEC/42/2018, dated 19/10/2018).

11.4.2. Inclusion Criteria and Exclusion criteria: (Refer General Materials and Methods)

11.4.3. Selection of Age Groups for the study

The study population was segmented into four age cohorts for analysis: adolescents (<18 years), young adults (18–25 years), middle-aged adults (26–50 years), and seniors (>50 years).

11.4.4. Sample size requirement

Using prevalence data from a multicentric study (Qi et al., 2015), calculated the required sample size of 304.12, which was rounded to 304 per group. The final dataset comprised 15,603 lipid profile samples, satisfying the required subgroup sample sizes (refer General Materials and Methods). HDL cholesterol was interpreted according to established guidelines: values <40 mg/dL in males and <43 mg/dL in females were considered low, 40–60 mg/dL in males and 43–60 mg/dL in females were considered normal, and levels >60 mg/dL were classified as high. These thresholds align with the recommendations of the American Heart Association (AHA) and ESC/EAS.

11.4.5. Methodology:

- a) Collect fasting HDL Cholesterol sample data from the Clinical Biochemistry Laboratory
- b) Evaluate the HDL Cholesterol distribution by histogram before and after outlier removal in samples partitioned according to age and gender.
- c) Analyse the number and percentage of samples in different histogram regions.

11.4.6. Assay for HDL Cholesterol: HDL was separated from LDL and VLDL through a precipitation method using dextran sulfate and magnesium chloride. Upon application of a magnetic field, the precipitated lipoproteins were removed, and the supernatant containing HDL cholesterol was collected (Refer General Materials and Methods).

11.4.7. Statistical Methods

The distribution of lipid concentration data was analyzed in SPSS version 19 using the Shapiro-Wilk and Jarque-Bera tests. Graphical visualization, including histograms and Normal Q-Q plots, was performed before and after outlier removal. Gender differences in lipid parameters were statistically tested using the Student t-test for parametric data or the Mann-Whitney U test for non-parametric data, in accordance with established guidelines (Altman, 1991; Gao & Smith, 2003).

11.5. Results

A. Distribution Characteristics of HDL Cholesterol in groups partitioned according to gender and age

The larger sample size observed with increasing age may be attributed to more frequent hospital visits by older individuals. The sample number was higher in males for the four age groups.

There were 97 male and 86 female samples in the age group <18 years. The female samples of age <18 years had higher mean HDL Cholesterol values (42.14 ± 10.48) than the male samples (39.73 ± 14.39) of same age group. The minimum to maximum values of HDL Cholesterol in male samples <18 years of age showed very large range (12 – 104 mg/dL) than in the female sample (20 – 74 mg/dL) (Table 11.1).

In the 18 – 25 years of age group, the female samples (n = 159) had higher mean HDL Cholesterol values (42.94 ± 12.21) than the male (n = 210) samples (39.02 ± 10.98). There were gender differences in HDL cholesterol values with the P value for Mann-Whitney U test is 0.002 (Table 11.1).

The mean values for HDL Cholesterol were higher in female (n = 1921) samples than the male (n = 3247) samples of age group between 26 – 50 years. The minimum to maximum range for HDL Cholesterol values in male samples (26 – 50 years of age) were from 10 to 176 mg/dL and were higher than that of the female sample 8 mg/dL – 93 mg/dL. There were gender differences in HDL Cholesterol values in the sample of age between 26 – 50 years (Mann Whitney U test $P = <0.001$) (Table 11.1).

There was significant gender difference between the HDL Cholesterol levels of clinically apparent healthy individuals of >50 years of age with Mann Whitney U test value ($P = <0.001$). The mean values for HDL Cholesterol were higher in female (n = 3958) samples (44.83 ± 13.74) than the male (n = 5925) samples (40.19 ± 12.38) of age group >50 years (Table 11.1).

Graphical Distribution Characteristics of HDL Cholesterol in groups after partitioning according to gender and age

In all partitioned groups (except 18 – 25 years), the Normal Q-Q plot of HDL Cholesterol distribution showed, very large deviation of the sample distribution away from the straight-line normal distribution in male samples than in total female sample (Fig. 11.1).

The graphical observation of Normal Q-Q plot and histogram showed an increased positive skewing in males and females above 25 years of age (Fig 11.1E to Fig 11.1H) and (Fig 11.2E to Fig 11.2H). The distribution also showed a horizontal compression in the samples due to positive skewing and presence of outliers.

In the two lower age groups, the positive skewing was lower as graphically observed in the Normal Q-Q plot and histogram (Fig11.1A to 11.1D; Fig 11.2A to 11.2D). Histograms of HDL Cholesterol distribution in male and female samples of age group between 18 – 25 years showed slight positive skewing (Fig 11.2C and Fig 11.2D). In samples of age group between 26 – 50 years and samples of age <50 years, male samples showed, high positive skewing than the female samples in HDL cholesterol values (Fig 11.2E, Fig 11.2F, Fig 11.2G and 11.2H).

Sample number expressed as percentage for each of the four groups also decreased with increase in concentration of HDL-C. As an example, in the age group <18 years, the percentage of samples in the abnormal lipid region (<40 mg/dL and <43.mg/dL in males and females) were 54.6% and 39.17%, respectively. As the HDL-C levels rose, the percentage of samples in various histogram regions dropped significantly from 54.63% to 39.17%, then to 4.12%, and finally to 2.06% among males under 18 years old.

Range between the last positively skewed HDL Cholesterol concentration and the last outlier defines the HDL Cholesterol concentration range for hyperalphalipoproteinemia.

In <18 years, range between the last positively skewed HDL Cholesterol concentration and the last outlier in males was 72mg/dL – 104 mg/dL and in females,

it was 65mg/dL – 82mg/dL. in 18 – 25 years, range between the last positively skewed HDL Cholesterol concentration and the last outlier in males was 69mg/dL – 82mg/dL and in females, it was 69mg/dL – 87mg/dL.

In 26 – 50 years, range between the last positively skewed HDL Cholesterol concentration and the last outlier in males was 102mg/dL – 176mg/dL and in females it was >93mg/dL. In >50 years, range between the last positively skewed HDL Cholesterol concentration and the last outlier in males was 110mg/dL – 189mg/dL and in female it was 105mg/dL – 113mg/dL (Table 11.2)

B. Distribution Characteristics of HDL Cholesterol in groups after outlier removal.

HDL Cholesterol distribution in male and female samples of age group between 18 – 25 years showed gender differences with Mann Whitney U test $P = 0.001$. In samples of age group between 26 – 50 years and samples of age >50 years, there were gender differences with Mann Whitney U test $P = <0.001$ (Table 11.3)

11.6. Discussions

The cut-off levels for low HDL-C were 40 mg/dL in males and 43 mg/dL in females. It is the lower levels of HDL-C that have increased risk for disease states. This is unlike other lipid profile analytes, where it is the increase in concentration that increases the risk for the disease. The lower level of HDL-C represents lower clearance of tissue cholesterol to the liver for excretion in bile and for conversion in to the bile salts. The result indicated that there were lower levels of HDL-C in male samples in the four age groups.

In the two lower age groups, there were less of positive skewing and fewer numbers of outliers. But in the higher age groups, the female samples had higher concentration of HDL-C (Table 11.1). There were greater positive skewing and a greater number of outliers in the two higher age groups (Fig 11.1E, 11.1F, 11.1G, 11.1H)

The higher level of HDL-C in males and females may be beneficial to the

patient but lower HDL-C is one of the several risk factors for atherosclerosis, myocardial infarction (Remaley et al., 2012). There was no noticeable relationship between the percentages of increased or decreased HDL-C with age. The higher mean cholesterol and higher 95% confidence interval of HDL-C in females is a prominent gender difference in HDL-C indicating the possibility of lower risk for atherosclerosis and myocardial infarction in females (Remaley et al., 2012).

In all age groups there were gender differences in distribution of HDL Cholesterol with higher mean HDL – Cholesterol values in females than in males. HDL-Cholesterol levels are higher in females than in males mainly due to following reasons a) Hormonal influences: Females have higher levels of estrogen, which plays an important role in HDL – Cholesterol production. Estrogen stimulates the liver to produce more HDL-Cholesterol and thereby regulating its synthesis. Studies have shown that estrogen therapy can increase HDL-Cholesterol levels in post-menopausal women (Walsh and Schiff, 1997). Progesterone also contributes to higher HDL levels in women (Kim and Kalkhoff, 2006). b) Lifestyle factors: women tend to eat more fiber-rich foods, fruits and vegetables, which can result in increase in HDL levels (Lichtenstein and Kennedy, 2003). Women more often engage in aerobic exercises such as jogging and walking, which can raise in HDL – Cholesterol levels (Haskell and Lee, 2007). c) Genetic variants that promote higher HDL-Cholesterol levels are more likely to be inherited by women than men (Kathiresan and Srivastava, 2012).

11.7. Conclusions

A statistical and clinical method of classification of HDL Cholesterol concentration was proposed. Graphical analysis by histogram and Normal Q-Q plot showed presence of positive skewing and was confirmed by Shapiro-Wilk and Jarque Bera test. In each of the gender age groups maximum sample number was seen in the low HDL Cholesterol region and minimum number in the outlier region (High HDL Cholesterol region). The mean HDL Cholesterol values were higher in female sample. Hormonal influence of HDL Cholesterol concentrations resulting in gender differences in individuals between 18 and 50 years of age is another area for future study.

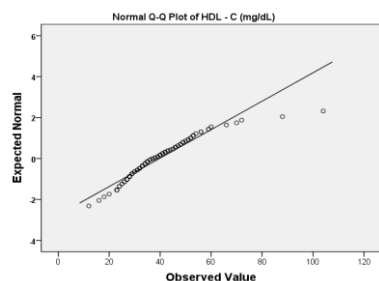
A. Distribution Characteristics of HDL Cholesterol in groups partitioned according to gender and age**Table 11.1.** Distribution of HDL cholesterol (mg/dL) in samples of age group <18 years, 18 – 25 years, 26 – 50 years and >50 years before outlier removal

Samples	Sample number (n)	Mean±SD 95%CI for mean Range: Min –Max	Median Skewness kurtosis	Shapiro – Wilk (Log transformation)		Gender difference, Mann-Whitney U Test (P)
				Before	After	
Samples (below 18 years of age)						
Male	97	39.73±14.39 36.83 – 42.63 12 – 104	37.00 1.428 4.089	<0.001 (<0.001)	0.549 (0.179)	0.039
Female	86	42.14±10.48 39.89 – 44.39 20 – 74	43.00 0.153 0.425	0.262 (0.697)	0.006 (0.040)	
Samples: Age group 18 – 25 years						
Male	210	39.02±10.98 37.52 – 40.51 10 – 82	39.00 0.179 1.058	0.031 (0.006)	(<0.001) (<0.001)	0.002
Female	159	42.94±12.21 41.04 – 44.86 12 – 87	43.00 0.298 0.437	0.083 (0.191)	0.001 (<0.001)	
Samples: Age group 26 – 50 years						
Male	3247	39.87±12.04 39.46 – 40.28 10 – 176	39.00 1.898 11.494	(<0.001) (<0.001)	(<0.001) (<0.001)	(<0.001)
Female	1921	44.00±12.22 43.46 – 44.55 8 – 93	43.00 0.596 0.912	(<0.001) (<0.001)	(<0.001) (<0.001)	

Samples	Sample number (n)	Mean±SD 95%CI for mean Range: Min –Max	Median Skewness kurtosis	Shapiro – Wilk (Log transformation)		Gender difference, Mann-Whitney U Test (P)
				Before	After	
Samples: Age group > 50 years						
Male	5925	40.19±12.38 38.87 – 40.51 8 – 189	39.00 1.414 7.332	(<0.001) (<0.001)	(<0.001) (<0.001)	(<0.001)
Female	3958	44.83±13.74 44.41 – 45.26 9 – 133	44.00 0.848 2.339	(<0.001) (<0.001)	(<0.001) (<0.001)	
Table 11.1 Continuation						

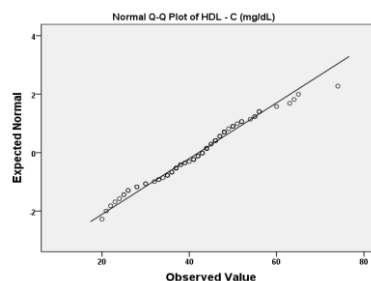
Figure. 11.1 Comparison of sample distribution of HDL Cholesterol (mg/dL) in gender age groups visualized by Normal Q-Q plot

1A.



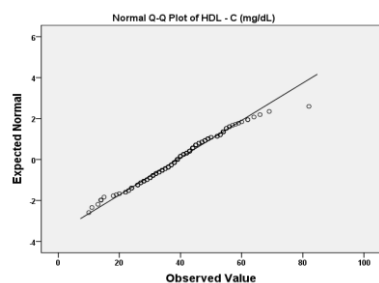
Male <18 years (n = 97)

1B.



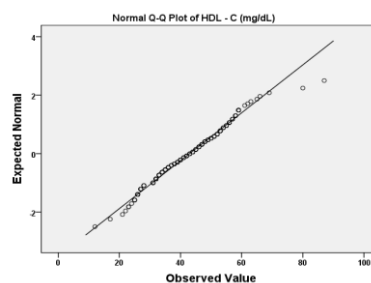
Female <18 years (n = 86)

1C.



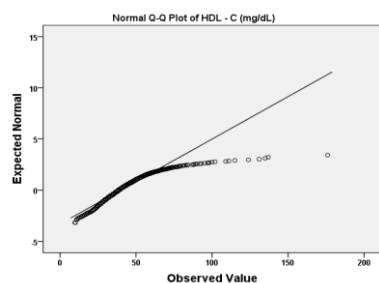
Male 18 – 25 years (n = 210)

1D.



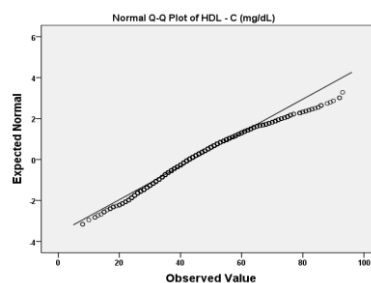
Female 18 – 25 years (n = 159)

1E.



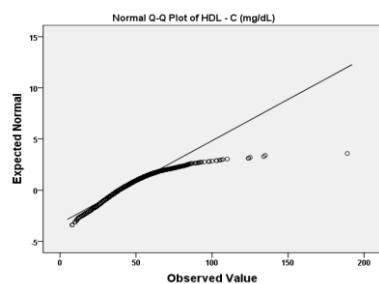
Male 26 – 50 years (n = 3247)

1F.



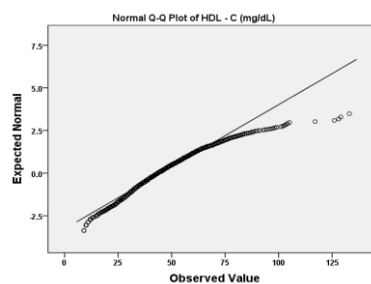
Female 26 – 50 years (n = 1921)

1G.



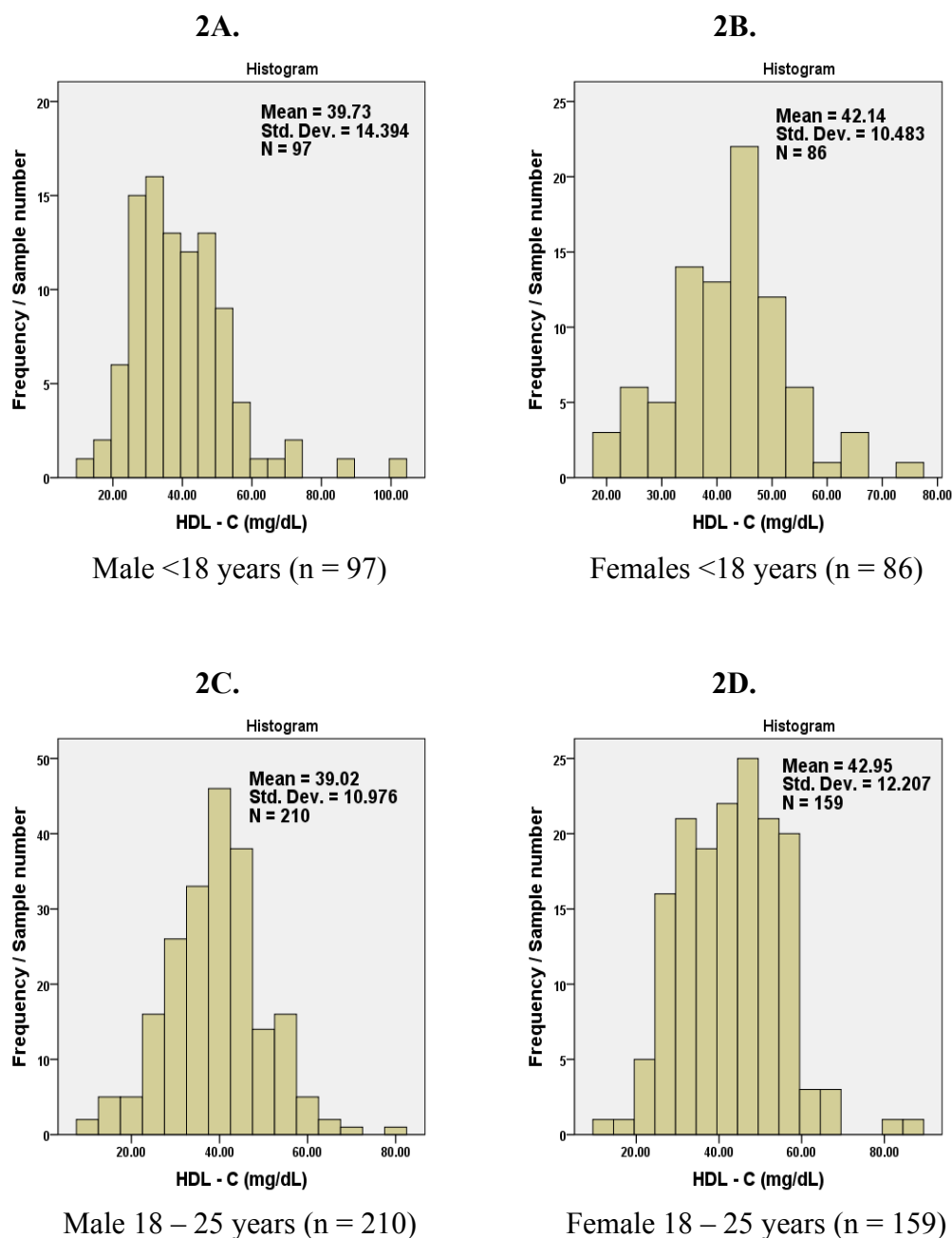
Male >50 years (n = 5925)

1H.

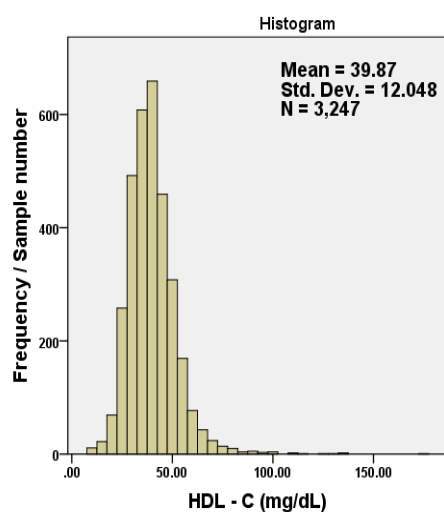


Female >50 years (n = 3958)

Figure.11. 2 Comparison of sample distribution of HDL Cholesterol (mg/dL) in gender-age groups before outlier removal visualized by histogram. (X axis- HDL- C (mg/dL), Y axis – Frequency of sample number)

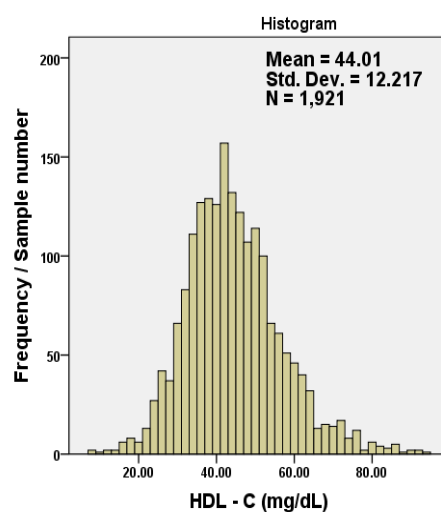


2E.



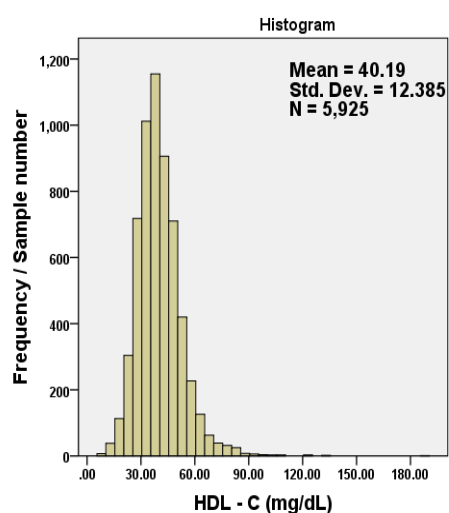
Male 26 – 50 years (n = 3247)

2F.



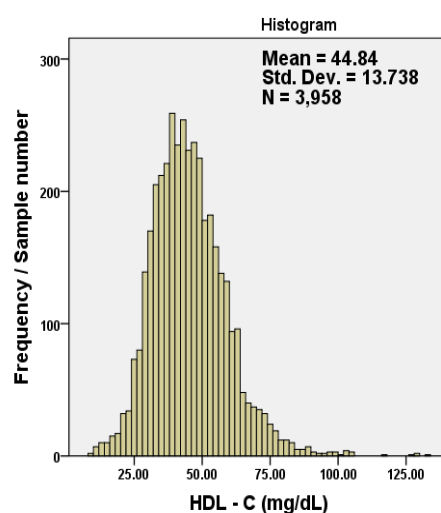
Female 26 – 50 years (n = 1921)

2G.



Male >50 years (n = 5925)

2H.



Female >50 years (n = 3958)

(Figure 11.2 continuation)

Table 11.2. The sample number and its percentage (%) in the histogram, distributed in the region with HDL Cholesterol <60 mg/dL **40 – 60 mg/dL (males) and 43 – 60 mg/dL (females) (Healthy region)**, HDL Cholesterol ≥ 60 mg/dL to last positively skewed sample (**Skewed region**), and last positively skewed to last outlier region (**Outlier region**) of HDL Cholesterol (mg/dL) in different age and gender groups. F and M are males and females

Age group Sample number and %	HDL Cholesterol (mg/dL)	Sample number and %	
	M:<40; F:<43		
	M: 40 – 60; F: 43 – 60		
	≥ 60 to last positively skewed sample in M & F		
	Outlier region in M & F	Male	Female
<18 years M, n = 97 (100%) F, n = 86(100%)	M:<40; F:<43	53 (54.63%)	41 (47.67%)
	M: 40 – 59; F: 43 – 59	38(39.17%)	40 (46.51%)
	M = 60 – 72 F = 60 – 65	4 (4.12%)	4 (4.65%)
	M – >72 – 104 F – >65 – 82	2 (2.06%)	1 (1.16%)
18 – 25 Years M, n = 210(100%) F, n = 159(100%)	M:<40; F:<43	113 (53.80%)	77 (48.42%)
	M: 40 – 59; F: 43 – 59	90 (42.85%)	74 (46.54%)
	M = 60 – 69 F = 60 – 69	6 (2.87%)	6 (3.77%)
	M – >69 – 82 F – >69 – 87	1(0.47%)	2 (1.25%)

Age group Sample number and %	HDL Cholesterol (mg/dL)	Sample number and %	
	M:<40; F:<43		
	M: 40 – 60; F: 43 – 60		
	≥60 to last positively skewed sample in M & F		
	Outlier region in M & F	Male	Female
26 – 50 years M, n = 3247(100%) F, n = 1921(100%)	M:<40; F:<43	1750 (53.89%)	945 (49.19%)
	M: 40 – 59; F: 43 – 59	1341 (41.29%)	784 (40.81%)
	M = 60 – 102 F = 60 – 93	148 (4.55%)	192 (9.99%)
	M – >102 – 176 F – >93	8 (0.24%)	0
>50 years M, n = 5925(100%) F, n = 3958(100%)	M:<40; F:<43	3137 (52.94%)	1855 (46.86%)
	M: 40 – 59; F: 43 – 59	2436 (41.11%)	1601 (40.44%)
	M = 60 – 110 F = 60 – 105	346 (5.83%)	497 (12.55%)
	M – >110 – 189 F – >105 – 133	6 (0.10%)	5 (0.12%)

Table 11.2 Continuation

B. Distribution Characteristics of HDL Cholesterol in groups after outlier removal.**Table 11.3.** Distribution characteristics and gender differences in HDL Cholesterol (mg/dL) after removal of outliers by histogram. F and M are males and females

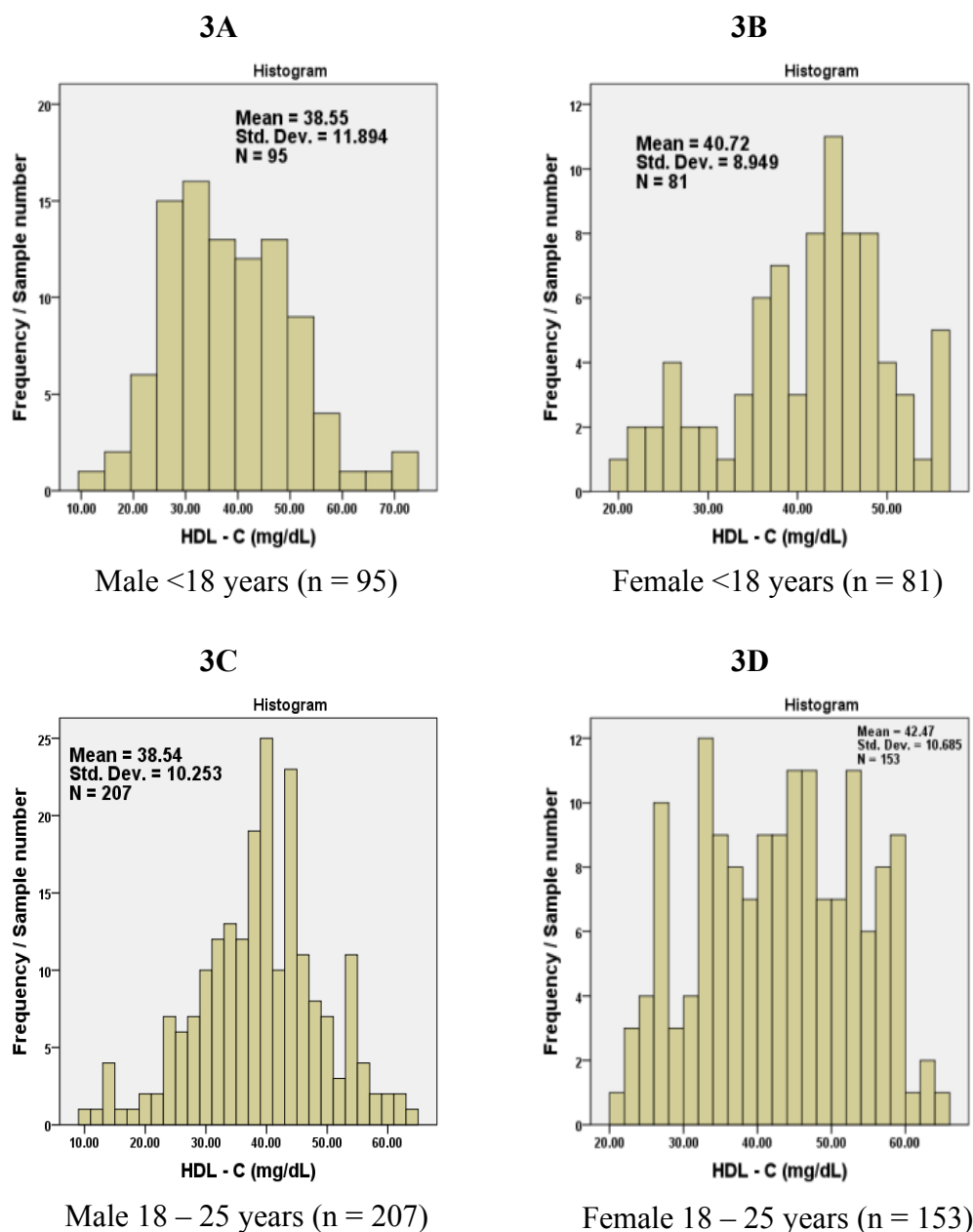
Sample number (n)		Outliers removed (cycles)	HDL Cholesterol Mean±SD 95%CI for mean Min –Max Median Skewness/ kurtosis	HDL Cholesterol Shapiro – Wilk, P (Jarque Bera)		Gender Difference, Student t test/Mann - Whitney U test (P)
Outlier removal				Log transformation		
Before	After			Before	After	
Samples, age <18 years						
M – 97	M – 95	2 (1)	38.54±11.89 36.12 – 40.97 12 – 72 37.00 0.452/0.047	0.201 (.209)	0.182 (0.068)	0.076
F – 86	F – 81	5 (2)	40.71±8.94 38.73 – 42.69 20 – 56 42.00 -0.487/-0.335	0.019 (0.165)	<0.001 (0.001)	
Samples, age 18 – 25 years						
M – 210	M – 207	3 (2)	38.53±10.25 37.13 – 39.94 10 – 64 39.00 -0.207/0.221	0.121 (0.414)	<0.001 (<0.001)	0.001
F – 159	F – 153	6 (2)	42.47±10.68 40.76 – 44.17 21 – 65 43.00 -0.052/-0.961	0.007 (0.048)	<0.001 (0.013)	

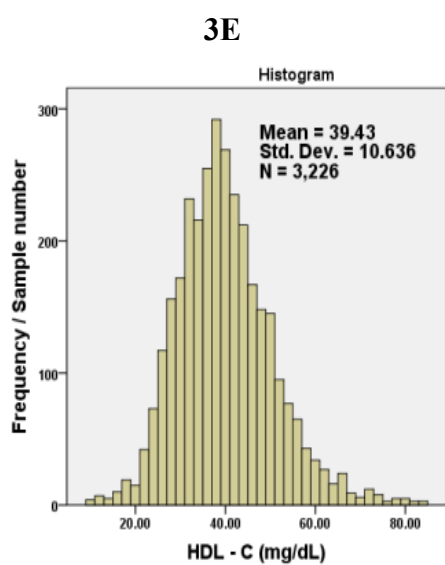
Table 11.3. (continued)

Distribution characteristics and gender differences in HDL Cholesterol (mg/dL) after removal of outliers by histogram. F and M are males and females

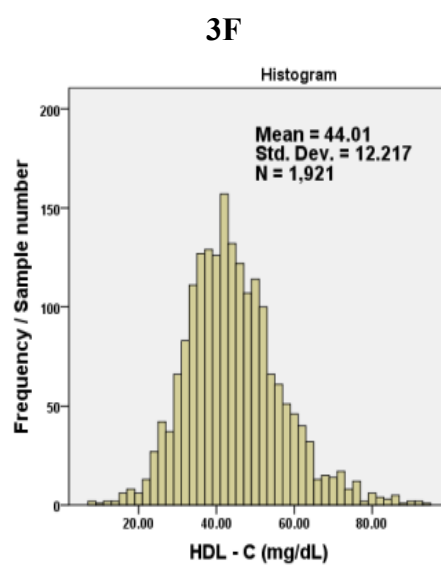
Sample number (n)		Outliers removed (cycles)	HDL Cholesterol Mean±SD 95%CI for mean Min –Max Median Skewness/ kurtosis	HDL Cholesterol Shapiro – Wilk, P (Jarque Bera)		Gender Difference, Student t test/Mann - Whitney U test (P)
Outlier removal				Log transformation		
Before	After			Before	After	
Samples, age 26 -50 years						
M –3247	M – 3226	21 (2)	39.43±10.63 39.06 – 39.79 10 – 84 38.00 0.623 /1.027	<0.001 (<0.001)	<0.001 (<0.001)	<0.001
F – 1921	F – 1921	0	44.00±12.21 43.45 – 44.55 8 – 93 43.00 0.596/0.912	<0.001 (<0.001)	<0.001 (<0.001)	
Samples, age > 50 years						
M –5925	M – 5900	25 (2)	39.90±11.52 39.61 – 40.20 8 - 87 39.00 0.677/1.097	<0.001 (<0.001)	<0.001 (<0.001)	<0.001
F – 3958	F – 3945	13 (1)	44.61±13.18 44.20 – 45.02 9 – 99 43.00 0.531/0.649	<0.001 (<0.001)	<0.001 (<0.001)	

Figure. 11.3. Histogram of the sample distribution of HDL Cholesterol (mg/dL) in males and females of various age groups after outlier removal. (X axis- HDL – C (mg/dL), Y axis – Frequency of sample number)

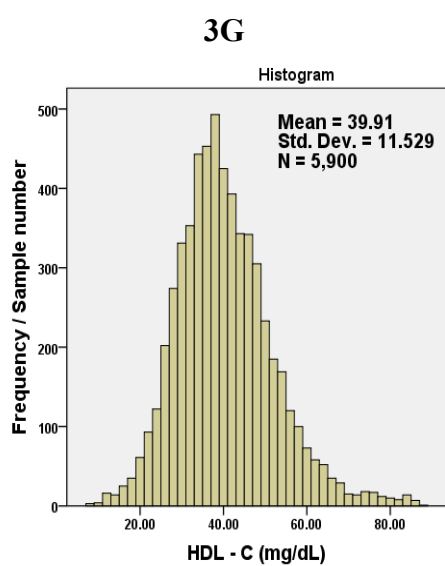




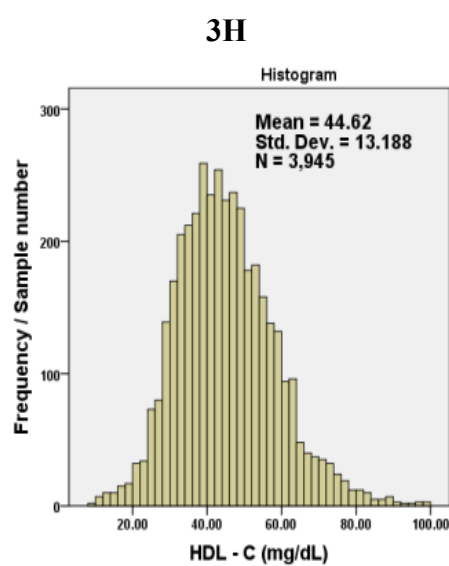
Male 26 – 50 years (n = 3226)



Female 26 – 50 years (n = 1921)



Male >50 years (n = 5900)



Female >50 years (n = 3945)

(Figure 11.3 continuation)

CHAPTER 12

**BOX-WHISKER PLOT USED FOR PARTITIONING HDL
CHOLESTEROL ACCORDING TO QUARTILES AND FENCES
TO IDENTIFY GENDER AND AGE-RELATED DIFFERENCES
AND SIMILARITIES – A CROSS-SECTIONAL STUDY.**

12.1. Abstract

Introduction: HDL cholesterol has importance in maintaining cholesterol homeostasis and preventing atherosclerotic vascular disease. In this study, Lipid profile data was collected from Clinical Biochemistry Laboratory and samples were partitioned according to age and gender. In each of the partitioned age and gender group, the HDL Cholesterol distribution, percentage and number of samples in healthy, positively skewed and outlier regions of Box-Whisker plot were studied.

Objectives: To evaluate the distribution, gender disparities, and the proportion of hypo/hyperalphalipoproteinaemia in relation to median, quartiles and fences using Box-Whisker plots.

Methods: An observational cross-sectional study of HDL cholesterol in normal and dyslipidemic states was carried out at Amala Institute of Medical Sciences via an indirect sampling approach. Chemistry analyses were done by autoanalyzer. The Shapiro-Wilk and Jarque-Bera tests were applied to assess distribution, which was further visualized by Box-Whisker plots. Gender differences in lipid concentrations were tested by Mann-Whitney U test or Student's t-test. Statistical analyses were performed by Statistical Package for Social Sciences (SPSS).

Results: Graphical analysis by Box-Whisker plot showed presence of positive skewing with large number of outliers with high HDL Cholesterol concentration particularly in older age groups. In each of the gender age groups maximum sample number was seen in the low HDL Cholesterol region and the mean HDL Cholesterol values were higher in female sample. Very few outliers were observed in two lower age groups. Positive skewing could be detected in the male sample <18 years and in the female samples 26 – 50 years. Samples in Lower near outlier region may be due to the presence of hypoalphalipoproteinemia. The gender differences were weak in the group <18 years after removal of outliers. Strong gender difference was seen in >18 years with Mann –Whitney U test - $P = <0.001$, after removal of outliers.

Conclusions: Higher number of outliers in the older age groups can be attributed to a greater number of individuals with elevated HDL Cholesterol levels (which are not

known to increase the risk for atherosclerosis). Elevated triglyceride can lead to the exchange of cholesterol ester for triglyceride between VLDL and HDL, potentially resulting in decreased HDL Cholesterol levels, which may be prevalent in populations with high rates of hypertriglyceridemia. As mean HDL-C is higher in females; there is decreased risk of myocardial infarction in females below 50 years of age. For detecting near and far outliers, Box-Whisker plots are more useful, whereas histograms are better suited for assessing the degree of positive skewness in the data.

12.2. Introduction

High Density lipoprotein is composed of apolipoproteins (primarily apoA1), phospholipids and cholesterol. HDL Cholesterol plays an important role in reverse cholesterol transport (RCT), carrying excess cholesterol from peripheral tissues to the liver for excretion (Zhou et al., 2015)). A higher HDL level is associated with a lower risk of cardiovascular disease.

HDL cholesterol is synthesized in liver and intestine (Remaley et al., 2012). HDL particle is distributed throughout the body, with higher concentrations found in the liver, spleen and lymph nodes (Zhou et al., 2015) and its levels are influenced by various factors, including genetics, diet, exercise and smoking (Rosenson et al., 2012).

Analyzing the distribution of HDL cholesterol levels by Box-Whisker plots is important for understanding pattern and trends in HDL Cholesterol levels, and thereby identifying individuals/population at risk of cardiovascular disease. Factors like age, sex, BMI, and physical activity can influence the distribution of HDL cholesterol.

12.3. Objectives

- a) Collection of Fasting HDL Cholesterol sample data from the Clinical Biochemistry Laboratory
- b) Evaluation of the HDL Cholesterol distribution by Box-Whisker plot before and after outlier removal in samples, partitioned according to age and gender
- c) Calculating the cut-off value between moderate and severe hyperalphalipoproteinemia, and analyzing the number and percentage of

samples in different Box-Whisker plot regions.

12.4. Materials and Methods

12.4.1. Research Design

This observational cross-sectional study on hyperalphalipoproteinemia was conducted at the Clinical Biochemistry Laboratory, Department of Biochemistry, Amala Institute of Medical Sciences, using an indirect sampling method. Indirect sampling was chosen over direct population sampling due to the need for a large sample size to detect rare lipid disorders efficiently, with reduced time and cost (Horowitz et al., 2012). Fasting lipid profile samples from outpatient and inpatient departments were continuously collected from September 2018 to March 2022 to ensure randomized data. The study received approval from the Institutional Research and Ethics Committees (Ref. AIMSIEC/42/2018, dated 19/10/2018) for data collection and analysis.

12.4.2. Inclusion Criteria and Exclusion criteria: (Refer General Materials and Methods)

12.4.3. Selection of Age Groups for the study

Samples were divided based on age into adolescents (under 18 years), young adults (18 to 25 years), middle-aged adults (26 to 50 years), and seniors (over 50 years).

12.4.4. Sample size requirement

Sample size estimation was performed by a statistician using prevalence figures from a multicentric dyslipidaemia study (Qi et al., 2015). Parameters included 20% margin of error, $\alpha = 0.05$, $p = 24\%$, $q = 1-p$, $Z(1-\alpha) = 1.96$, and 80% power, resulting in a sample size of 304.12, rounded to 304 per group. The final sample of 15,603 fulfilled the sample size requirements for each subgroup (see General Materials and Methods). HDL cholesterol values were classified as low (<40 mg/dL in males, <43 mg/dL in females), normal (40–60 mg/dL in males, 43–60 mg/dL in females), and high (>60 mg/dL) according to the National Heart Association (AHA)

and ESC/EAS guidelines (Procedure manual Vitros). The sample set used in this study was same as in chapter 11

12.4.5. Methodology

- a) Collect fasting HDL Cholesterol sample data from the Clinical Biochemistry Laboratory
- b) Assess distribution using Box-Whisker plots before and after outlier removal in samples, partitioned according to age and gender.
- c) Analyze the number and percentage of samples in different Box-Whisker plot regions.

12.4.6. Assay for HDL Cholesterol: Refer General Materials and Methods

12.4.7. Statistical Methods

The Shapiro-Wilk and Jarque-Bera tests were performed in SPSS version 19 to evaluate distribution, with Box-Whisker plots illustrating data before and after outlier elimination. Gender differences in lipid concentrations were studied by Student's t-test and Mann-Whitney U test (Altman, 1991; Gao and Smith, 2003).

12.5. Results

A. Distribution and gender differences before removal of outliers

Outliers were very few in the Box-Whisker plot of two lower age groups. But in higher age group there was large number of positive outliers. Positive skewing could be detected in the male sample of age group <18 years and in the female age group 26 – 50 years (Fig.12.1A and 12.1F). There were large number of near outliers and a smaller number of far outliers in the two age groups greater than 25 years in males and females.

Of the eight partitioned regions of Box-Whisker plot in the age group below 18 years, the lower near outliers and lower far outliers did not have any samples. The middle four region of Box-Whisker plot had around 19.7% to 26.8% of the total

samples in the group <18 years. There were some negative outliers in the age groups above 18 years. In the two higher age groups there were far positive outliers (Table 12.1).

The Shapiro-Wilk (Jarque Bera test) showed normal distribution before log transformation in the two lower age groups. In the two higher age groups, the distribution was non-Gaussian ($P < 0.001$) due to positive skewing and presence of outliers (Table 12.2).

B. Distribution and gender differences after removal of outliers

The gender differences were weak in the group <18 years. But there were very strong gender differences in all the three higher age groups. The female sample had higher mean HDL-C than the male sample. There was no clear age-related variation in the median (Table 12.2).

The non-Gaussian distribution could not be well appreciated by Box-Whisker plot. A close examination showed positive skewing in males <18 years and in males above 50 years.

The male samples of 18 – 25 years showed a negative skewing (Fig.12.2).

12.6. Discussions

The increased number of outliers in the higher age groups were due to increased number of HDL-C with higher HDL values which are not known to increase the risk for atherosclerosis. Increased triglyceride can result in exchange of cholesterol ester with triglyceride between VLDL and HDL. As large percentage of individuals in a population have hypertriglyceridemia. There can be cases of decreased HDL-C in the population. As HDL-C is higher in females; there is decreased risk of myocardial infarction in females below 50 years of age (The INTERHEART study, 2004). As the outliers were few in number, after removal of outliers there is small decrease in mean HDL-C value. But even after removal of outliers the mean HDL-C value is higher in females of all age groups. This leads to strong gender difference in HDL-C especially in the three old age groups before and after removal of outliers.

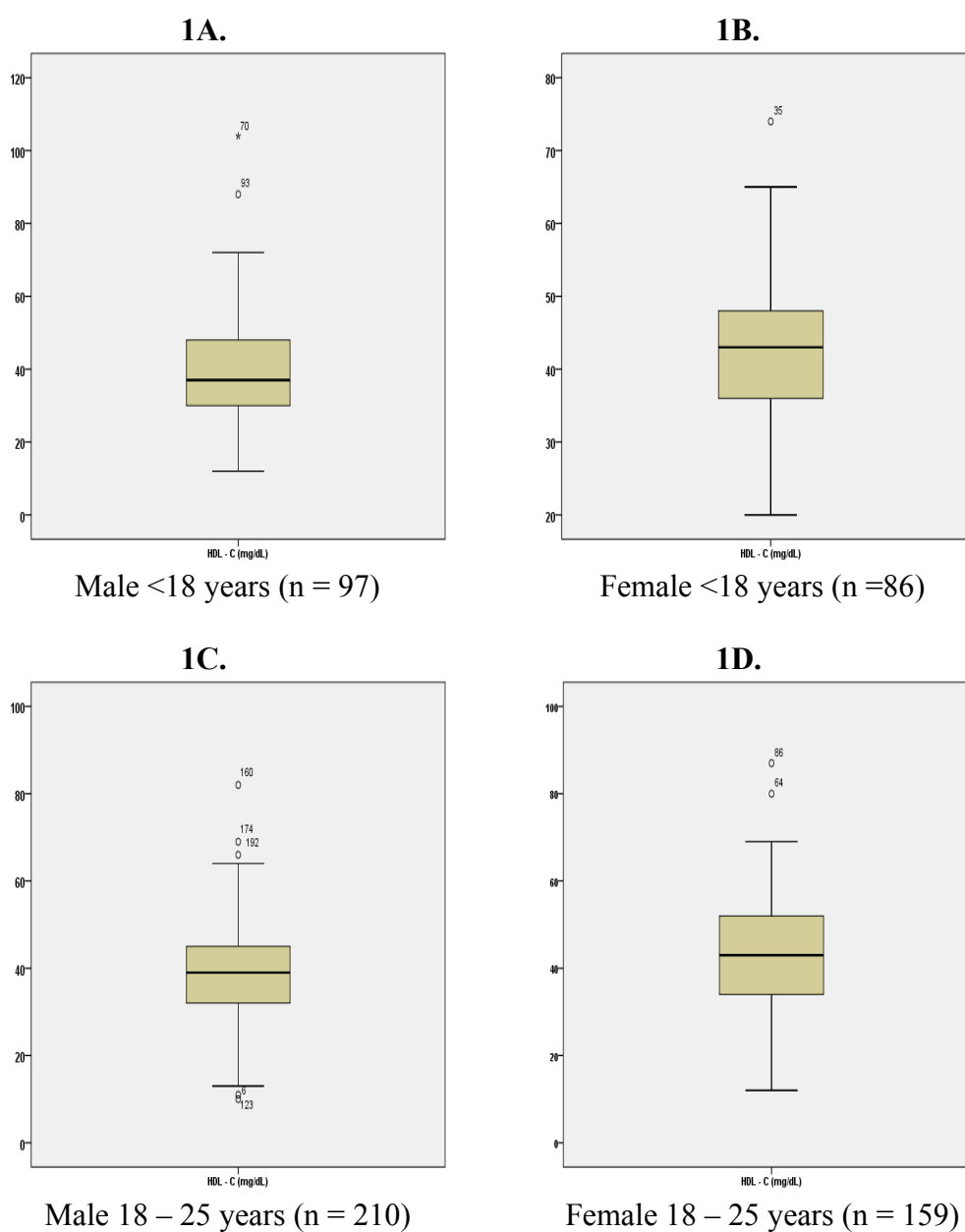
The positive skewing is better appreciated by histogram than Box-Whisker plot. The Box-whisker plot shows some weak positive skewing in the Box region and in the Whisker region in some of the age groups. Therefore, Box-Whisker plot is more useful in defining the near and far outliers, while histogram can be used for better approach of positive skewing.

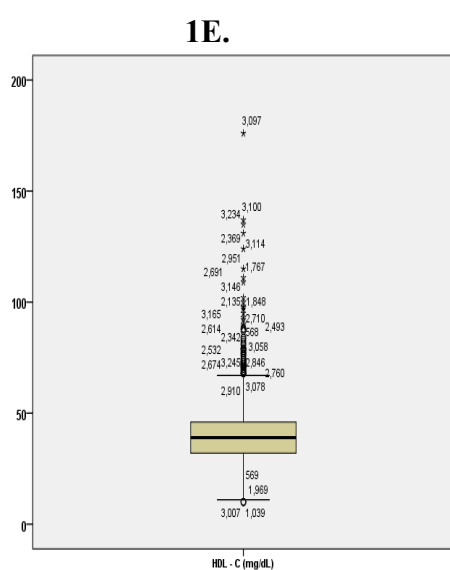
12.7. Conclusions

A statistical and clinical method of classification of HDL Cholesterol concentration was proposed. Graphical analysis by Box-Whisker plot showed presence of positive skewing with large number of outliers with high HDL Cholesterol concentration particularly in older age groups. In each of the gender age groups maximum sample number was seen in the low HDL Cholesterol region and the mean HDL Cholesterol values were higher in female sample.

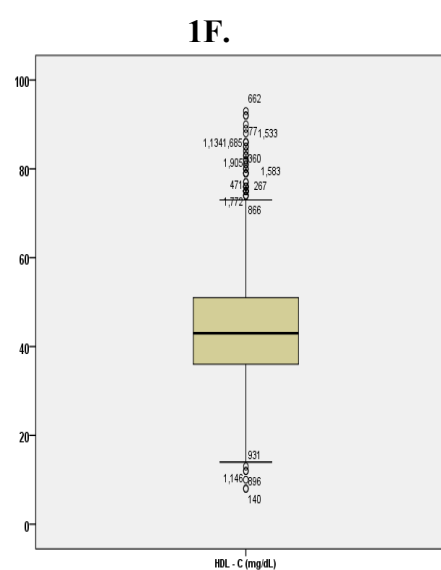
A. Distribution and gender differences before removal of outliers

Figure.12. 1. Box Whisker plot of the sample distribution of serum HDL Cholesterol (mg/dL) in males and females of various age groups before outlier removal.

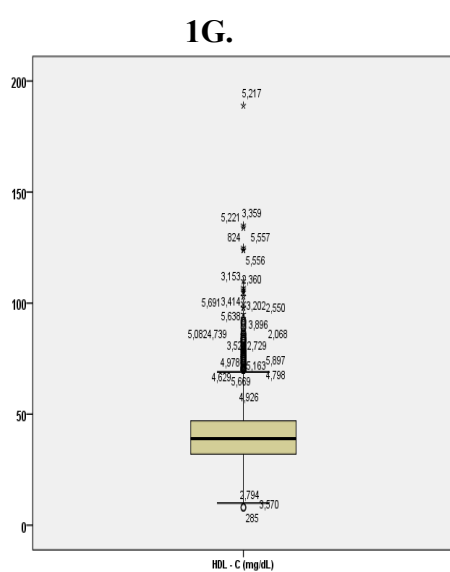




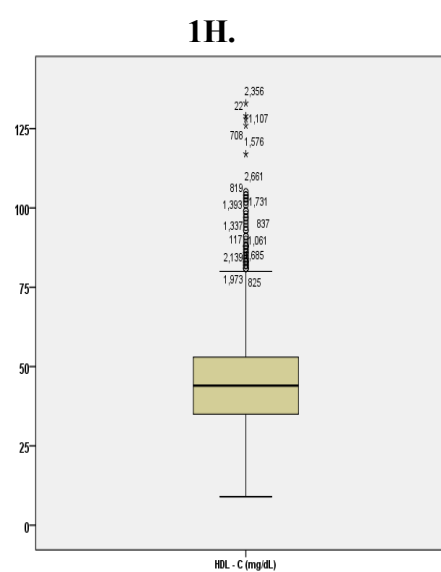
Male 26 – 50 years (n = 3247)



Female 26 – 50 years (n = 1921)



Male >50 years (n = 5925)



Female >50 years (n = 3958)

(Figure.12.1 Continuation)

Table 12.1. The number of samples calculated from Box Whisker plot in the skewed and outlier region of HDL Cholesterol (mg/dL) distribution data in samples of different age groups.

Partitioned regions of Box Whisker plot	HDL Cholesterol concentration (mg/dL)		Sample number in the partitioned regions	
	M	F	M	F
<18 years: Male (n = 97); Female (n = 86): Median: Male – 37; Female – 43				
Lower Near outliers ($F_1 - f_1$)	(-24 – 3)	(0 – 18)	0	0
Lower Far outliers ($<F_1$)	$< (-24)$	$< (0)$	0	0
Lower Whisker ($a_1 - Q_1$)	12 – 30	20 – 36	26 (26.8%)	23 (26.7%)
$Q_1 - Q_2$	30 – 37	36 – 43	23 (23.7%)	21 (24.4%)
$Q_2 - Q_3$	37 – 48	43 – 48	26 (26.8%)	24 (27.9%)
Upper Whisker ($Q_3 - a_3$)	48 – 72	48 – 65	20 (20.6%)	17 (19.7%)
Upper Near outliers ($f_3 - F_3$)	75 – 102	66 – 84	2 (2.0%)	1 (1.1%)
Upper Far outliers ($>F_3$)	>102	>84	0	0
18 – 25 Years: Male (n = 210); Female (n = 159): Median: Male – 39; Female – 43				
Lower Near outliers ($F_1 - f_1$)	(-7 – 13)	(-20 – 7)	3	0
Lower Far outliers ($<F_1$)	(-7)	(-20)	0	0
Lower Whisker ($a_1 - Q_1$)	14 – 32	12 – 34	51 (24.2%)	44 (27.6%)
$Q_1 - Q_2$	32 – 39	34 – 43	59 (28.0%)	37 (23.2%)
$Q_2 - Q_3$	39 – 45	43 – 52	51 (24.2%)	40 (25.1%)
Upper Whisker ($Q_3 - a_3$)	45 – 64	52 – 69	43 (20.4%)	36 (22.6%)
Upper Near outliers ($f_3 - F_3$)	65 – 84	79 – 106	3 (1.4%)	2 (1.2%)
Upper Far outliers ($>F_3$)	>84	>106	0	0

Table 12.1. (continuation)

Partitioned regions of Box Whisker plot	HDL Cholesterol concentration (mg/dL)		Sample number in the partitioned regions	
	M	F	M	F
26 – 50 years: Male (n = 3247); Female (n = 1921): Median: Male – 39; Female – 43				
Lower Near outliers ($F_1 - f_1$)	(-10 – 11)	(-9 – 14)	8	7
Lower Far outliers ($<F_1$)	(-10)	(-9)	0	0
Lower Whisker ($a_1 - Q_1$)	12 – 32	15 – 36	844 (25.9%)	526 (27.3%)
$Q_1 - Q_2$	32 – 39	36 – 43	898 (27.6%)	471 (24.5%)
$Q_2 - Q_3$	39 – 46	43 – 51	748 (23.0%)	465 (24.2%)
Upper Whisker ($Q_3 - a_3$)	46 – 67	51 – 73	674 (20.7%)	411 (21.3%)
Upper Near outliers ($f_3 - F_3$)	67 – 88	74 – 96	57 (1.7%)	41 (2.1%)
Upper Far outliers ($>F_3$)	>88	>96	18	0
> 50 years: Male (n = 5925); Female (n = 3958): Median: Male – 39; Female – 44				
Lower Near outliers ($F_1 - f_1$)	(-13 – 10)	(-19 – 8)	7	0
Lower Far outliers ($<F_1$)	(-13)	(-19)	0	0
Lower Whisker ($a_1 - Q_1$)	11 – 32	9 – 35	1526 (25.7%)	1006 (25.4%)
$Q_1 - Q_2$	32 – 39	35 – 44	1604 (27.0%)	1097 (27.7%)
$Q_2 - Q_3$	39 – 47	44 – 53	1441 (24.3%)	925 (23.3%)
Upper Whisker ($Q_3 - a_3$)	47 – 61	53 – 79	1065 (17.9%)	865 (21.8%)
Upper Near outliers ($f_3 - F_3$)	62 – 77	80 – 107	211 (3.5%)	60 (1.5%)
Far outliers on right ($>F_3$)	>77	>107	71 (1.1%)	5 (0.1%)

B. Distribution and gender differences after removal of outliers**Table 12.2.** Distribution of HDL Cholesterol (mg/dL) after removing outliers using Box Whisker plot in samples of age<18, 18 – 25 years, 26 - 50 years and >50 years (M – male, F – female)

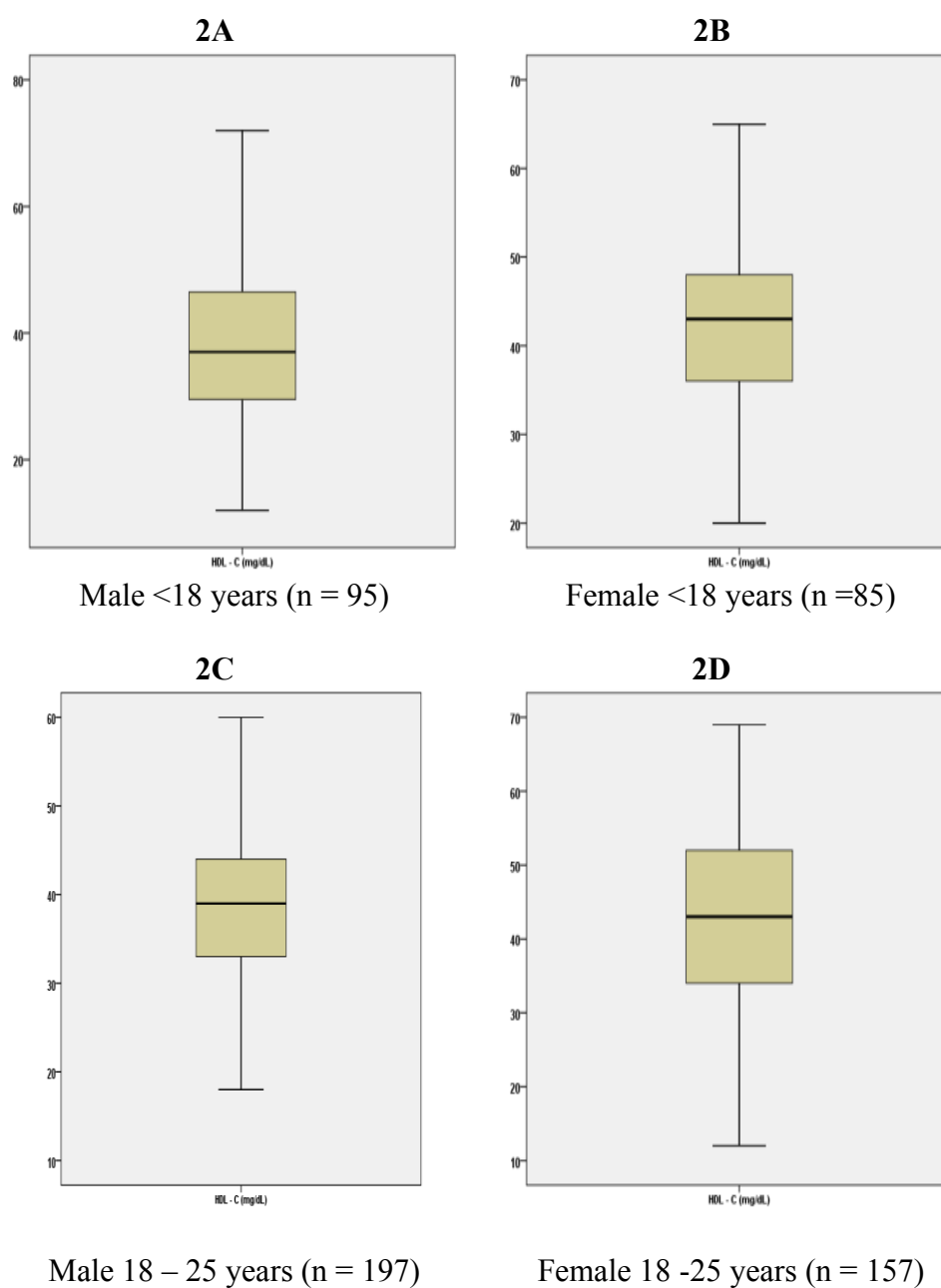
Sample number (n)		Total number of outliers removed (cycles)	Mean±SD 95%CI for mean Min –Max Median Skewness/ kurtosis	Shapiro – Wilk P (Jarque Bera)		Gender difference, Student’s t test/ Mann -Whitney U test
Outlier removal				Log transformation		
Before	After			Before	After	
Samples, age <18 years						
M – 97	M –95	2 (1)	38.54±11.89 36.12 – 40.97 12 – 72 37.00 0.452/0.047	0.201 (0.209)	0.182 (0.068)	0.052
F – 86	F – 85	1 (1)	41.76±9.94 39.61 – 43.91 20 – 65 43.00 -0.103/-0.068	0.288 (0.900)	0.001 (0.012)	
Samples, age 18 – 25 years						
M – 210	M – 197	13 (3)	39.07±8.82 37.83 – 40.31 18 – 60 39.00 0.041/-0.359	0.208 (0.537)	0.001 (0.005)	<0.001
F – 159	F – 157	2 (1)	42.43±11.37 40.63 – 44.22 12 – 69 43.00 -0.090/-0.640	0.180 (0.218)	<0.001 (<0.001)	

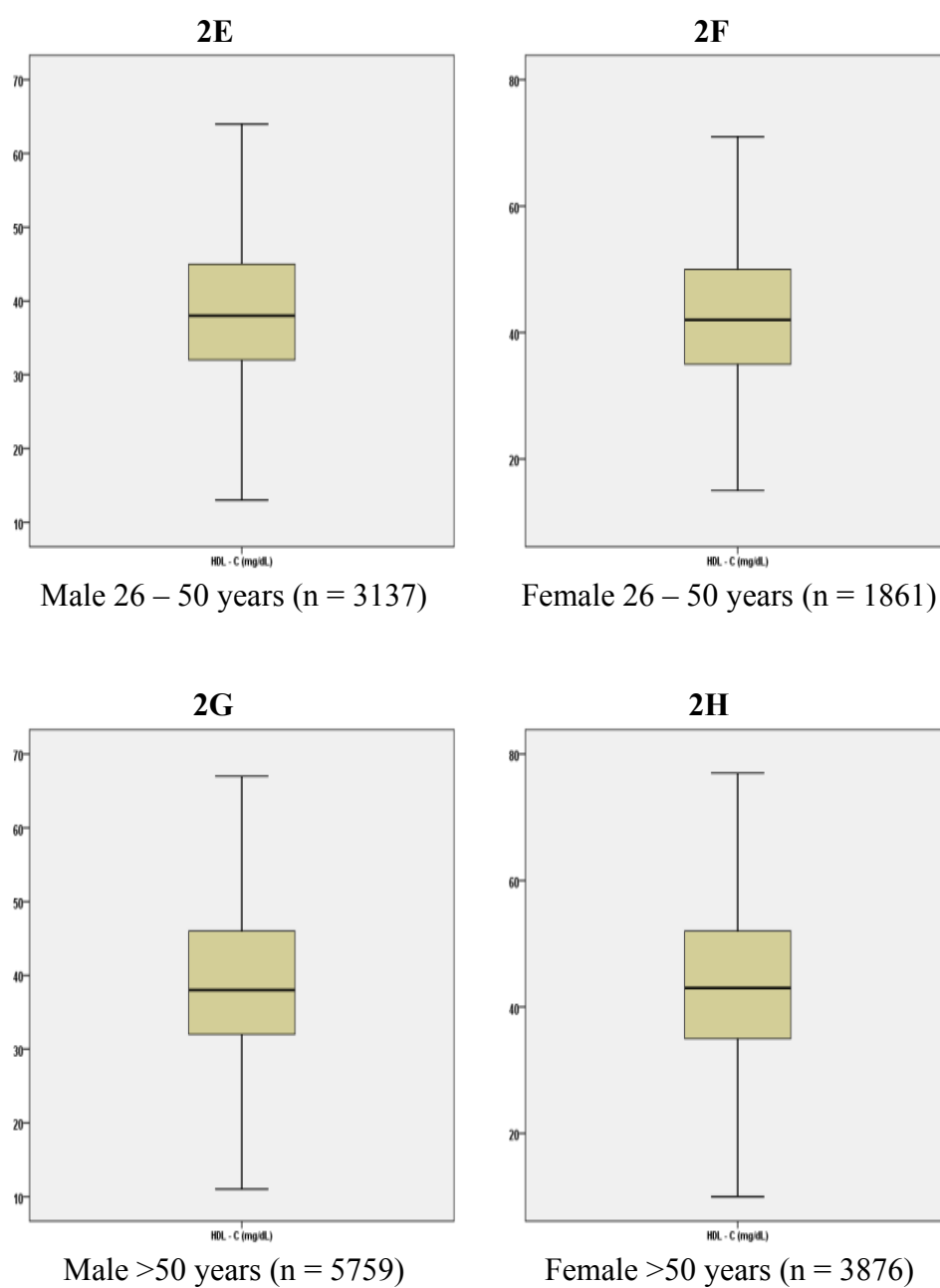
Table 12.2. (Continued)

Distribution of HDL Cholesterol (mg/dL) after removing outliers using Box Whisker plot in samples of age<18, 18 – 25 years, 26 - 50 years and >50 years (M – male, F – female)

Sample number (n)		Total number of outliers removed (cycles)	Mean±SD 95%CI for mean Min –Max Median Skewness/ kurtosis	Shapiro – Wilk (Jarque Bera)		Gender difference, Student's t test/ Mann -Whitney U test
Outlier removal				Log transformation		
Before	After			Before	After	
Samples, age 26 – 50 years						
M – 3247	M – 3137	110 (2)	38.74±9.34 38.41 – 39.07 13 – 64 38.00 0.205/-0.255	<0.001 (<0.001)	<0.001 (<0.001)	<0.001
F – 1921	F – 1861	60 (2)	43.13±42.65 42.65 – 43.62 15 - 71 42.00 0.203/-0.228	<0.001 (<0.001)	<0.001 (<0.001)	
Samples, age > 50 years						
M – 5925	M – 5759	166 (2)	39.11±10.21 38.85 – 39.38 11 – 67 38.00 0.214/-0.169	<0.001 (<0.001)	<0.001 (<0.001)	<0.001
F – 3958	F – 3879	79 (2)	43.96±12.18 43.58 – 44.35 10 – 77 43.00 0.218/-0.181	<0.001 (<0.001)	<0.001 (<0.001)	

Figure.12.2. Box Whisker plot of HDL Cholesterol (mg/dL) after outlier removal in males and females of various age groups





(Figure.12.2 continuation)

CHAPTER 13

CORRELATION ANALYSIS OF LIPID PROFILE ANALYTES WITH THYROID FUNCTION, LIVER AND RENAL FUNCTION, GLUCOSE LEVELS AND BODY MASS INDEX.

13.1. Abstract

Introduction: Lipid profile data was collected from Clinical Biochemistry Laboratory and samples were partitioned according to age and gender. Correlation analysis of lipid profile measurands with glucose, TSH, liver function and kidney function test parameters were done.

Objective: To investigate the correlation of Lipid profile measurands with other biochemical and physical parameters like Glucose, Liver and Renal Functions, Thyroid Function and BMI in different age and gender groups.

Methods: This is an observational cross-sectional study of normal and dyslipidemia conditions at Amala Institute of Medical Sciences, utilizing an indirect sampling approach. Enzymatic dry chemistry assays were performed on the Ortho Clinical Diagnostics Vitros 5.1 FS autoanalyzer. Correlations were analyzed by Spearman's correlation for non-parametric data and Pearson's correlation for parametric data. Distribution characteristics were computed using SPSS.

Results: There is positive correlation of Triglycerides with fasting glucose in adult males and females. Triglycerides correlate positively with TSH in males of all age groups. Total Cholesterol correlates positively with TSH in males and females above 25 years. ALT positively correlated with Total Cholesterol, triglycerides and LDL-C in adult males. The positive correlation between AST, ALKP and triglycerides in females but not in males may be attributed to sex-specific differences (estrogens) in lipid metabolism and liver enzyme activity. Triglycerides correlate positively with Total Cholesterol and negatively correlate with HDL-C in males and females of all age groups. There were Positive correlation between Triglyceride and BMI.

Conclusions: The correlation studies demonstrate significant relationship between biochemical parameters such as TSH, ALT, ALP and glucose with lipid profile analytes. These correlations highlight the interconnectedness of metabolic parameters and their association with cardiovascular risk. Comprehensive metabolic assessment and targeted interventions, including lifestyle modifications and pharmacological treatments are crucial for managing metabolic health and cardiovascular risk. Future

research is needed to understand this relationship and develop effective management strategies, potentially enabling personalized medicine approaches that account for an individual's unique metabolic profile and treatment strategies.

13.2. Introduction

Correlation studies are done to understand the relationship between two different sets of data. Despite the importance of lipid profile in cardiovascular disease risk assessment, the association of lipid profile parameters such as serum Triglycerides (TG), Total Cholesterol (TC), HDL Cholesterol (HDL-C) and LDL Cholesterol (LDL-C), and related parameters such as age, body mass index, sex, waist circumference, fasting blood glucose, blood pressure, smoking status and physical activity are not fully understood. A comprehensive analysis of these correlations is essential for developing effective strategies for CVD prevention and management.

Type 2 diabetes mellitus is a metabolic disorder characterized by hyperglycemia, and risk factors include increasing age, Asian (especially Indian) ethnicity (Nayak et al., 2011; Qiao et al., 2003), hypertension, sedentary lifestyle, obesity, and family history of diabetes. Diabetic individuals typically have an abnormal lipid profile which is referred to as diabetic dyslipidemia. It is characterized primarily by low levels of HDL Cholesterol (<45 mg/dL) along with high Triglyceride levels (>250 mg/dL) and LDL Cholesterol levels in normal level.

Abnormal lipid levels cause complication in liver metabolism and further results in hepatic tissue damage and leads to liver diseases like non-alcoholic fatty liver diseases (Kathak et al., 2022). Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) are liver function enzymes, which are the important for studying disease severity in chronic liver diseases (Liu et al., 2018). Hypothyroidism is one of the causes for developing secondary dyslipidemia (Remaley et al., 2012). In some studies, it is mentioned that serum uric acid had positive correlation with serum Triglyceride, Total Cholesterol and LDL Cholesterol and had negative correlation with HDL Cholesterol (Tariq et al., 2022).

The sampling in this method is Indirect sampling from Clinical Biochemistry

Laboratory as the sample size requirements are very high. Indirect sampling represents the population base of the particular hospital. Indirect sampling is cost effective and large sample size can be obtained in a shorter duration.

13.3. Objectives

- a) Collect fasting Lipid profile data along with data related to biochemical and physical parameters from the Clinical Biochemistry Laboratory.
- b) Correlation analysis of lipid profile measurands with clinical parameters like Glucose, TSH, liver function and kidney function test parameters.
- c) Correlation analysis of Serum Triglycerides with HDL Cholesterol and Total Cholesterol
- d) Correlation analysis of Total Cholesterol with LDL Cholesterol.

13.4. Materials and Methods

13.4.1 Research Design

This is an **Observational Cross-Sectional study** of dyslipidemia conditions presented at Amala Institute of Medical Sciences by **Indirect sampling** method.

13.4.2. Selection of Age Groups for the study

Total samples collected include males and females >18 years of age. The sample was partitioned according to age into four groups: < 18 years, 18 – 25 years (young adults), 26 – 50 years (middle age adults) and >50 years (Senior adults). These ranges of classification were selected from the lipid profile variations with age in the population of study, and occurrence of dyslipidemia diseases which are grouped into primary and secondary dyslipidemia.

13.4.3. Direct versus Indirect sampling

Indirect sampling was preferred to direct sampling due to the large sample requirements for both common and rare lipid profile conditions and abnormal levels. Indirect sampling was done from hospital (Refer General Materials and Methods)

13.4.4. Sample Collection

The period of sample collection was from September 2018 to March 2022 with a break when hospital was closed due to Covid.

The sample number for total and partitioned samples was;

Total samples; n = 15603

Total male samples; n = 9479

Total female samples; n = 6124

Total males <18 years of age; n = 97

Total females <18 years of age; n = 86

Total male samples 18 – 25 years of age; n = 210

Total female samples 18 – 25 years of age; n = 159

Total male samples 26 - 50 years of age; n = 3247

Total female samples 26 - 50 years of age; n = 1921

Total male samples >50 years of age; n = 5925

Total female samples >50 years of age; n = 3958

13.4.5. Selection and Assay of the Lipid profile Analytes

Lipid profile analytes selected were serum Total triglycerides, Total cholesterol, HDL cholesterol and LDL cholesterol. Chemistry tests with Ortho Clinical Diagnostics (OCD) Vitros 5.1 FS autoanalyzer (Refer General Materials and Methods)

13.4.6. Statistical Methods

SPSS Version 19 was used for calculating correlation. Correlation between different measurands were done by Pearson's and Spearman's correlation method (Refer General Materials and Methods).

13.5. Results

13.5.1. Correlation between Glucose (mg/dL) and Lipid Profile measurands

13.5.1.a. In Total Samples (Male: n = 6553; Female: n = 4114)

There was positive correlation between glucose and serum triglycerides in both male and female samples with correlation coefficient $r = 0.103$ and $r = 0.259$ in males and females, respectively with $P = <0.001$. There was negative correlation between LDL cholesterol and glucose in both male and female samples with $r = -0.083$ and $r = -0.084$, respectively ($P = <0.001$) (Table 13.1).

Negative correlation between glucose and Total cholesterol was observed in males ($r = -0.036$; $P = 0.004$). But, in females there were no significant correlation between glucose and Total cholesterol (Table 13.1)

In females, there was negative correlation between glucose and HDL cholesterol with $r = -0.064$ and $P = <0.001$. There was no significant correlation between fasting glucose and HDL cholesterol in males (Table 13.1).

13.5.1. b. In age group 18 – 25 years (Male: n = 143; Female: n = 100)

In females, Fasting Glucose positively correlated with Total Cholesterol ($r = 0.294$; $P = 0.003$), serum Triglycerides ($r = 0.383$, $P = <0.001$), LDL Cholesterol ($r = 0.256$, $P = 0.010$) and negatively correlated with HDL Cholesterol, with $r = -0.197$ and $P = 0.050$ (Table 13.1).

In males, there was no correlation between lipid profile analytes and Fasting Glucose (Table 13.1).

13.5.1. c. In age group 26 - 50 years (Male: n = 2504; Female: n = 1421)

In female samples Fasting Glucose positively correlated with serum triglyceride ($r = 0.307$, $P = <0.001$) and negatively correlated with HDL ($r = -0.113$, $P = <0.001$). There was no significant correlation between fasting glucose and total cholesterol and also between fasting glucose and LDL Cholesterol in females of age group 26 – 50 years (Table 13.1).

In males, fasting glucose negatively correlated with total cholesterol and LDL cholesterol with $r = -0.040$ ($P = 0.046$) and -0.105 ($P = <0.001$), respectively. There was positive correlation between fasting glucose and serum triglycerides in males with coefficient of correlation $r = 0.115$ and $P = <0.001$. There was no significant correlation between fasting glucose and HDL cholesterol in males of age group 26 – 50 years (Table 13.1).

13.5.1. d. In age group >50 years (Male: n = 3906; Female: n = 2593)

Positive correlation between fasting glucose and serum triglyceride in both male and female samples with $r = 0.157$ and $r = 0.203$, respectively and significance of correlation was $P = <0.001$ in both samples (Table 13.1)

Fasting glucose positively correlated with Total cholesterol in males ($r = 0.038$, $P = 0.017$). In females, fasting glucose negatively correlated with Total cholesterol ($r = -0.040$, $P = 0.042$), HDL cholesterol ($r = -0.063$, $p = 0.001$) and LDL cholesterol ($r = -0.075$, $P = <0.001$). There was no significant correlation between fasting glucose and HDL cholesterol, and between fasting glucose and LDL cholesterol in males of age group > 50 years (Table 13.1).

13.5.2. Correlation between Thyroid Stimulating Hormone (mIU/L) and lipid profile measurands

13.5.2. a. In total samples (Male: n = 2391; Female: n = 1894)

In both male and female samples, TSH positively correlated with Total cholesterol (males: $r = 0.119$, $P = <0.001$; females: $r = 0.091$, $P = <0.001$), serum triglycerides (males: $r = 0.170$, $P = <0.001$; females: $r = 0.122$, $P = <0.001$) and LDL cholesterol (males: $r = 0.078$, $P = <0.001$; females: $r = 0.071$, $P = 0.002$) (Table 13.2).

There was no significant correlation between TSH and HDL cholesterol in both male and female samples (Table 13.2).

13.5.2. b. In age group 18 – 25 years (Male: n = 94; Female: n = 76)

There was correlation between TSH and serum triglycerides in male sample with coefficient of correlation $r = 0.247$ and $P = 0.017$ (Table 13.2). There was no significant correlation for TSH with Total cholesterol, LDL cholesterol and HDL cholesterol in both male and females of age group 18 – 25 years (Table 13.2).

13.5.2. c. In age group 26 - 50 years (Male: n = 1127; Female: n = 780)

In both male and female samples, TSH positively correlated with Total cholesterol (males: $r = 0.153$, $P = <0.001$; females: $r = 0.121$, $P = 0.001$), serum triglycerides (males: $r = 0.153$, $P = <0.001$; females: $r = 0.088$, $P = 0.014$) and LDL cholesterol (males: $r = 0.120$, $P = <0.001$; females: $r = 0.128$, $P = <0.001$) (Table 13.2).

There was no significant correlation between TSH and HDL cholesterol in both male and female samples of age group 26 – 50 years (Table 13.2)

13.5.2. d. In age group > 50 years (Male: n = 1170; Female: n = 1038)

In both male and female samples, TSH positively correlated with Total cholesterol (male: $r = 0.085$, $P = 0.004$; females: $r = 0.076$, $P = 0.015$), and serum triglycerides (male: $r = 0.717$, $P = <0.001$; females: $r = 0.160$, $P = <0.001$) (Table 13.2),

There was no significant correlation between TSH and HDL cholesterol in both male and female samples of age group >50 years. There was no significant correlation between TSH and LDL cholesterol in both male and female samples of age group >50 years (Table 13.2)

13.5.3. Correlation between Alanine transaminase (ALT) and lipid profile measurands**13.5.3. a. In total samples (Male: n = 3474; Female: n = 1726)**

ALT positively correlated with serum triglycerides in both male ($r = 0.221$) and female ($r = 0.146$) samples with significance of correlation $P = <0.001$. ALT

positively correlated with Total cholesterol ($r = 0.226$) and LDL cholesterol ($r = 0.186$) in males with significance of correlation $P = <0.001$ (Table 13.3).

There was no correlation between ALT and HDL cholesterol in both male and female samples (Table 13.3).

13.5.3. b. In age group 18 – 25 years (Male: n = 102; Female: n = 72)

ALT values showed positive correlation with Total cholesterol ($r = 0.306$, $P = 0.002$), serum triglyceride ($r = 0.316$, $P = 0.001$) and LDL cholesterol ($r = 0.218$, $P = 0.028$) in males (Table 13.3).

There was negative correlation between ALT and HDL cholesterol with $r = -0.273$ and $P = 0.021$ in females. There was no correlation between ALT and HDL cholesterol in males

In female samples, ALT values have no correlation with total cholesterol, serum triglyceride and LDL cholesterol (Table 13.3).

13.5.3. c. In age group 26 - 50 years (Male: n = 1488; Female: n = 652)

In both male and female samples ALT showed positive correlation with Total cholesterol (male $r = 0.202$, $P = <0.001$; female $r = 0.094$, $P = 0.016$) and serum triglycerides (male $r = 0.189$, $P = <0.001$; female $r = 0.248$, $P = <0.001$) (Table 13.3).

ALT positively correlated with LDL cholesterol with $r = 0.146$ and $P = <0.001$ in males. There was no correlation between ALT and HDL cholesterol in both male and female samples (Table 13.3).

13.5.3. d. In age group > 50 years (Male: n = 1884; Female: n = 1002)

ALT positively correlated with Total cholesterol ($r = 0.087$, $P = <0.001$), serum triglyceride ($r = 0.111$, $P = <0.001$) and LDL cholesterol ($r = 0.072$, $P = 0.002$) in male samples. There was no correlation between ALT and HDL cholesterol in male >50 years of age.

There was no significant correlation between ALT and lipid profile measurands

in females >50 years of age (Table 13.3).

13.5.4. Correlation between Aspartate Transferase (AST) and lipid profile measurands

13.5.4. a. In total samples (Male: n = 2866; Female: n = 1414)

There was positive correlation between AST and serum triglycerides in both male ($r = 0.097$) and female ($r = 0.117$) samples with significance of correlation $P = <0.001$.

AST positively correlated with Total cholesterol ($r = 0.123$), HDL cholesterol ($r = 0.088$) LDL cholesterol ($r = 0.080$) in males with significance of correlation $P = <0.001$ in males (Table 13.4). AST positively correlated with Total cholesterol ($r = 0.057$, $P = 0.033$) and HDL cholesterol ($r = 0.055$, $P = 0.040$) in females. There was no correlation between AST and LDL cholesterol in female samples (Table 13.4).

13.5.4. b. In age group 18 – 25 years (Male: n = 95; Female: n = 63)

AST values showed positive correlation with serum triglyceride ($r = 0.377$, $P = 0.007$) in females. There was no correlation between AST and Total cholesterol, LDL cholesterol and HDL cholesterol in females (Table 13.4). In male samples, AST values have no correlation with lipid profile measurands (Table 13.4).

13.5.4. c. In age group 26 - 50 years (Male: n = 1334; Female: n = 560)

In both male and female samples AST showed positive correlation with total cholesterol (male $r = 0.124$, $P = <0.001$; female $r = 0.149$, $P = <0.001$) and serum triglycerides (male $r = 0.119$, $P = <0.001$; female $r = 0.202$, $P = <0.001$) (Table 13.4).

There was positive correlation between AST and HDL cholesterol in male sample with $r = 0.084$ and $P = 0.002$. There was no correlation between AST and HDL cholesterol in female samples (Table 13.4).

AST positively correlated with LDL cholesterol with $r = 0.054$ ($P = 0.049$) and $r = 0.107$ ($P = 0.011$) in males and females, respectively (Table 13.4).

13.5.4. d. In age group > 50 years (Male: n = 1437; Female: n = 791)

AST positively correlated with HDL cholesterol in male ($r = 0.097$, $P = <0.001$) and in female sample ($r = 0.083$, $P = 0.019$) (Table 13.4).

AST positively correlated with total cholesterol in male samples ($r = 0.060$, $P = 0.022$). There was no significant correlation between AST and Total cholesterol in females >50 years of age (Table 13.4).

There was no significant correlation between AST and serum triglycerides in both male and female samples >50 years of age (Table 13.4).

13.5.5. Correlation between Alkaline Phosphatase and Lipid Profile measurands.**13.5.5. a. In total samples (Male: n = 2176; Female: n = 1083)**

There was positive correlation between Alkaline phosphatase values with serum triglyceride in female samples with $r = 0.159$ and $P = <0.001$. There was no significant correlation between Alkaline phosphatase and other lipid measurands like Total cholesterol, LDL cholesterol and HDL cholesterol in both male and females (Table 13.5).

13.5.5. b. In age group 18 – 25 years (Male: n = 73; Female: n = 49)

In males, there were positive correlation between Alkaline phosphatase and Total cholesterol ($r = 0.292$, $P = 0.012$). In females, Alkaline phosphatase positively correlated with serum triglycerides ($r = 0.306$, $P = 0.032$) and negatively correlated with HDL cholesterol ($r = -0.358$, $P = 0.012$) (Table 13.5).

LDL cholesterol does not have significant correlation with Alkaline phosphatase in both males and females (Table 13.5).

13.5.5. c. In age group 26 - 50 years (Male: n = 1014; Female: n = 420)

Alkaline Phosphatase in both male and female samples showed significant positive correlation with Total cholesterol (males: $r = 0.066$, $P = 0.037$; females: $r = 0.120$, $P = 0.014$) and with serum triglycerides (males: $r = 0.089$, $P = 0.004$; females:

$r = 0.184$, $P = <0.001$) (Table 13.5).

Alkaline phosphatase values had no significant relationship with HDL cholesterol and LDL cholesterol in both the male and female samples (Table 13.5).

13.5.5. d. In age group > 50 years (Male: n = 1089; Female: n = 614)

In both male and female samples, there was no significant relationship between alkaline phosphatase and most lipid profile measurands (Table 13.5).

13.5.6. Correlation between Serum Creatinine and Lipid Profile measurands

13.5.6. a. In total samples (Male: n = 5108; Female: n = 2631)

Total cholesterol negatively correlated with serum creatinine with correlation coefficient $r = -0.133$ and $r = -0.097$ in males and females, respectively with significance of correlation $P = <0.001$ (Table 13.6).

Serum creatinine negatively correlated with serum triglycerides in males with correlation coefficient $r = -0.030$ ($P = 0.030$), but positively correlated in females with correlation coefficient $r = 0.070$, $P = <0.001$ (Table 13.6).

Serum creatinine negatively correlated with HDL Cholesterol (males: $r = -0.046$, $P = 0.001$; females: $r = -0.042$, $P = 0.033$) and LDL cholesterol (males: $r = -0.099$, $P = <0.001$; females: $r = -0.118$, $P = <0.001$) (Table 13.6).

13.5.6. b. In age group 18 – 25 years (Male: n = 97; Female: n = 76)

In males, serum creatinine positively correlated with Total cholesterol ($r = 0.269$, $P = 0.008$), serum Triglycerides ($r = 0.282$, $P = 0.005$) and LDL Cholesterol ($r = 0.248$, $P = 0.014$) (Table 13.6).

13.5.6. c. In age group 26 - 50 years (Male: n = 1765; Female: n = 815)

There was negative correlation between serum creatinine and HDL cholesterol with correlation coefficient $r = -0.062$ and $P = 0.009$ in males (Table 13.6). In females of age group 26 – 50 years, there was no significant correlation between serum creatinine and lipid profile measurands (Table 13.6).

13.5.6. d. In age group > 50 years (Male: n = 3246; Female: n = 1740)

Serum creatinine negatively correlated with Total cholesterol in males and in females with $r = -0.092$ and $r = -0.112$, respectively ($P = <0.001$). Serum creatinine negatively correlated with HDL cholesterol in males ($r = -0.038$, $P = 0.030$) and in females (-0.082 , $P = 0.001$).

There was negative correlation between serum creatinine and LDL cholesterol in male ($r = -0.097$) and females ($r = -0.117$) with significance of correlation $P = <0.001$ (Table 13.6).

13.5.7. Correlation between Serum Uric Acid and lipid profile measurands**13.5.7. a. In total samples (Male: n = 1332; Female: n = 508)**

There was positive correlation between Total cholesterol and uric acid in males with $r = 0.143$ and $P = <0.001$. Uric acid positively correlated with serum triglycerides with correlation coefficient $r = 0.128$ in males and $r = 0.222$ in females with significance of correlation $P = <0.001$.

Uric acid negatively correlated with HDL cholesterol in male ($r = -0.093$, $P = 0.001$) and in female ($r = -0.169$, $P = <0.001$) samples (Table 13.7).

Uric acid positively correlated with LDL cholesterol in males with $r = 0.138$ and $P = <0.001$ (Table 13.7).

13.5.7. b. In age group 18 – 25 years (Male: n = 13; Female: n = 7)

Uric acid positively correlated with serum triglycerides ($r = 0.929$, $P = 0.003$) and negatively correlated with HDL cholesterol in females ($r = -0.857$, $P = 0.014$) of the age group 18 – 25 years (Table 14.7).

13.5.7. c. In age group 26 - 50 years (Male: n = 723; Female: n = 236)

There was positive correlation between uric acid and Total cholesterol with $r = 0.147$ and $P = <0.001$ in males (Table 13.7).

In both males and females of age group 26 – 50 years, there was significant

positive correlation between uric acid and serum triglycerides (Table 13.7).

Uric acid values negatively correlated with HDL cholesterol in females ($r = -0.251$, $P = <0.001$). In males there was positive correlation between uric acid and LDL cholesterol with coefficient of correlation $r = 0.138$ and $P = <0.001$ (Table 13.7).

13.5.7. d. In age group > 50 years (Male: n = 596; Female: n = 265)

Uric acid positively correlated with serum triglycerides in males ($r = 0.101$, $P = 0.014$) and in females ($r = 0.121$, $P = 0.048$). Uric acid negatively correlated with HDL cholesterol in males with $r = -0.117$, $P = 0.004$ (Table 13.7).

13.5.8. Correlation between serum triglycerides and HDL cholesterol

13.5.8.a Correlation between serum triglycerides with HDL Cholesterol in total samples

In total sample, Serum Triglyceride data negatively correlated with HDL Cholesterol in both male ($n = 9479$) and females ($n = 6124$) with $r = -0.254$ ($P = <0.001$) and $r = -0.340$ ($P = <0.001$), respectively (Table 13.8).

13.5.8.b. Correlation between serum triglycerides with HDL Cholesterol in samples of age group <18 years.

In age group < 18 years, Serum Triglyceride data negatively correlated with HDL Cholesterol in both male ($n = 97$) and females ($n = 86$) with correlation coefficient $r = -0.388$ ($P = <0.001$) and $r = -0.374$ ($P = <0.001$), respectively (Table 13.8 and Fig 13.1).

13.5.8.c. Correlation between Serum triglycerides with HDL Cholesterol in samples of age group 18 - 25 years.

Serum Triglyceride data negatively correlated with HDL Cholesterol in male ($n = 210$) and females ($n = 159$) of age group 18 – 25 years, with correlation coefficient $r = -0.193$ ($P = 0.005$) and $r = -0.349$ ($P = <0.001$), respectively (Table 13.8 and Fig 13.1).

13.5.8.d. Correlation between Serum triglycerides with HDL Cholesterol in samples of age group 26 – 50 years.

Serum Triglyceride data negatively correlated with HDL Cholesterol in males and females of age group 26 - 50 years, with $r = -0.293$ ($P = <0.001$) and $r = -0.386$ ($P = <0.001$), respectively (Table 13.8 and Fig 13.1).

13.5.8.e. Correlation between Serum triglycerides with HDL Cholesterol in samples of age group >50 years.

In age group >50 years, Serum Triglyceride data negatively correlated with HDL Cholesterol in both male ($n = 5925$) and females ($n = 3958$) with $r = -0.243$ ($P = <0.001$) and $r = -0.325$ ($P = <0.001$), respectively (Table 13.8 and Fig 13.1).

13.5.9. Correlation between Serum triglycerides with Total Cholesterol in total samples and samples in different age groups

13.5.9.a Correlation between Serum triglycerides with Total Cholesterol in total samples.

In total sample, Serum Triglyceride data positively correlated with Total Cholesterol in both male ($n = 9479$) and females ($n = 6124$) with $r = 0.407$ ($P = <0.001$) and $r = 0.273$ ($P = <0.001$), respectively (Table 13.8).

13.5.9.b. Correlation between serum triglycerides with Total Cholesterol in samples of age group <18 years.

In age group <18 years, Serum Triglyceride data positively correlated with Total Cholesterol in both male ($n = 97$) and females ($n = 86$) with $r = 0.402$ ($P = <0.001$) and $r = 0.395$ ($P = <0.001$), respectively (Table 13.8 and Fig 13.2).

14.5.9.c. Correlation between serum triglycerides with Total Cholesterol in samples of age group 18 - 25 years.

Serum Triglyceride data positively correlated with Total Cholesterol in males ($n = 210$) and females ($n = 159$) in age group 18 – 25 years with $r = 0.521$ ($P = <0.001$) and $r = 0.414$ ($P = <0.001$), respectively (Table 13.8 and Fig 13.2).

13.5.9.d. Correlation between Serum triglycerides with Total Cholesterol in samples of age group 26 – 50 years.

Serum Triglyceride data positively correlated with Total Cholesterol in both males and females in age group 26 – 50 years with correlation coefficient $r = 0.352$ ($P = <0.001$) and $r = 0.278$, ($P = <0.001$), respectively (Table 13.8 and Fig 13.2).

13.5.9.e. Correlation between serum triglycerides with Total Cholesterol in samples of age group >50 years.

Serum Triglyceride data positively correlated with Total Cholesterol in both males and females in age group >50 years with $r = 0.355$ ($P = <0.001$) and $r = 0.274$ ($P = <0.001$), respectively (Table 13.8 and Fig 13.2).

13.5.10. Correlation between Total Cholesterol with LDL Cholesterol in total samples and samples in different age groups

13.5.10.a Correlation between Total Cholesterol with LDL Cholesterol in total samples

In total sample, Total Cholesterol data showed strong positive correlation with LDL Cholesterol in both male ($n = 9441$) and females ($n = 6113$) with $r = 0.917$ ($P = <0.001$) and $r = 0.935$ ($P = <0.001$), respectively (Table 13.9).

13.5.10.b. Correlation between Total Cholesterol with LDL Cholesterol in samples of age group <18 years.

In samples of age group <18 years, Total Cholesterol data showed strong positive correlation with LDL Cholesterol in both male ($n = 95$) and females ($n = 86$) with $r = 0.861$ ($P = <0.001$) and $r = 0.912$ ($P = <0.001$), respectively (Table 13.9 and

Fig 13.3).

13.5.10.c. Correlation between Total Cholesterol with LDL Cholesterol in samples of age group 18 - 25 years.

In samples of age group 18 – 25 years, Total Cholesterol data showed strong positive correlation with LDL Cholesterol in both male (n = 209) and females (n = 159) with $r = 0.910$ ($P = <0.001$) and $r = 0.916$ ($P = <0.001$), respectively (Table 13.9 and Fig 13.3).

13.5.10.d. Correlation between Total Cholesterol with LDL Cholesterol in samples of age group 26 – 50 years.

In samples of age group 26 - 50 years, Total Cholesterol data showed strong positive correlation with LDL Cholesterol in both male (n = 3229) and females (n = 1921) with correlation coefficient $r = 0.892$ ($P = <0.001$) and $r = 0.918$ ($P = <0.001$), respectively (Table 13.9 and Fig 13.3).

13.5.10.e. Correlation between Total Cholesterol with LDL Cholesterol in samples of age group >50 years.

In samples of age group >50 years, Total Cholesterol data showed strong positive correlation with LDL Cholesterol in both male (n = 5908) and females (n = 3958) with correlation coefficient $r = 0.921$ ($P = <0.001$) and $r = 0.940$ ($P = <0.001$), respectively (Table 13.9 and Fig 13.3).

13.5.11. Correlation of BMI with lipid profile analytes

There is positive correlation of Serum triglycerides with BMI (Table 13.10)

13.6. Discussions

There is **positive correlation of Triglycerides with glucose** in adult males and females. The triglyceride- glucose index is a reliable marker for **insulin resistance** and has been linked to an increased risk of cardiovascular diseases and mortality (lopez-Jaramillo et al, 2023).

Triglycerides correlate positively with TSH in males of all age groups. Both elevated levels of triglycerides and TSH levels have been linked to metabolic syndrome, studies suggest that abnormal TSH levels can impact lipid metabolism, potentially affecting triglyceride levels indirectly (Hussain et al, 2019).

Total Cholesterol correlates positively with TSH in males and females above 25 years. This relationship indicates that higher TSH levels, often indicative of hypothyroidism, are linked to increased Total Cholesterol levels (American Thyroid Association study).

ALT positively correlated with Total Cholesterol, triglycerides and LDL-C in adult males. This may be attributed to the role of the liver in lipid metabolism, insulin resistance and metabolic syndrome (Kim et al., 2017; Schindhelm et al, 2007).

The positive correlation between AST, ALP and triglycerides in females but not in males may be attributed to sex-specific differences (estrogen) in lipid metabolism and liver enzyme activity (Wang et al, 2019).

AST, ALP and ALT are analytes related to liver dysfunction. Even a minor influence in liver dysfunction such as increase in ALT can influence and cause increase in triglyceride levels. The possible liver dysfunction may be fatty liver, steatohepatitis, cirrhosis liver, acute and chronic hepatitis.

Serum Creatinine was not correlating with lipid profile reagents. There was a positive correlation between uric acid and Triglycerides and a negative correlation between Uric acid and HDL Cholesterol. Although there is no direct relationship between hypertriglyceridemia and high uric acid level, they are linked by a common denominator: a diet enriched in fructose. Fructose not only increases endogenous production of uric acid but also induces de novo lipid synthesis, thereby resulting in enhanced hepatic output of triglyceride rich lipoprotein (VLDL). Fructose accelerates purine de novo synthesis by increasing mammalian target of rapamycin expression (mTOR).

Triglycerides correlate positively with TC and negatively correlate with HDL-C in males and females of all age groups. Elevated TG can be associated with changes

in lipoprotein metabolism, leading to increased levels of Total Cholesterol (Berglund et al, 2012). Insulin resistance can lead to increased TG production and decreased clearance contributing to elevated Total Cholesterol levels (Yuang et al, 2007).

Triglycerides and HDL-C are metabolically connected. Elevated TG levels can lead to changes in HDL-C composition and function, contributing to decreased HDL Cholesterol levels (Wang et al, 2020). There was strong positive correlation between Total Cholesterol and LDL Cholesterol in all gender-age groups

Positive correlation between TG and BMI - Excess body fat (BMI) especially around abdominal area can lead to insulin resistance, metabolic syndrome and increased Triglyceride production.

13.7. Conclusions

The correlation studies demonstrate significant relationship between biochemical parameters with lipid profile analytes. These correlations highlight the interconnectedness of metabolic parameters and their association with cardiovascular risk. Comprehensive metabolic assessment and targeted interventions, including lifestyle modifications and pharmacological treatments are crucial for managing metabolic health and cardiovascular risk. Further research is needed to fully understand this relationship and develop effective management strategies, potentially enabling personalized medicine approaches that account for an individual's unique metabolic profile and treatment strategies.

Table 13.1. Correlation between Fasting Glucose (mg/dL) and lipid profile analytes(mg/dL) in males and females in the four age groups, and in the Total samples.

Analytes	Correlation coefficient r (Spearman's rho ρ)		Significance of correlation p	
	Male	Female	Male	Female
Total adult samples (Male: n=6553; Female: n=4114)				
Glucose and TC	-0.036	-0.029	0.004	0.059
Glucose and TG	0.103	0.259	(<0.001)	(<0.001)
Glucose and HDL- C	0.005	-0.064	0.713	(<0.001)
Glucose and LDL-C	-0.083	-0.084	(<0.001)	(<0.001)
Age 18-25 years (Male: n=143; Female: n=100)				
Glucose and TC	0.144	0.294	0.087	0.003
Glucose and TG	0.120	0.383	0.153	(<0.001)
Glucose and HDL- C	0.011	-0.197	0.895	0.050
Glucose and LDL-C	0.038	0.256	0.652	0.010
Age 26-50 years (Male: n=2504; Female: n=1421)				
Glucose and TC	-0.040	0.028	0.046	0.283
Glucose and TG	0.115	0.307	(<0.001)	(<0.001)
Glucose and HDL- C	0.017	-0.113	0.381	(<0.001)
Glucose and LDL-C	-0.105	-0.021	(<0.001)	0.434
Age >50 years (Male: n=3906; Female: n=2593)				
Glucose and TC	0.038	-0.040	0.017	0.042
Glucose and TG	0.157	0.203	(<0.001)	(<0.001)
Glucose and HDL- C	-0.015	-0.063	0.352	0.001
Glucose and LDL-C	-0.002	-0.075	0.925	(<0.001)

Table 13.2. Correlation between Thyroid stimulating hormone (mIU/L) and lipid profile analytes

Analytes	Correlation coefficient r (Spearman's rho ρ)		Significance of correlation p	
	Male	Female	Male	Female
Total adult samples (Male: n=2391; Female: n=1894)				
TSH and TC	0.119	0.091	(<0.001)	(<0.001)
TSH and TG	0.170	0.122	(<0.001)	(<0.001)
TSH and HDL – C	0.017	<0.001	0.410	0.996
TSH and LDL – C	0.078	0.071	(<0.001)	0.002
Age 18-25 years (Male: n=94; Female: n=76)				
TSH and TC	0.186	<0.001	0.073	0.998
TSH and TG	0.247	0.124	0.017	0.285
TSH and HDL – C	-0.084	0.088	0.422	0.450
TSH and LDL – C	0.108	-0.053	0.298	0.649
Age 26-50 years (Male: n=1127; Female: n=780)				
TSH and TC	0.153	0.121	(<0.001)	0.001
TSH and TG	0.153	0.088	(<0.001)	0.014
TSH and HDL – C	0.001	0.002	0.971	0.965
TSH and LDL – C	0.120	0.128	(<0.001)	(<0.001)
Age >50 years (Male: n=1170; Female: n=1038)				
TSH and TC	0.085	0.076	0.004	0.015
TSH and TG	0.717	0.160	(<0.001)	(<0.001)
TSH and HDL – C	0.037	0.001	0.201	0.975
TSH and LDL – C	0.039	0.038	0.177	0.220

(TSH – Thyroid Stimulating Hormone)

Table 13.3. Correlation between ALT (U/L) and lipid profile analytes (mg/dL).

Analytes	Correlation coefficient r (Spearman's rho ρ)		Significance of correlation p	
	Male	Female	Male	Female
Total adult samples (Male: n=3474; Female: n=1726)				
ALT and TC	0.226	0.027	(<0.001)	0.254
ALT and TG	0.221	0.146	(<0.001)	(<0.001)
ALT and HDL – C	0.018	-0.006	0.280	0.811
ALT and LDL – C	0.186	-0.002	(<0.001)	0.944
Age 18-25 years (Male: n=102; Female: n=72)				
ALT and TC	0.306	0.078	0.002	0.513
ALT and TG	0.316	0.156	0.001	0.190
ALT and HDL – C	0.029	-0.273	0.770	0.021
ALT and LDL – C	0.218	0.201	0.028	0.090
Age 26-50 years (Male: n=1488; Female: n=652)				
ALT and TC	0.202	0.094	(<0.001)	0.016
ALT and TG	0.189	0.248	(<0.001)	(<0.001)
ALT and HDL – C	0.022	-0.026	0.406	0.506
ALT and LDL – C	0.146	0.040	(<0.001)	0.306
Age >50 years (Male: n=1884; Female: n=1002)				
ALT and TC	0.087	-0.016	(<0.001)	0.621
ALT and TG	0.111	0.057	(<0.001)	0.073
ALT and HDL – C	0.017	0.026	0.452	0.413
ALT and LDL – C	0.072	-0.030	0.002	0.347

(ALT – Alanine transaminase)

Table 13.4. Correlation between AST (U/L) and lipid profile analytes (mg/dL)

Analytes	Correlation coefficient r (Spearman's rho ρ)		Significance of correlation p	
	Male	Female	Male	Female
Total adult samples (Male: n=2866; Female: n=1414)				
AST and TC	0.123	0.057	<0.001	0.033
AST and TG	0.097	0.117	<0.001	<0.001
AST and HDL – C	0.088	0.055	<0.001	0.040
AST and LDL – C	0.080	0.025	<0.001	0.356
Age 18-25 years (Male: n=95; Female: n=63)				
AST and TC	0.066	0.113	0.525	0.379
AST and TG	-0.054	0.377	0.603	0.007
AST and HDL – C	0.194	-0.218	0.059	0.087
AST and LDL – C	0.017	0.143	0.874	0.262
Age 26-50 years (Male: n=1334; Female: n=560)				
AST and TC	0.124	0.149	<0.001	<0.001
AST and TG	0.119	0.202	<0.001	<0.001
AST and HDL – C	0.084	0.029	0.002	0.492
AST and LDL – C	0.054	0.107	0.049	0.011
Age >50 years (Male: n=1437; Female: n=791)				
AST and TC	0.060	0.008	0.022	0.815
AST and TG	0.022	-0.003	0.407	0.936
AST and HDL – C	0.097	0.083	<0.001	0.019
AST and LDL – C	0.041	-0.005	0.125	0.878

(AST – Aspartate transaminase)

Table 13.5. Correlation between Alkaline phosphatase (U/L) and lipid profile analytes (mg/dL)

Analytes	Correlation coefficient r (Spearman's rho ρ)		Significance of correlation p	
	Male	Female	Male	Female
Total adult samples (Male: n = 2176; Female: n = 1083)				
ALP and Total cholesterol	0.004	0.011	0.867	0.729
ALP and triglycerides	0.038	0.159	0.073	(<0.001)
ALP and HDL – C	-0.009	-0.014	0.660	0.649
ALP and LDL – C	-0.021	-0.029	0.316	0.335
Age 18-25 years (Male: n = 73; Female: n = 49)				
ALP and Total cholesterol	0.292	-0.051	0.012	0.730
ALP and triglycerides	0.160	0.306	0.177	0.032
ALP and HDL – C	-0.126	-0.358	0.288	0.012
ALP and LDL – C	0.140	-0.080	0.237	0.583
Age 26-50 years (Male: n = 1014; Female: n = 420)				
ALP and Total cholesterol	0.066	0.120	0.037	0.014
ALP and triglycerides	0.089	0.184	0.004	(<0.001)
ALP and HDL – C	0.028	-0.013	0.375	0.790
ALP and LDL – C	0.023	0.076	0.460	0.119
Age >50 years (Male: n = 1089; Female: n = 614)				
ALP and Total cholesterol	-0.024	-0.038	0.422	0.343
ALP and triglycerides	0.025	0.078	0.415	0.054
ALP and HDL – C	-0.037	-0.036	0.221	0.368
ALP and LDL – C	-0.034	-0.050	0.259	0.217

(ALP- Alkaline phosphatase)

Table 13.6. Correlation between Serum Creatinine and lipid profile analytes

Analytes (mg/dl)	Correlation coefficient r (Spearman's rho ρ)		Significance of correlation p	
	Male	Female	Male	Female
Total adult samples (Male: n=5108; Female: n= 2631)				
Creatinine and Total Cholesterol	-0.113	-0.097	(<0.001)	(<0.001)
Creatinine and Triglycerides	-0.030	0.070	0.030	(<0.001)
Creatinine and HDL – C	-0.046	-0.042	0.001	0.033
Creatinine and LDL – C	-0.099	-0.118	(<0.001)	(<0.001)
Age 18-25 years (Male: n=97; Female: n= 76)				
Creatinine and Total Cholesterol	0.269	0.109	0.008	0.349
Creatinine and Triglycerides	0.282	0.131	0.005	0.259
Creatinine and HDL – C	-0.020	-0.019	0.847	0.869
Creatinine and LDL – C	0.248	0.074	0.014	0.523
Age 26-50 years (Male: n=1765; Female: n= 815)				
Creatinine and Total Cholesterol	0.007	0.048	0.784	0.173
Creatinine and Triglycerides	-0.017	0.015	0.479	0.679
Creatinine and HDL – C	-0.062	0.057	0.009	0.101
Creatinine and LDL – C	0.042	0.046	0.075	0.190
Age >50 years (Male: n=3246; Female: n= 1740)				
Creatinine and Total Cholesterol	-0.092	-0.112	(<0.001)	(<0.001)
Creatinine and Triglycerides	0.013	0.043	0.445	0.071
Creatinine and HDL – C	-0.038	-0.082	0.030	0.001
Creatinine and LDL – C	-0.097	-0.117	(<0.001)	(<0.001)

Table 13.7. Correlation between Uric acid and lipid profile analytes

Analytes (mg/dL)	Correlation coefficient r (Spearman's rho ρ)		Significance of correlation p	
	Male	Female	Male	Female
Total adult samples (Male: n = 1332; Female: n = 508)				
Uric acid and Total Cholesterol	0.143	0.060	(<0.001)	0.179
Uric acid and Triglycerides	0.128	0.222	(<0.001)	(<0.001)
Uric acid and HDL – C	-0.093	-0.169	0.001	(<0.001)
Uric acid and LDL – C	0.138	0.040	(<0.001)	0.363
Age 18-25 years (Male: n = 13; Female: n = 7)				
Uric acid and Total Cholesterol	0.219	0.321	0.473	0.482
Uric acid and Triglycerides	-0.285	0.929	0.345	0.003
Uric acid and HDL – C	0.261	-0.857	0.389	0.014
Uric acid and LDL – C	0.327	0.214	0.276	0.645
Age 26-50 years (Male: n = 723; Female: n = 236)				
Uric acid and Total Cholesterol	0.147	0.056	(<0.001)	0.395
Uric acid and Triglycerides	0.116	0.267	0.002	(<0.001)
Uric acid and HDL – C	-0.073	-0.251	0.050	(<0.001)
Uric acid and LDL – C	0.138	0.035	(<0.001)	0.592
Age >50 years (Male: n = 596; Female: n = 265)				
Uric acid and Total Cholesterol	0.029	0.066	0.481	0.282
Uric acid and Triglycerides	0.101	0.121	0.014	0.048
Uric acid and HDL – C	-0.117	-0.088	0.004	0.154
Uric acid and LDL – C	0.040	0.064	0.335	0.302

Table 13.8. Correlation of Serum triglycerides with HDL Cholesterol and Total Cholesterol

Analytes (mg/dl)	Correlation coefficient r (Spearman's rho ρ)		Significance of correlation p	
	Male	Female	Male	Female
Total samples (Male: n=9479; Female: n=6124)				
Serum triglyceride and HDL Cholesterol	-0.254	-0.340	<0.001	<0.001
Serum triglyceride and Total Cholesterol	0.407	0.273	<0.001	<0.001
Age <18 years (Male: n=97; Female: n=86)				
Serum triglyceride and HDL Cholesterol	-0.388	-0.374	<0.001	<0.001
Serum triglyceride and Total Cholesterol	0.402	0.395	<0.001	<0.001
Age 18-25 years (Male: n=210; Female: n=159)				
Serum triglyceride and HDL Cholesterol	-0.193	-0.349	0.005	<0.001
Serum triglyceride and Total Cholesterol	0.521	0.414	<0.001	<0.001
Age 26-50 years (Male: n=3247; Female: n=1921)				
Serum triglyceride and HDL Cholesterol	-0.293	-0.386	<0.001	<0.001
Serum triglyceride and Total Cholesterol	0.352	0.278	<0.001	<0.001
Age >50 years (Male: n=5925; Female: n=3958)				
Serum triglyceride and HDL Cholesterol	-0.243	-0.325	<0.001	<0.001
Serum triglyceride and Total Cholesterol	0.355	0.274	<0.001	<0.001

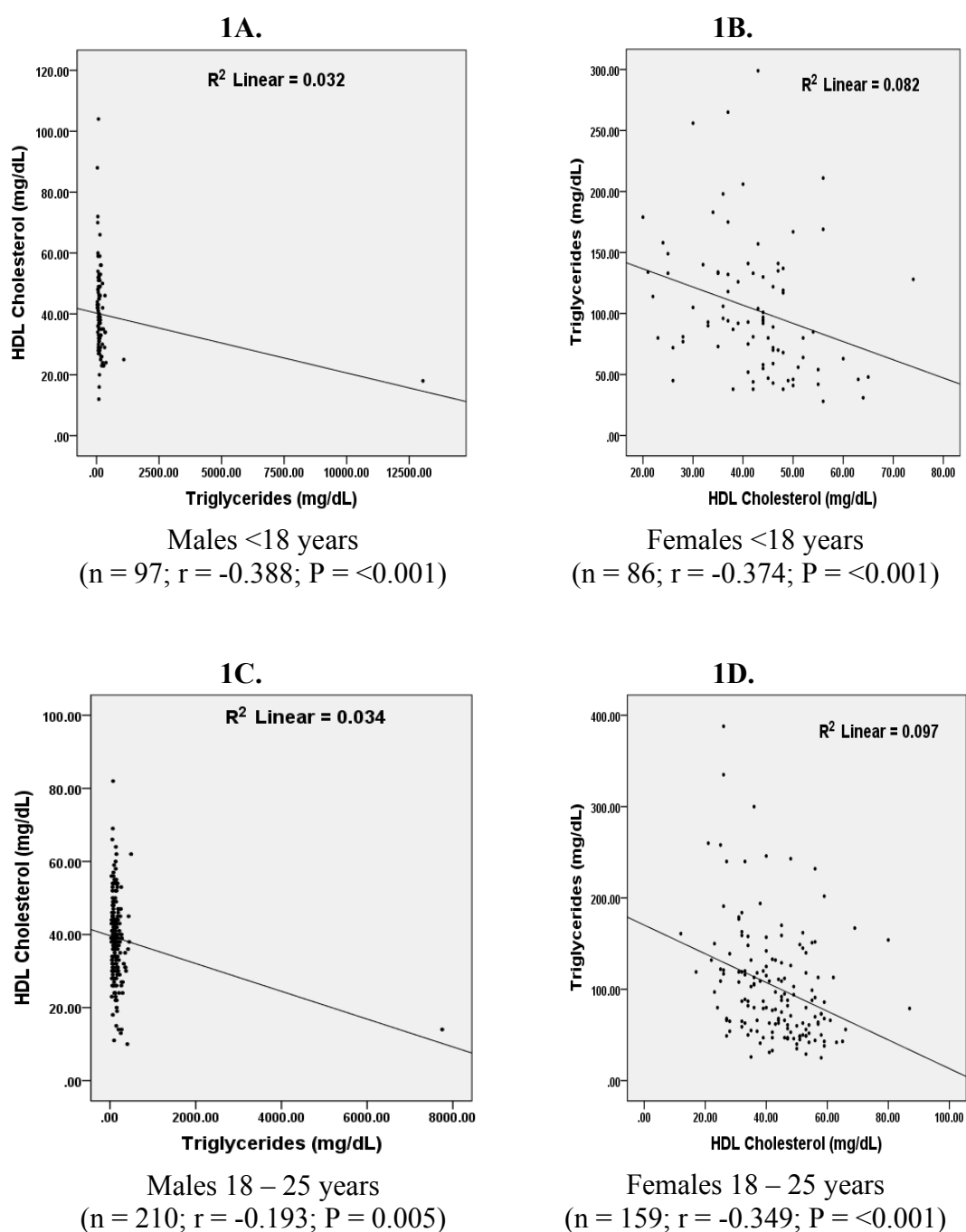
Table 13.9. Correlation between Total Cholesterol and LDL Cholesterol

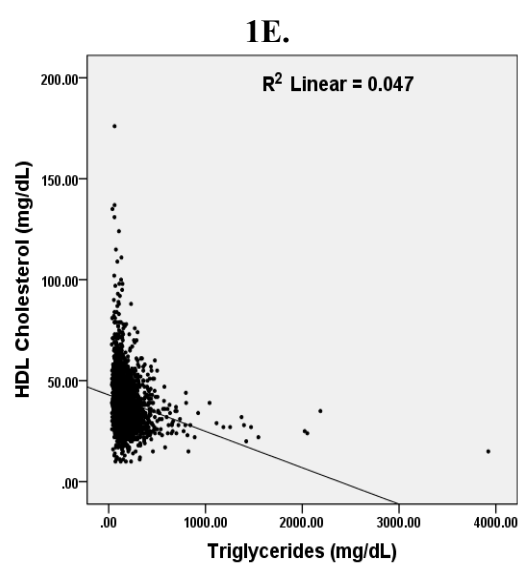
Analytes (mg/dL)	Correlation coefficient r (Spearman's rho ρ)		Significance of correlation p	
	Male	Female	Male	Female
Total samples (Male: n = 9441; Female: n = 6113)				
Total Cholesterol and LDL Cholesterol	0.917	0.935	<0.001	<0.001
Age <18 years (Male: n = 95; Female: n = 86)				
Total Cholesterol and LDL Cholesterol	0.861	0.912	<0.001	<0.001
Age 18-25 years (Male: n = 209; Female: n = 159)				
Total Cholesterol and LDL Cholesterol	0.910	0.916	<0.001	<0.001
Age 26-50 years (Male: n = 3229; Female: n = 1921)				
Total Cholesterol and LDL Cholesterol	0.892	0.918	<0.001	<0.001
Age >50 years (Male: n = 5908; Female: n = 3958)				
Total Cholesterol and LDL Cholesterol	0.921	0.940	<0.001	<0.001

Table 13.10. Correlation between BMI and lipid profile analytes

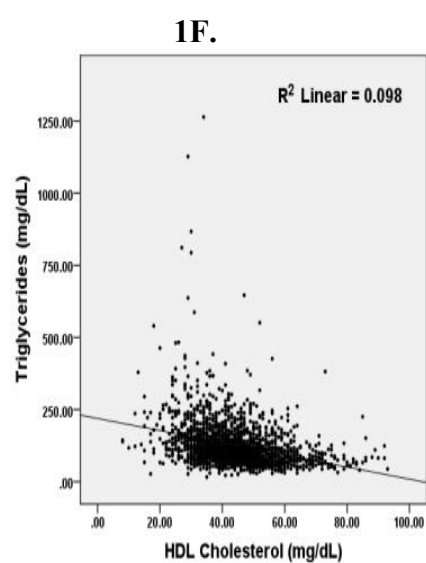
Analytes (mg/dL)	Correlation coefficient r (Spearman's rho ρ)		Significance of correlation p	
	Male	Female	Male	Female
Total adult samples (Male: n = 92; Female: n = 172)				
BMI and Total cholesterol	0.058	0.249	0.583	0.001
BMI and triglycerides	0.264	0.479	0.011	<0.001
BMI and HDL	-0.082	-0.289	0.440	<0.001
BMI and LDL	-0.016	0.246	0.877	0.001
Age 18-25 years (Male: n = 20; Female: n = 42)				
BMI and Total cholesterol	0.169	-0.007	0.475	0.953
BMI and triglycerides	0.461	0.161	0.041	0.308
BMI and HDL	-0.139	-0.262	0.560	0.094
BMI and LDL	-0.144	0.120	0.543	0.450
Age 26-50 years (Male: n = 43; Female: n = 97)				
BMI and Total cholesterol	0.096	0.072	0.542	0.482
BMI and triglycerides	0.141	0.333	0.366	0.001
BMI and HDL	-0.102	-0.333	0.517	0.001
BMI and LDL	0.061	0.081	0.698	0.429
Age >50 years (Male: n = 27; Female: n = 30)				
BMI and Total cholesterol	-0.210	-0.047	0.293	0.804
BMI and triglycerides	0.204	0.056	0.307	0.770
BMI and HDL	-0.210	-0.329	0.294	0.076
BMI and LDL	-0.249	0.058	0.210	0.752

Figure 13.1. Correlations between Serum triglyceride and HDL Cholesterol in different gender-age groups

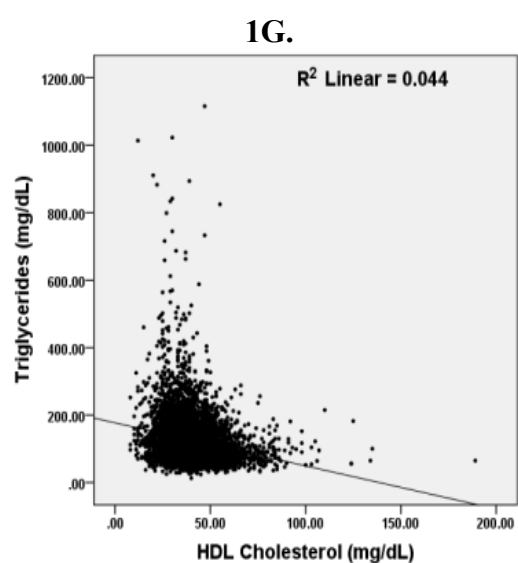




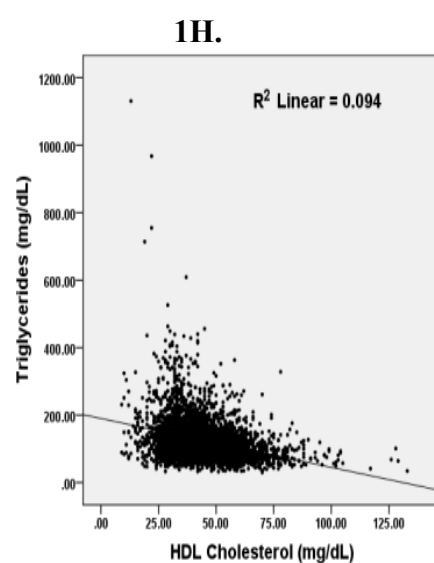
Males 26 – 50 years
(n = 3247; r = -0.293; P = <0.001)



Females 26 – 50 years
(n = 1921; r = -0.386; P = <0.001)



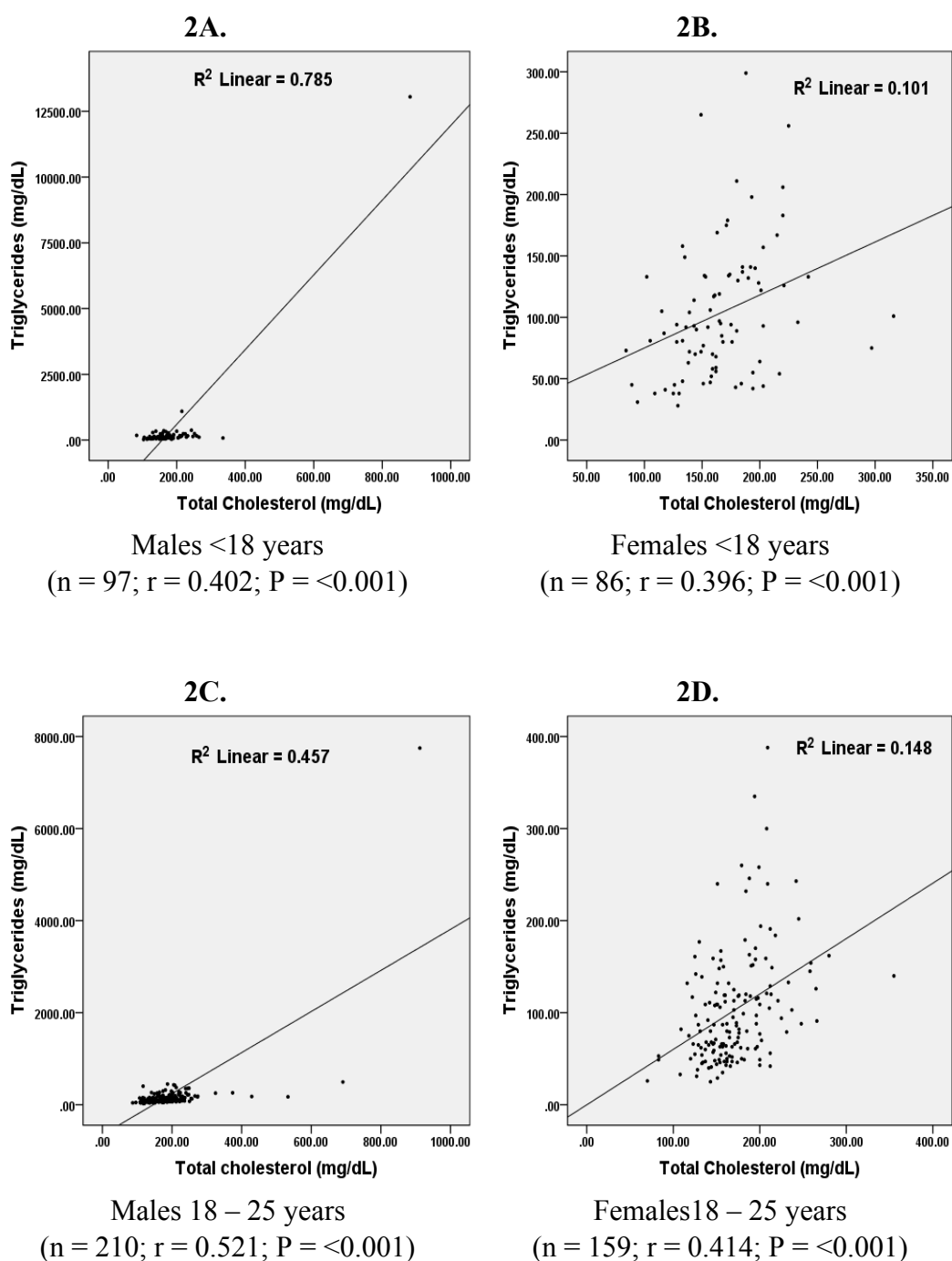
Males >50 years
(n = 5925; r = -0.243; P = <0.001)

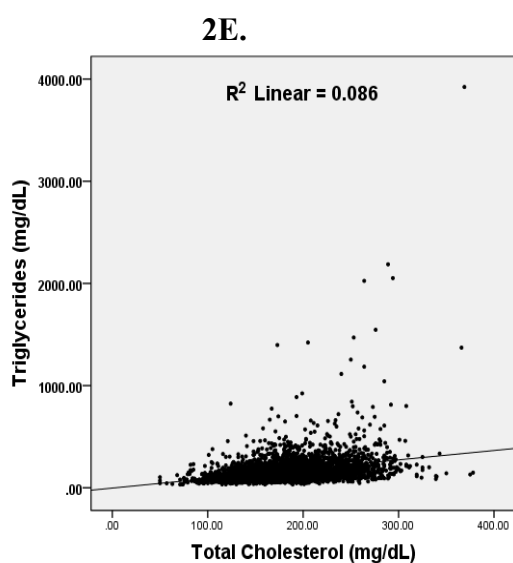


Females >50 years
(n = 3958; r = -0.325; P = <0.001)

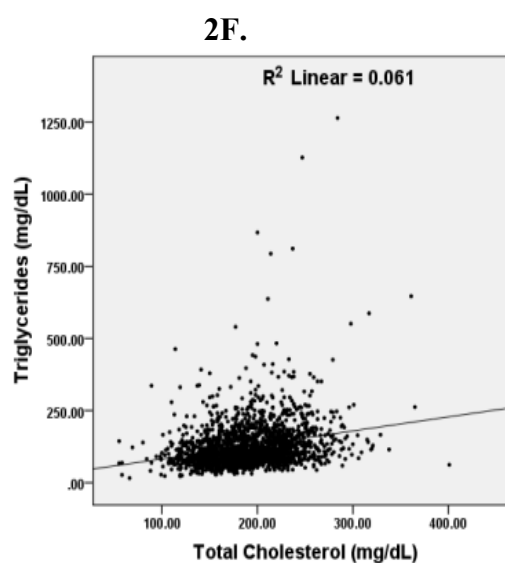
(Figure 13.1 Continuation)

Figure 13.2. Correlations between Serum triglyceride and Total Cholesterol in different gender-age groups

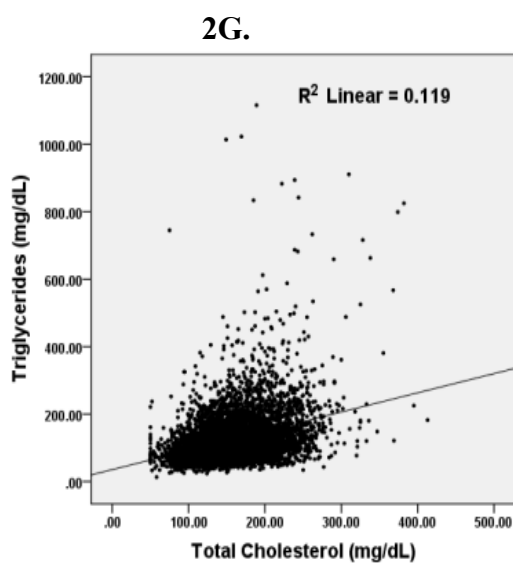




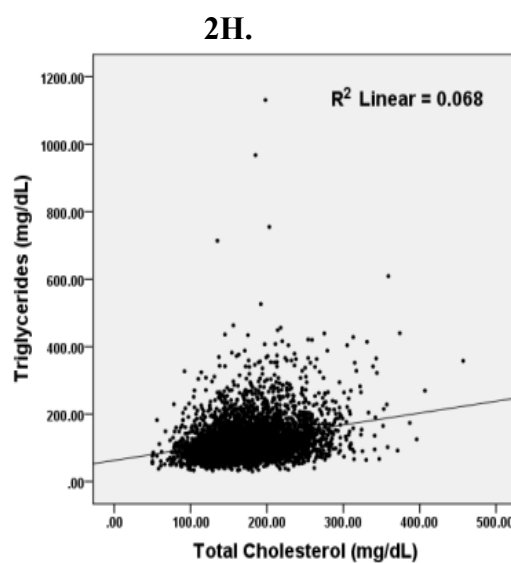
Males 26 – 50 years
(n = 3247; r = 0.352; P = <0.001)



Females 26 – 50 years
(n = 1921; r = 0.278; P = <0.001)



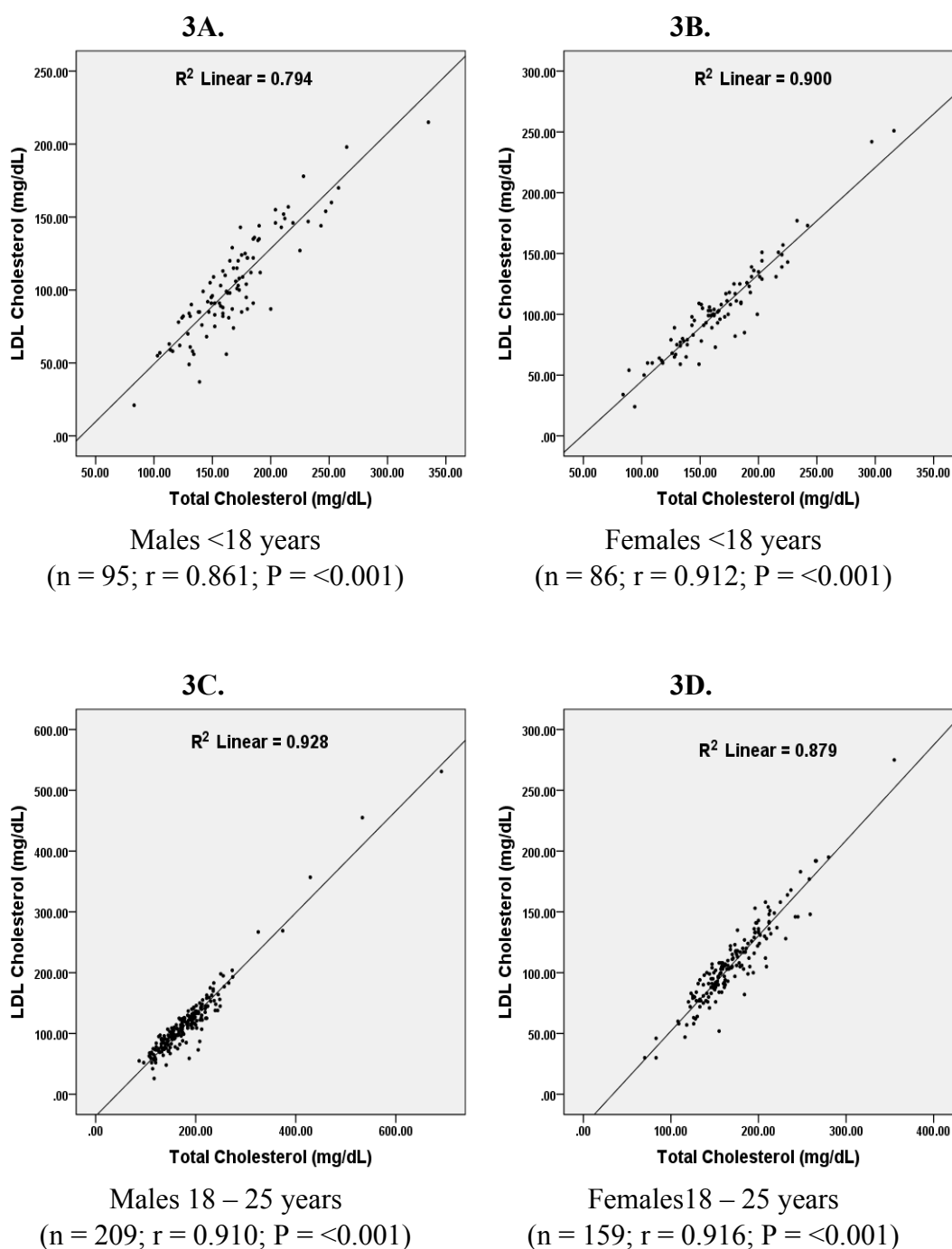
Males >50 years
(n = 5925; r = 0.355; P = <0.001)

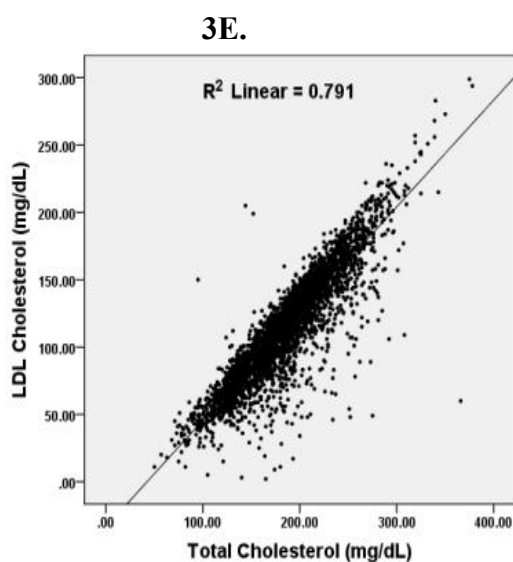


Females >50 years
(n = 3958; r = 0.274; P = <0.001)

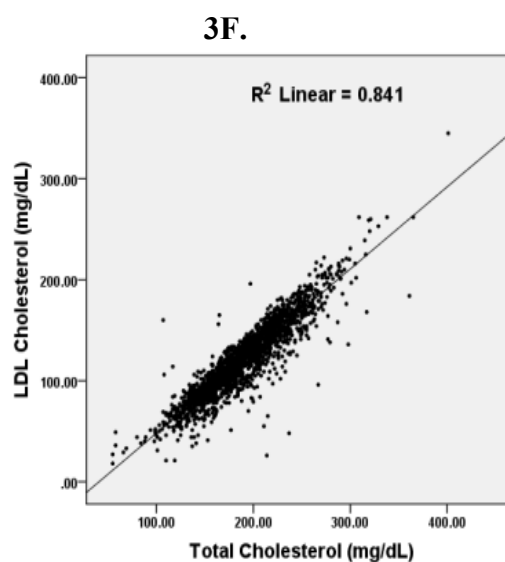
(Figure 13.2. continuation)

Figure 13.3. Correlations between Total Cholesterol and LDL Cholesterol in different gender-age groups

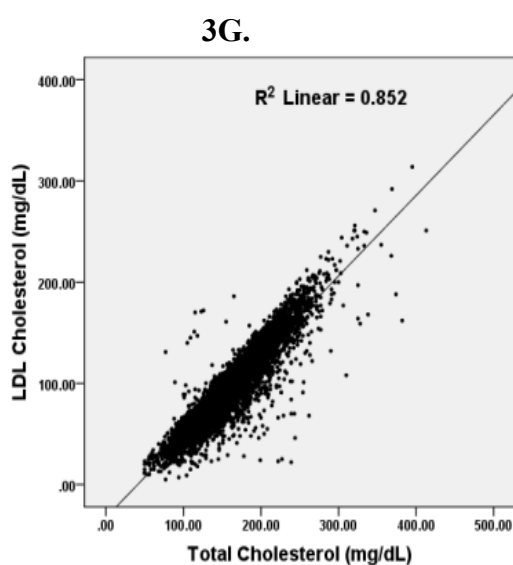




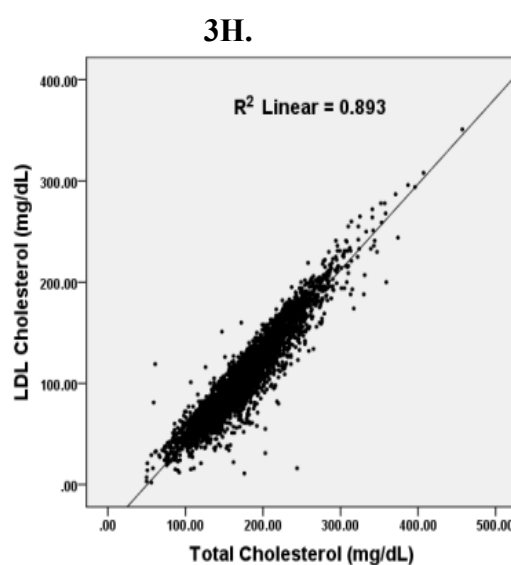
Males 26 – 50 years
(n = 3229; r = 0.892; P = <0.001)



Females 26 – 50 years
(n = 1921; r = 0.918; P = <0.001)



Males >50 years
(n = 5908; r = 0.921; P = <0.001)



Females >50 years
(n = 3958; r = 0.940; P = <0.001)

(Figure.13.3. continuation)

CHAPTER 14

INDIRECT PREVALENCE AND RELATIVE PREVALENCE OF HEALTHY AND DYSLIPIDEMIA CONDITIONS IN ISOLATED AND COMBINED TYPES OF LIPID PROFILE

14.1. Abstract

Introduction: Indirect prevalence of dyslipidemia is the percentage of dyslipidemia samples to the total sample presented at an institution for a particular duration of time.

Objective; To study the Indirect and Relative prevalence of dyslipidemia cases.

Methods: Lipid profile data was collected from Clinical Biochemistry Laboratory continuously for a fixed time period of time. Samples were partitioned according to age and gender. Indirect prevalence and relative prevalence of normal and dyslipidemia cases presented in hospital were studied. This is an Observational Cross-Sectional study of normal and dyslipidemia conditions presented at Amala Institute of Medical Sciences by **Indirect Sampling** method. Chemistry tests were done with Vitros 5.1 FS autoanalyzer from Ortho Clinical Diagnostics (OCD). Statistical Package for Social Sciences (SPSS) software was used for calculating distribution characteristics.

Results: Among all the adult individuals within different gender and age groups studied, dyslipidemia cases were more prevalent in males of the age group 26 – 50 years (83.71%). The females in age group <18 years were found to be the healthiest group without dyslipidemia (33.72%). The prominent dyslipidemia in the age group 18 – 25 years was isolated decrease in HDL Cholesterol with indirect prevalence of 30.82% in females. This is followed by isolated serum triglyceride increase with indirect prevalence of 14.76% in males. In the age group 26 – 50 years, the prominent lipid abnormality was combined LDL Cholesterol and Total Cholesterol increase (23.99% in females). With in age group >50 years, the highly prevalent lipid abnormality in the individuals was isolated HDL Cholesterol decrease (31.25% in males) followed by combined LDL Cholesterol and total cholesterol increase (17.79% in females). Among all dyslipidemia cases, the relative prevalence of isolated decrease in HDL Cholesterol was higher in all gender age groups. The relative prevalence of isolated HDL Cholesterol decrease was highest in females (45.61%) of age <18 years.

Conclusions: Most prevalent dyslipidemia is Isolated HDL- C decrease in males and females of all age groups except 26 – 50 years. In 26 – 50 years males, the most

prevalent dyslipidemia is Isolated TG increase. But in Females, Combined LDL-C and TC increase is the most prevalent dyslipidemia. Second Prevalent Dyslipidemia is isolated TG increase in males and females of <18 yrs, 26 – 50 yrs and in males of 18 – 25 yrs and >50 years. But females of age group 18 – 25 yrs and >50 years has combined TC and LDL-C increase as the second most prevalent dyslipidemia. Triglycerides >885mg/dL is the rare form of dyslipidemia. The prevalence of dyslipidemia is highest in males of 26 – 50 years. The healthiest lipid profile was observed in the age group females <18 years.

14.2. Introduction

Lipid profile data from plasma or serum sample includes Triglycerides (TG), Total Cholesterol (TC), HDL Cholesterol (HDL-C) and LDL Cholesterol (LDL-C). These measurands are commonly assayed in a Clinical Biochemistry Laboratory. The lipid profile analytes are causes of common disease conditions that can be identified very early and corrected to a significant extend. But seldom is the lipid profile data used for further diagnostic and therapeutic purposes. The scope for such diagnostic analysis is much more than that is done regularly in the clinics.

Dyslipidemia is the abnormal level of cholesterol and triglycerides in blood and are the major factors for the development of atherosclerosis, stroke, type 2 diabetes and other cardiovascular diseases (Remaley et al., 2012). **Prevalence** of dyslipidemia conditions is the percentage of each dyslipidemia in the population where the sampling is done. **Relative prevalence** is the percentage of each dyslipidemia condition in the sample population in which the total number of dyslipidemia cases is considered as 100%.

In this study, the **sampling is Indirect**, from a hospital screening database and not directly from the population. The increase in prevalence of dyslipidemia cases is a major public health problem worldwide and it varies according to cultural, socioeconomic and ethnic characteristics.

Dyslipidemias can be due to hyper- or hypo- lipoproteinemia. Hyperlipidemias maybe monogenic or polygenic (Table 14.1.) and has been redefined

on the basis of recent genetic data and lipid concentrations (Johansen et al., 2011; Kathiresan et al., 2011; Talmud et al., 2013; Johansen and Hegele, 2011). Fasting Triglyceride level ≥ 10 mmol/L (≥ 885 mg/dL) is likely to be due to monogenic causes with familial hetero- and homozygous autosomal recessive characteristics. Individuals with triglyceride levels between 1.7 – 10 mmol/L (150 - 885mg/dL) are considered to be the result of multiple genetic and environment effects that are heterozygous with cumulative burden (Mach et al., 2020; Dron and Hegele, 2020). Additive effects of these genes cause higher genetic risk score (Johansen and Hegele, 2012). Family history of the disease states or data analysis in family members may be used to further understand the disease states, its dominant or recessive nature. Non fasting triglyceride concentration increases cardiovascular disease risk and is evident from large, prospective studies (Nordestgaard et al., 2007; Bansal et al., 2007).

The lipoproteins responsible for these conditions are postprandial Triglyceride rich IDL and VLDL, and may not be due to chylomicrons (Norata et al., 2007; Mamo et al., 1998). Non fasting, postprandial remnant cholesterol containing lipoproteins also increase cardiovascular risk (Nordestgaard and Freiberg, 2011).

In a study conducted by the ICMR-INDIAB, the percent prevalence and relative prevalence (n = 2042) for following conditions were: 1) Isolated and Combined Hypercholesterolemia (13.9% & 17.7%); 2) Isolated and Combined Hypertriglyceridemia (29.5%; 37.34%); 3) Isolated and Combined Low HDL Cholesterol (72.3%; 91.5%); 4) High LDL cholesterol (11.8%; 14.93%), respectively. The total of individual prevalence and relative prevalence are >100 , as some individuals have more than one disease state (Joshi et al., 2014).

14.3. Objectives

- a) Collection of fasting lipid profile data from the Clinical Biochemistry Laboratory
- b) Calculation of Indirect Prevalence and Relative Prevalence of dyslipidemia cases presented in the hospital.

14.4. Materials and methods

14.4.1. Research Design

This is an **Observational Cross-Sectional study** of dyslipidemia conditions presented at Amala Institute of Medical Sciences by **Indirect sampling** method. The lipid profile data was studied by:

- A) Lipid profile data collection from Clinical Biochemistry Laboratory.
- B) Grouping of normal and dyslipidemia samples for Indirect Prevalence and Relative Prevalence calculation in the various gender-age groups.

14.4.2. Sample size requirement

Sample size was calculated from an earlier report on prevalence of dyslipidaemia in a multicentric study. From the sample size and prevalence data, the relative prevalence of common dyslipidaemia was calculated.

$$n = \frac{Z^2_{(1-\alpha)}pq}{d^2}$$

d = 20% prevalence; $\alpha = 0.05$; p = 24%; q = 1- p; $Z_{(1-\alpha)}$ value = 1.96; power 80%; n = 304.12.

Samples size rounded off to 304 (Qi et al., 2015). Final sample size n = 15603 (Refer General Materials and Methods)

14.4.3. Selection of Age Groups for the study

Total samples collected include males and females of all gender – age group. This sample was partitioned according to age into four groups, below 18 years (adolescents), 18 to <25 years (young adults), 25 to <50 years (middle age adults) and 50 and above years (senior adults). These ranges of classification were selected from the lipid profile variations with age in the population of study, and occurrence of lipid profile diseases which are grouped into primary and secondary dyslipidemias.

14.4.4. Direct versus Indirect sampling

Indirect sampling was preferred to direct sampling due to the large sample requirements for the different groups including both common and rare lipid profile conditions with abnormal levels (Refer General Materials and Methods).

14.4.5. Sample Collection

The period of sample collection was from September 2018 to March 2022 with a break when hospital was closed due to COVID. For every sample with Lipid profile data collected, its associated other biochemical test 's results values (TSH, glucose, liver function tests, etc.) were also collected from the Clinical Biochemistry Laboratory (Refer general Materials and Methods)

14.4.6. Selection of the Lipid profile data

Lipid profile analytes selected were Total Triglycerides, Total Cholesterol, HDL Cholesterol and LDL Cholesterol. LDL Cholesterol was assayed by direct assay of LDL cholesterol or by calculation by Friedwald equation. $TC = LDL-C + HDL-C + (Total\ TG/5)$ (Remaley et al., 2012)

14.4.7. Assays of Lipid Profile analytes (Refer General Materials and Method)

The cut-off values selected for normal healthy sample were: Total Cholesterol (<200 mg/dL), Triglycerides (<150 mg/dl), LDL Cholesterol (<130 mg/dl) and HDL Cholesterol (40 mg/dL - 59 mg/dL in males, HDL-C 43 mg/dL -59 mg/dL in females (Joshi et al., 2014; Mach et al., 2020, Vitros FS procedure manual).

14.4.8. Indirect and Relative Prevalence

a) Prevalence is the proportion of a population who have a specific characteristic (such as abnormal value, subclinical disorder or a disease) in a given time period. Prevalence of a disease is the number of cases of a disease, number of infected people or number of people with some other attribute present during a particular interval of time. Prevalence may also be called proportion. It is the measure of disease states at a given moment in time. Example, prevalence of Type 2 Diabetes Mellitus globally in

2022 was 830 million (WHO Statistics).

b) Indirect prevalence – it is the prevalence of a particular disease obtained from randomized data collection from an institution such as school, medical college or clinical laboratory.

This data represents individually and approximately the data that is obtained from population. The sample data collection has to be examined to rule out any serious bias.

Indirect and relative prevalence calculation (Sterne and Kirkwood, 2003)

a) Prevalence of dyslipidemia % = $\frac{\text{Number of samples with dyslipidemia}}{\text{Total number of samples collected}} \times 100$

b) Prevalence of isolated/combined dyslipidemia cases %

$$= \frac{\text{Number of samples with isolated/combined dyslipidemia}}{\text{Total number of samples collected}} \times 100$$

c) Relative prevalence of a particular disease condition such as hyper beta lipoproteinemia or increase in LDL-C (%)

$$= \frac{\text{Prevalence of increased LDL-C condition}}{\text{prevalence of dyslipidemia}} \times 100$$

Prevalence calculation should be differentiated with relative prevalence calculated from a histogram or Box-Whisker plot (refer chapter 5 to 12). For example, histogram for distribution of triglyceride may be grouped in to healthy region <150 mg/dL, positively skewed region with moderate prevalence of hypertriglyceridemia (which is the sample distributed above healthy concentration to last positively skewed sample). The outlier regions are represented by high concentration of triglyceride which is of rare occurrence. The moderately prevalent region represents multifactorial disorders. The rarely prevalent condition of outlier region represents mainly inherited metabolic disorders which are single gene defects and can be evaluated by pedigree analysis. The multifactorial disorders are evaluated by pedigree analysis of data distribution.

14.4.9. Grouping of samples for prevalence calculation

14.4.9.1. Total sample collected was partitioned in to different **groups based on gender** into:

- a) Total males
- b) Total females

14.4.9.2. Total males and females were further **grouped as adults and adolescents**:

- a) Males <18 years
- b) Males $\geq$ 18 years
- c) Females <18 years
- d) females $\geq$ 18 years

14.4.9.3. The males and females were further **grouped into four age groups**:

- a) Males <18 years
- b) Males 18 – 25years of age
- c) Males 26 – 50 years of age
- d) Males >50 years of age
- e) Females <18 years
- f) Females 18– 25myears of age
- g) Females 26 – 50 years of age
- h) Females >50 years of age

14.4.10. Lipid Profile Groups

The lipid profile in male and female samples in Total, >18 years, and in all the four age groups were further subdivided, **according to concentration of lipids**, into:

I. Normal Lipid Profile Groups (a to e)

- a) **All analytes Normal Lipid profile group:** Samples with serum Triglyceride <150 mg/dL, Total Cholesterol <200 mg/dL, HDL-C: 40 – 59 mg/dL in males and HDL-C: 43– 59 mg/dL in females, LDL-C <130 mg/dL. All lipid profile parameters are within Normal Reference Interval.
- b) **Normal Triglyceride (<150 mg/dL) group:** samples with serum Triglyceride <150 mg/dL and all other lipid profile analytes may be either of Normal or Abnormal concentrations.
- c) **Normal Total Cholesterol (<200 mg/dL) group:** samples with Total Cholesterol <200 mg/dL and all other lipid profile analytes may be either in Normal or in Abnormal concentrations.
- d) **Normal HDL Cholesterol group:** samples with HDL-C (40 – 59 mg/dL in males and 43– 59 mg/dL in females) and all other lipid profile analytes may be either in Normal or at abnormal concentration.
- e) **Normal LDL Cholesterol group:** samples with LDL-C <130 mg/dL and all other lipid profile analytes may be either in normal or in abnormal concentration.

II. Isolated, Increased or Decreased Triglyceride or Cholesterol Groups (f to k)

- f) **Serum Triglyceride >885 mg/dL group:** Rare diseases caused by single gene defects.
- g) **Isolated serum Triglyceride increase group:** samples with serum Triglyceride values ≥ 150 mg/dL but, Total Cholesterol and LDL-C values are in their normal reference range.
- h) **Isolated Total Cholesterol increase group:** samples with Total Cholesterol values ≥ 200 mg/dL but, serum Triglycerides, LDL-C values are in their normal reference range.

- i) **Isolated HDL Cholesterol increase group:** samples with HDL Cholesterol values ≥ 60 mg/dL but, all other lipid profile analytes are in their normal reference range.
- j) **Isolated HDL Cholesterol decrease group:** samples with HDL Cholesterol values < 40 mg/dL in males and < 43 mg/dL in females, but all other lipid profile analytes are in their Normal Reference range.
- k) **Isolated LDL Cholesterol increase group:** samples with only LDL Cholesterol values ≥ 130 mg/dL but, serum Triglycerides and Total cholesterol values are in their normal reference range.

III. Combined Increased or Decreased Triglyceride or Cholesterol groups (l to q)

- l) **Combined HDL Cholesterol decrease group:** Samples with HDL Cholesterol values < 40 mg/dL in males and < 43 mg/dL in females but, one or more of the other lipid profile analytes may be in the abnormal concentration.
- m) **Combined serum Triglyceride and Total Cholesterol increase (TG ≥ 150 mg/dL; TC ≥ 200 mg/dL) group:** Samples with serum Triglycerides ≥ 150 mg/dL and Total Cholesterol ≥ 200 mg/dL, but LDL-C concentrations are in their Normal Reference Range. HDL Cholesterol may or may not be in the Reference range.
- n) **Combined LDL Cholesterol and Total Cholesterol increase (LDL-C ≥ 130 mg/dL; TC ≥ 200 mg/dL) group:** Samples with LDL-Cholesterol ≥ 130 mg/dL and Total Cholesterol ≥ 200 mg/dL but, serum Triglycerides values are in their Normal Reference Range. HDL Cholesterol may or may not be in the Reference range.
- o) **Combined LDL-C ≥ 130 mg/dL; TC ≥ 200 mg/dL; TG ≥ 150 mg/dL increase group:** Samples with LDL Cholesterol ≥ 130 mg/dL, Total Cholesterol ≥ 200 mg/dL and serum Triglyceride ≥ 150 mg/Dl. HDL Cholesterol may or may not be in the Reference range.

- p) **Combined LDL Cholesterol and serum Triglyceride (LDL-C ≥ 130 mg/dL; TG ≥ 150 mg/dL) increase group:** Samples with LDL Cholesterol ≥ 130 mg/dL, serum Triglyceride ≥ 150 mg/dL but Total Cholesterol values are in their normal Reference range.
- q) **Combined serum Triglyceride increase and HDL-C decrease (TG ≥ 150 ; HDL-C: < 40 mg/dL in males and < 43 mg/dl in females) group:** Samples with serum triglyceride ≥ 150 mg/dL, HDLC: < 40 mg/dL in males and < 43 mg/dl in females. Total Cholesterol and LDL cholesterol are in normal reference range.

14.5. Results

Various serum lipids were increased in isolation or in combination with other lipids.

14.5.1. The number of samples in Total and in Partitioned groups.

14.5.1.1 In Total samples

Data analyses of 15603 lipid profile samples were done. Out of total 15603 samples; there were 183 samples in the age group < 18 years and there were 15420 adult samples of age ≥ 18 years. There were 97 male samples and 86 female samples < 18 years of age.

14.5.1.2. Samples in Partitioned Groups

Out of 15420 adult samples of age ≥ 18 years, there were 9382 adult male samples and 6038 adult female samples. (Table 14.2)

There were 369 samples between age group 18 – 25 years. Out of which, there were 210 male samples and 159 female samples. The number of samples between 26 – 50 years was 5168. Out of which, there were 3247 male samples and 1921 female samples in the age group 26 – 50 years. The number of samples in age group > 50 years was 9883. Out of which, there were 5925 male samples and 3958 female samples. (Table 14.2)

14.5.2. Number of samples in normal lipid profile group

14.5.2.1. In Total samples

Total male and female samples were 9479 and 6124, respectively. The number of male samples with normal lipid profile was 2159, and there were 1341 female samples with normal lipid profile values (Table 14.3).

14.5.2.2. Samples in different age groups

There were 28 male samples and 29 female samples with normal lipid profile in the samples age group **<18 years**. There were 52 male samples and 50 female samples with normal lipid profile in the samples age group **18 – 25 years**. There were 529 male samples and 362 female samples with normal lipid profile in the samples age group **26 – 50 years**. There were 1550 male samples and 900 female samples with normal lipid profile in the samples age group **>50 years** (Table 14.3).

14.5.3. Number of samples within the isolated Dyslipidemia groups (Table 14.3)

14.5.3.1. In Total samples (Males: n = 9479; Females: n = 6124)

In the total sample there were 1737 male samples and 790 female samples with isolated serum **Triglyceride increase**. There were 108 male samples and 130 female samples with isolated increase in **Total Cholesterol** values. Isolated increase in **LDL Cholesterol** were 310 male and 221 female samples. There were 292 male samples and 375 female samples within the **isolated HDL Cholesterol increase** group. There were 2478 male and 1362 female samples within the **isolated HDL cholesterol decrease** group (Table 14.3).

14.5.3.2. In Groups Partitioned according to age

14.5.3.2.a. In <18 years age group (Males: n = 97; Females: n = 86)

The number of male and female samples with **isolated serum Triglyceride** increase in the age group <18 years was 16 and 8, respectively. There were no male and female sample within the **isolated Total Cholesterol** increase group. The number of male and female samples with **isolated LDL Cholesterol** increase in the age group <18 years was 5 and 3, respectively. Within the **isolated HDL Cholesterol increase** group, there were 4 male and 5 female samples. There were 24 male and 26 female samples in **isolated HDL cholesterol decrease** group (Table 14.3).

14.5.3.2.b. In 18 – 25 years age group (Males: n = 210; Females: n = 159)

The number of male and female samples with **isolated serum Triglyceride** increase in the age group 18 – 25 years was 31 and 18, respectively. There were 4 male samples and 1 female sample within **isolated Total Cholesterol** increase group. The number of male and female samples with **isolated LDL Cholesterol** increase in the age group 18 – 25 years was 8 and 7, respectively. Within the **isolated HDL Cholesterol increase** group, there were only 2 samples of the male and female groups. There were 56 male and 49 female samples in **isolated HDL Cholesterol decrease** group (Table 14.3).

14.5.3.2.c. In 26 – 50 years age group (Males: n = 3247; Females: n = 1921)

There were 690 male samples and 219 female samples with isolated serum Triglyceride increase in the samples age group 26 – 50 years. In isolated Total cholesterol increase group, there were 43 male and 44 female samples. There were 114 male and 103 female samples in the isolated LDL increase group. There were 65 male and 69 female samples in isolated HDL Cholesterol increase group. The number of samples in isolated HDL Cholesterol decrease group in male and female samples was 546 and 387, respectively (Table 14.3).

14.5.3.2.d. In >50 years age group (Males: n = 5925; Females: n = 3958)

There were 1000 male samples and 545 female samples with isolated serum triglyceride increase in the samples of the age group >50 years. In isolated Total Cholesterol increase group, there were 61 male and 85 female samples. There were 183 male and 108 female samples in the isolated LDL increase group. There were 221 male and 299 female samples in isolated HDL Cholesterol increase group. The number of samples in isolated HDL Cholesterol decrease group in male and female samples was 1852 and 900, respectively (Table 14.3).

14.5.4. Number of samples within the combined Dyslipidemia groups

14.5.4.1. In Total samples (Males: n = 9479; Females: n = 6124)

There were 345 male and 166 female samples in the combined serum triglyceride and total cholesterol increase ($TG \geq 150$; $TC \geq 200$) group. The number of samples in combined LDL cholesterol and Total cholesterol increase ($LDL \geq 130$; $TC \geq 200$) group in male and female samples were 1101 and 1196, respectively. The number of samples in combined LDL cholesterol and serum triglyceride increase ($LDL \geq 130$; $TG \geq 150$) group was 34 and 18 in males and females, respectively. There were 915 male and 525 female samples in the combined LDL cholesterol, Total cholesterol and serum triglyceride increase ($LDL \geq 130$; $TC \geq 200$; $TG \geq 150$) group. There were 1273 male and 590 female samples in the combined HDL cholesterol decrease and serum triglyceride increase ($HDL:M < 40$; $F < 43$; $TG \geq 150$) group (Table 14.3).

14.5.4.2. In Groups Partitioned according to age

14.5.4.2.a. In <18 years age group (Males: n = 97; Females: n = 86)

The number of male and female samples with combined serum Triglyceride and Total Cholesterol increase ($TG \geq 150$; $TC \geq 200$) in the age group <18 years was 4 and 1, respectively. There were 8 male samples and 10 female samples within combined Total cholesterol and LDL cholesterol increase ($LDL \geq 130$; $TC \geq 200$) group. There were 1 male samples within the combined LDL cholesterol and serum

triglyceride increase ($\text{LDL} \geq 130$; $\text{TG} \geq 150$) group. The number of male and female samples in combined LDL cholesterol, Total cholesterol and serum triglyceride increase ($\text{LDL} \geq 130$; $\text{TC} \geq 200$; $\text{TG} \geq 150$) group was 7 in males and 4 in females. Within the combined HDL decrease and serum triglyceride increase ($\text{HDL: M} < 40$; $\text{F} < 43$; $\text{TG} \geq 150$) group, there were 15 male and 5 female samples (Table 14.3).

14.5.4.2.b. In 18 – 25 years age group (Males: n = 210; Females: n = 159)

The number of male and female samples with combined serum triglyceride and total cholesterol increase ($\text{TG} \geq 150$; $\text{TC} \geq 200$) in the age group 18 – 25 years was 8 and 4, respectively. There were 23 male samples and 21 female samples within combined Total cholesterol and LDL cholesterol increase ($\text{LDL} \geq 130$; $\text{TC} \geq 200$) group. There were two male samples within the combined LDL cholesterol and serum triglyceride increase ($\text{LDL} \geq 130$; $\text{TG} \geq 150$) group. The number of male and female samples in combined LDL cholesterol, Total cholesterol and serum triglyceride increase ($\text{LDL} \geq 130$; $\text{TC} \geq 200$; $\text{TG} \geq 150$) group was 24 in males and 7 in females. Within the combined HDL decrease and serum triglyceride increase ($\text{HDL:M} < 40$; $\text{F} < 43$; $\text{TG} \geq 150$) group, there were 22 male and 13 female samples (Table 14.3).

14.5.4.2.c. In 26 – 50 years age group (Males: n = 3247; Females: n = 1921)

There were 201 male samples and 66 female samples with combined serum triglyceride and Total cholesterol increase ($\text{TG} \geq 150$; $\text{TC} \geq 200$) group in the samples age group 26 – 50 years. In combined LDL cholesterol and Total cholesterol increase ($\text{LDL} \geq 130$; $\text{TC} \geq 200$) group, there were 497 male and 461 female samples. There were 20 male and 10 female samples in the combined LDL cholesterol and serum triglyceride increase ($\text{LDL} \geq 130$; $\text{TG} \geq 150$) group. There were 542 male and 200 female samples in combined LDL cholesterol, Total cholesterol and serum triglyceride increase ($\text{LDL} \geq 130$; $\text{TC} \geq 200$; $\text{TG} \geq 150$) group. The number of samples in combined HDL decrease and serum triglyceride increase ($\text{HDL:M} < 40$; $\text{F} < 43$; $\text{TG} \geq 150$) group in male and female samples was 522 and 173, respectively (Table 14.3).

14.5.4.2.d. In >50 years age group (Males: n = 5925; Females: n = 3958)

There were 132 male samples and 95 female samples with combined serum triglyceride and Total cholesterol increase ($TG \geq 150$; $TC \geq 200$) in the samples age group >50 years. In combined LDL cholesterol and Total cholesterol increase ($LDL \geq 130$; $TC \geq 200$) group, there were 573 male and 704 female samples. There were 11 male and 8 female samples in the combined LDL cholesterol and serum triglyceride increase ($LDL \geq 130$; $TG \geq 150$) group. There were 342 male and 314 female samples in combined LDL cholesterol, Total cholesterol and serum triglyceride increase ($LDL \geq 130$; $TC \geq 200$; $TG \geq 150$) group. The number of samples in combined HDL Cholesterol decrease and serum triglyceride increase ($HDL: M < 40$; $F < 43$; $TG \geq 150$) group in male and female samples was 714 and 399, respectively (Table 14.3).

To summarize, there were more number males than females in each of the four age groups. There were more males with isolated triglyceride increase in the total sample and in different age groups. The maximum number of samples with dyslipidemia was in HDL Cholesterol decrease group. It was difficult to make strong conclusions from the number of samples from normal and abnormal lipid profile groups, as the sample number varies according to age, gender and whether it is isolated/combined dyslipidemia. Therefore, the percentage indirect prevalence and relative prevalence were calculated to make more strong and more defined conclusion about the prevalence of dyslipidemia.

14.5.5. Indirect Prevalence of healthy and unhealthy lipid profile variables (Table 14.4).

14.5.5.1. Indirect prevalence of Normal or healthy Lipid profile

The female samples in the age group <18 years were found to be with the highest percentage (33.72%) of healthy samples with no lipid abnormality. The male samples in the age group 26 – 50 years were found to be the group with least percentage (16.29%) of healthy samples with no lipid abnormality (Table 14.4).

In the age group 18 – 25 years, 31.45% of females and 24.76% males had normal lipid profile. Among all adult age groups, females aged 18 – 25 had the highest percentage (31.45%) of healthy lipid profiles. In the age group >50 years, about 26.16% males and 22.74% females were with Normal lipid profiles (Table 14.4)

14.5.5.2. Indirect Prevalence of Dyslipidemia

Among all four age groups studied, dyslipidemia cases were more prevalent in males of the age group 26 – 50 years (83.71%). Among the two adult age groups <50 years, the dyslipidemia samples were more in males of the age group 18 – 25 years (75.24%) and in the age group 26 – 50 years (83.71%), compared to the females of the same age. But, in the individuals >50 years of age, dyslipidemia was more prevalent among the females (77.26%) than that of the males (Table 14.4).

14.5.5.2.a. Isolated Dyslipidemia

Among all dyslipidemia groups, **isolated HDL Cholesterol decrease** was the most prevalent individual lipid disorder and it was higher in male samples of the age group >50 years (31.25%).

Isolated increase in Triglyceride was higher in males than in females in all age groups. Isolated increase in serum Triglyceride was highest in males within age group 26 – 50 years (21.25%) and least in female samples within age group 18 – 25 years (11.32%) (Table 14.4)

Prevalence of samples with **serum Triglyceride >885mg/dL** was less than 1% in adults.

Isolated Total Cholesterol increase (2.29%) and **isolated LDL Cholesterol increase** (5.36%) was most prevalent in female samples within the age group 26 – 50 years. **Isolated HDL increase** was highest in female samples in all the four age groups. Isolated HDL cholesterol increase was highest in female samples within age group >50 years (7.55%) and least in males in the age group 18 – 25 years (0.95%) (Table 14.4).

14.5.5.2.b. Combined Dyslipidemia

Highest indirect prevalence of combined serum **Triglyceride and Total Cholesterol** was among male samples in age group 26 – 50 years (6.19%). Combined LDL cholesterol and Total cholesterol increase was prevalent in females in all age groups with highest percentage in the age group 26 – 50 years (23.99%) (Table 14.4). There were less than 1% of samples with combined LDL cholesterol and serum

triglyceride increase in both male and females in all adult age groups (Table 14.4).

The prevalence of combined serum **Triglyceride, Total Cholesterol and LDL Cholesterol** increase was **higher in males** in age group 26 – 50 years (16.69%) and in age group 18 – 25 years (11.43%). But >50 years of age, combined serum Triglyceride, Total Cholesterol and LDL Cholesterol increase was **higher in females** (7.93%). **Combined HDL decrease and serum Triglyceride increase** was more prevalent in male samples in all age groups and was highest in males in age group 26 – 50 years (16.08%) (Table 14.4).

14.5.6. Indirect prevalence of dyslipidemia in total sample and in adults ≥ 18 years

Indirect prevalence of dyslipidemia cases in total sample ($n = 15603$) was 77.57%. Indirect prevalence of dyslipidemia was 77.22% and 78.1% in total male and female samples respectively.

In total adult samples of age ≥ 18 years ($n = 15420$), the indirect prevalence of dyslipidemia was 77.67%. the indirect prevalence of dyslipidemia in male and female samples of age ≥ 18 years was 77.29% and 78.27%, respectively (Table 14.5)

14.5.7. Relative Prevalence of different dyslipidaemia in the Total Dyslipidaemia sample

Total numbers of samples collected were 15603. Out of this, the number of adult (≥ 18 years) individuals with abnormal lipid profiles was 11927. Relative prevalence of individuals in different age groups with at least one abnormality in lipid profile parameters (excluding the Normal groups), were grouped into different dyslipidaemia groups. Relative Prevalence of different dyslipidaemia conditions in the total dyslipidaemia sample population was studied.

14.5.7.1. Relative Prevalence of different dyslipidaemia in gender-age groups (Table 14.6).

a) Group <18 years (abnormal samples, n = 183)

The most prevalent lipid abnormality was isolated HDL decrease (45.61% in females) followed by isolated serum triglyceride increase (23.19% in males) in the age group <18 years.

b) In age group 18 – 25 years (abnormal samples, n = 267)

The most prevalent lipid abnormality was isolated HDL decrease (44.95% in females) followed by isolated serum triglyceride increase (19.62% in males) in the age group 18 – 25 years.

c) In age group 26 – 50 years (abnormal samples, n = 4227)

The most prevalent lipid abnormality in 26 – 50 years age group, was combined LDL cholesterol and Total cholesterol increase (29.57% in females). The isolated serum triglyceride increase is the second most prevalent lipid disorder (25.39% in males) in the same age group.

d) In age group >50 years (abnormal samples, n=7433)

The most prevalent lipid abnormality was isolated HDL decrease (42.33% in males) followed by combined LDL cholesterol and total cholesterol increase (23.02% in males) in the >50 years age group.

14.6. Discussions

Approximately 84% of general population in age group between 26 – 50 years, covered in this study have abnormalities in at least one of the lipid profile parameters analyzed.

Sample number increased with advancing age in both men and women. The higher number of hospital visits at older ages contributed to this trend. There were more male samples in all the four age groups indicating increased hospital visits among males.

Isolated triglyceride increase was found to be high in males of all four age groups compared to females. The increase in triglycerides might be inversely related to decrease in HDL cholesterol. Therefore, the isolated decrease in HDL cholesterol was found to be highest in males above 50 years. These results indicated the inverse relationship between triglycerides and HDL cholesterol.

The combined increase of LDL cholesterol and Total cholesterol was higher in females of all age groups indicating the direct correlation of LDL cholesterol with total cholesterol. Combined HDL cholesterol decrease associated with TG increase was higher in males compared to females of all age groups.

Combined triglyceride and total cholesterol increase was higher in males compared to females below 50 years and nearly equal in males and females above 50 years. Isolated LDL Cholesterol increase is more in females below 50 years but in the age group >50 years, it was higher in males.

Though atherosclerosis is more common in males below 50 years, there was no general pattern of increase or decrease in above pro atherosclerotic lipids. These results indicated that lipids alone are not predisposing factors of atherosclerosis. There are other factors such as hsCRP, Oxidative stress, dietary habits, exercise and stress may also influence atherosclerosis (Remaley et al., 2012).

14.7. Conclusions

Most prevalent dyslipidemia is Isolated HDL- C decrease in males and females of all age groups except 26 – 50 years. In 26 – 50 years males, the most prevalent dyslipidemia is Isolated TG increase. But in Females, Combined LDL-C and Total Cholesterol increase is the most prevalent dyslipidemia. Second Prevalent Dyslipidemia is isolated TG increase in males and females of <18 yrs, 26 – 50 yrs and in males of 18 – 25 yrs and >50 years. But females of age group 18 – 25 yrs and >50 years has combined TC and LDL-C increase as the second most prevalent dyslipidemia. Triglycerides >885mg/dL is the rare form of dyslipidemia. The prevalence of dyslipidemia is highest in males of 26 – 50 years. The healthiest lipid profile was observed in the age group females <18years.

Table 14.1. The different dyslipidemia, their Fredrickson phenotype, altered lipids, lipoproteins, their genetic basis, major clinical observations and interpretations.

Hyperlipidemia/ Hyper- Lipoproteinemia	Fredrickson hyper lipo-proteinemia Phenotype	Lipids altered	Lipoproteins altered, Major clinical symptoms	Genetic basis, Family history (f/h)
Familial hyper- chylomicronemia	Type 1	TG>10 mmol/L (885 mg/dl)	Chylomicrons increased Milky plasma. Tendon xanthomas	f/h monogenic autosomal recessive, h/o pancreatitis
Familial hyper- cholesterolemia	Type 2A	TC>200 mg/dl LDL>130mg/dl	LDL increased. Risk for atherosclerosis & coronary disease. Tendon xanthoma	Monogenic, autosomal codominant, due to LDL receptor or ligand (apoB) defect or factors influencing these, f/h of heart disease
Combined hyper- lipoproteinemia	Type 2B	TC>200 mg/dl, TG>10 mmol/l	VLDL & LDL increased.	Polygenic
Dysbeta- lipoproteinemia	Type 3	TC>200 mg/dl, TG>10 mmol/l	IDL Increased. Palmar Xanthomas	Polygenic
Primary & Secondary hyper TG	Type 4	TG>10 mmol/l	VLDL Increased. Diabetes, obesity Hypothyroidism. Risk for pancreatitis	Polygenic. f/h of diabetes type 2, obesity, hypothyroidism
Mixed Hyper- Triglyceridemia	Type 5	TC>200 mg/dl, TG> 10mmol/l	VLDL and Chylomicrons increased	Polygenic

Table 14.2. Total samples, in the four age groups and the gender groups.

Groups based on age	Gender		Total (n)
	Males(n)	Females(n)	
Samples, age <18 years	97	86	183
Samples, age 18-25 years	210	159	369
Samples, age 26-50 years	3247	1921	5168
Samples, age >50 years	5925	3958	9883
Total adult samples	9479	6124	15603

Table.14.3. Number of samples in normal and abnormal lipid profile groups

Groups	Total samples (n = 15603)		Samples, age <18 years (n = 183)		Samples, age 18-25 years (n = 369)		Samples, age 26-50 years (n = 5168)		Samples, age >50 years (n = 9883)	
	M	F	M	F	M	F	M	F	M	F
Gender										
Total	9479	6124	97	86	210	159	3247	1921	5925	3958
Normal Lipid profile	2159	1341	28	29	52	50	529	362	1550	900
Normal, TG <150	6448	4625	69	73	145	130	1794	1426	4440	2996
TG > 885	23	4	2	0	1	0	15	2	5	2
Isolated, TG ≥150	1737	790	16	8	31	18	690	219	1000	545
Combined, TG ≥150; TC ≥200	345	166	4	1	8	4	201	66	132	95
Normal, TC <200	7010	4107	78	71	151	126	1964	1150	4817	2760
Isolated, TC ≥200	108	130	0	0	4	1	43	44	61	85
Normal, LDL <130	7081	4153	74	69	152	124	2056	1142	4799	2818
Isolated, LDL ≥130	310	221	5	3	8	7	114	103	183	108
Combined, LDL ≥130; TC ≥200	1101	1196	8	10	23	21	497	461	573	704
Combined, LDL ≥130; TG ≥150	34	18	1	0	2	0	20	10	11	8

Groups	Total samples (n = 15603)		Samples, age <18 years (n = 183)		Samples, age 18-25 years (n = 369)		Samples, age 26-50 years (n = 5168)		Samples, age >50 years (n = 9883)	
	M	F	M	F	M	F	M	F	M	F
Combined, LDL$\geq$130; TC$\geq$200; TG$\geq$150	915	525	7	4	24	7	542	200	342	314
Normal, HDL: M: 40-59; F: 43-59	3905	2499	38	40	90	74	1341	784	2436	1601
HDL Increase: HDL $\geq$ 60	521	707	6	5	7	8	156	192	352	502
Isolated HDL Increase: HDL $\geq$ 60	292	375	4	5	2	2	65	69	221	299
HDL Decrease: HDL: M <40; F < 43	5053	2918	53	41	113	77	1750	945	3137	1855
Isolated HDL Decrease: HDL: M <40; F < 43	2478	1362	24	26	56	49	546	387	1852	900
Combined, HDL:M <40; F<43; TG $\geq$ 150	1273	590	15	5	22	13	522	173	714	399

Table 14.3 (continuation)

Table.14.4. Indirect Prevalence of healthy and unhealthy (isolated and combined) lipid profile variables in males and females of the sample population. (P=Prevalence, S No: =Sample Number)

Analytes (Cutoff-mg/dl)	Samples, age <18 years (n = 183)		Samples, age 18-25 years (n=369)		Samples, age 26-50 years (n=5168)		Samples, age >50 years (n=9883)	
	P = S No: / Total sample Age <18 yrs (%)		P = S No: / Total sample Age 18-25 yrs (%)		P = S No: / Total sample Age 26-50 yrs (%)		P = S No: / Total sample Age >50 yrs (%)	
	Male (n = 97)	Female (n = 86)	Male (n=210)	Female (n=159)	Male (n=3247)	Female (n=1921)	Male (n=5925)	Female (n=3958)
Total TG								
Isolated, TG ≥ 150	16.49%	9.30%	14.76%	11.32%	21.25%	11.40%	16.88%	13.77%
TG > 885	2.06%	0	0.48%	0	0.46%	0.10%	0.08%	0.05%
Combined, TG ≥ 150; TC ≥ 200	4.12%	1.16%	3.81%	2.51%	6.19%	3.43%	2.22%	2.40%
TC								
Isolated, TC ≥ 200	0	0	1.90%	0.63%	1.32%	2.29%	1.03%	2.15%
LDL- C								
Isolated, LDL ≥ 130	5.15%	3.48%	3.81%	4.40%	3.51%	5.36%	3.09%	2.73%
Combined, LDL ≥ 130; TC ≥ 200	8.24%	11.62%	10.95%	13.21%	15.31%	23.99%	9.67%	17.79%
Combined, LDL ≥ 130; TG ≥ 150	1.03%	0	0.95%	0	0.61%	0.52%	0.18%	0.20%
Combined, LDL ≥ 130; TC ≥ 200; TG ≥ 150	7.21%	4.65%	11.43%	4.40%	16.69%	10.41%	5.77%	7.93%

Analytes (Cutoff-mg/dl)	Samples, age <18 years (n = 183)		Samples, age 18-25 years (n=369)		Samples, age 26-50 years (n=5168)		Samples, age >50 years (n=9883)	
	P = S No: / Total sample Age <18 yrs (%)		P = S No: / Total sample Age 18-25 yrs (%)		P = S No: / Total sample Age 26-50 yrs (%)		P = S No: / Total sample Age >50 yrs (%)	
	Male (n = 97)	Female (n = 86)	Male (n=210)	Female (n=159)	Male (n=3247)	Female (n=1921)	Male (n=5925)	Female (n=3958)
HDL - C								
Isolated HDL Increase: HDL $\geq$ 60	4.12%	5.81%	0.95%	1.26%	2.00%	3.59%	3.73%	7.55%
Isolated HDL Decrease: HDL: M <40; F < 43	24.74%	30.23%	26.66%	30.82%	16.81%	20.15%	31.25%	22.74%
Combined, HDL: M <40; F <43; TG $\geq$ 150	15.46%	5.81%	10.47%	8.18%	16.08%	9.01%	12.05%	9.07%
Total prevalence								
Prevalence of healthy lipid profile samples	28.87%	33.72%	24.76%	31.45%	16.29%	18.84%	26.16%	22.74%
Prevalence of abnormal lipid profile samples	71.13%	66.28%	75.24%	68.55%	83.71%	81.16%	73.84%	77.26%

Table 14.4 (continuation)

Table.14.5. Calculation of indirect prevalence of dyslipidemia in the total sample and in adults

Groups	Total samples (n = 15603)	Total male samples (n = 9479)	Total female samples (n = 6124)	≥18 total samples (n = 15420)	≥18 male samples (n = 9382)	≥18 female samples (n = 6038)
Indirect prevalence of dyslipidemia	77.57%	77.22%	78.1%	77.67%	77.29%	78.27%
Prevalence of healthy lipid profile samples	22.43%	22.78%	21.9%	22.33%	22.71%	21.73%

Table.14.6. Relative Prevalence of different dyslipidemia conditions (combined/isolated) in the individuals within the dyslipidemia sample population.

Analytes (Cutoff- mg/dl)	Abnormal Samples, age <18 years (n= 183)		Abnormal Samples, age 18- 25 years (n= 267)		Abnormal Samples, age 26- 50 years (n= 4227)		Abnormal Samples, age >50 years (n=7433)	
	RP = S No: / Total abnormal samples, age < 18 yrs (%)		RP = S No: / Total abnormal samples, age 18-25 yrs (%)		RP = S No: / Total abnormal samples, age 26- 50yrs (%)		RP = S No: /Total abnormal samples, age>50yrs (%)	
	Male (n=69)	Female (n=57)	Male (n=158)	Female (n=109)	Male (n=2718)	Female (n=1559)	Male (n=4375)	Female (n=3058)
Total TG								
Isolated, TG ≥150	23.19%	14.03%	19.62%	16.51%	25.39%	14.05%	22.86%	17.82%
TG > 885	2.89%	0	0.63%	0	0.55%	0.13%	0.11%	0.07%
Combined, TG≥150; TC ≥200	5.79%	1.75%	5.06%	3.67%	7.39%	4.23%	3.01%	3.11%
TC								
Isolated, TC ≥200	0	0	2.53%	0.91%	1.58%	2.82%	1.39%	2.78%

(RP=Relative Prevalence, S No: = Sample Number)

Analytes (Cutoff-mg/dl)	Abnormal Samples, age <18 years (n= 183)		Abnormal Samples, age 18-25 years (n= 267)		Abnormal Samples, age 26-50 years (n= 4227)		Abnormal Samples, age >50 years (n=7433)	
	RP = S No: / Total abnormal samples, age < 18 yrs (%)		RP = S No: / Total abnormal samples, age 18-25 yrs (%)		RP = S No: / Total abnormal samples, age 26-50yrs (%)		RP = S No: /Total abnormal samples, age>50yrs (%)	
	Male (n=69)	Female (n=57)	Male (n=158)	Female (n=109)	Male (n=2718)	Female (n=1559)	Male (n=4375)	Female (n=3058)
LDL- C	7.24%	5.26%	5.06%	6.42%	4.19%	6.61%	4.18%	3.53%
Isolated, LDL$\geq$130								
Combined, LDL$\geq$130; TC$\geq$200	11.59%	17.54%	14.56%	19.26%	18.29%	29.57%	13.09%	23.02%
Combined, LDL$\geq$130; TG$\geq$150	1.44%	0	1.27%	0	0.73%	0.64%	0.25%	0.26%
Combined, LDL$\geq$130; TC$\geq$200; TG$\geq$150	10.14%	7.01%	15.19%	6.42%	19.94%	12.83%	7.82%	10.27%
HDL - C	5.79%	8.77%	1.27%	1.83%	2.39%	4.43%	5.05%	9.78%
Isolated HDL Increase: HDL $\geq$ 60								
Isolated HDL Decrease: HDL: M <40; F < 43	34.78%	45.61%	35.44%	44.95%	20.08%	24.82%	42.33%	29.43%
Combined, HDL:M<40;F<43; TG$\geq$150	21.73%	8.77%	13.92%	11.92%	19.21%	11.09%	16.32%	11.74%

(Table 14.6 continuation)

CHAPTER 15
REFERENCE INTERVAL CALCULATION OF LIPID PROFILE
ANALYTES

15.1. Abstract

Introduction: A reference interval is set of values used to interpret laboratory results. It represents range of values expected for a healthy population, typically defined as central 95% of values from a reference population. Reference interval may vary by age, gender and population.

Objective: To calculate reference interval for lipid profile analytes in different age gender groups

Methods: lipid profile data was collected from clinical biochemistry laboratory and partitioned in to different age and gender groups. Samples having abnormal values or outliers for other diseases and for the lipid profile analytes were removed. The 90% and 95% reference interval was calculated for each individual lipid profile analyte in each age/ gender groups.

Results: The 90% Reference Interval for Total Cholesterol in adult males and females were 96 mg/dL – 246 mg/dL and 113mg/dL – 254mg/dL, respectively. The 90% Reference Interval for Serum Triglycerides in adult males and females were 55 mg/dL – 209 mg/dL and 48 mg/dL – 225 mg/dL, respectively. The 90% Reference Interval for LDL Cholesterol in adult males and females were 42 mg/dL – 169 mg/dL and 52 mg/dL – 180 mg/dL, respectively. The 90% Reference Interval for HDL-Cholesterol in adult males and females were 24 mg/dL – 56 mg/dL and 26 mg/dL – 64 mg/dL, respectively.

Conclusions: The 90% reference interval calculation was the most acceptable as data is positively skewed.

15.2. Introduction

Reference values are defined as quantitative data obtained from a single individual or group of individuals that correspond to a defined healthy state and it is useful for clinical diagnosis of disease (Horowitz et al., 2018). Defining the reference individual and selection of a group of reference individuals is the first step for establishing a reference value or reference interval. Population reference values or

intervals are difficult to calculate and therefore a sample group is selected to represent the reference population. As it is difficult to select a reference population, a subset population is selected random from the reference population.

A reference limit, which is a percentile of the biological quantity of the measurand, the estimated interval is bound within limits within which the 'true' value is located with a specified confidence called the "confidence interval". Typically, a 95% confidence interval is calculated.

The term reference distribution is used for the reference values in the sample of the reference individuals. The arithmetic mean ($\bar{x}$) and standard deviation (s_x) are measures of the distribution and location of the values (x) of the measurands. the capital alphabets X , $\bar{X}$ and S_x represent the population reference values, mean, and standard deviation. These population parameters of the measurand are difficult to obtain. Therefore, the sample parameters are taken from the reference sample population.

The reference interval is calculated from a Gaussian distribution of the measurand from a sample population. Inter percentile interval method is used to estimate the reference interval. This method is simple; more commonly used and is recommended by IFCC. The typical reference inter percentile interval is 2.5-percentile to 97.5-percentile. This method defines the reference interval as the central 95%-interval bound by the 2.5- and 97.5 – percentiles.

The inter percentile interval is determined using a parametric or a non-parametric method.

- a) **Parametric method:** The parametric method for determination of percentiles and their confidence interval assumes a Gaussian distribution and is based on estimation of mean and standard deviation. In the parametric method from the Gaussian distribution, the interpercentile reference limits are calculated as values located two standard deviations below and above the mean (Horowitz et al., 2018)

If the reference distribution does not have Gaussian distribution, a mathematical function may be used that transforms the data to a distribution that approximates a Gaussian distribution as observed graphically. As an example, a positively skewed distribution may be made nearly symmetrical or Gaussian by use of data transformation such as reciprocal method or square-root method may be used rarely. After transformation, the central 95%-interval is taken to represent the reference interval.

- b) Non parametric method: The non-parametric method does not make any assumption regarding the distribution and, also does not use estimates of distribution parameters. the percentiles are determined by cutting off the required percentage of values in each tail of the subset of reference distribution. This excluded percentile is typically 2.5% at each end, taking the middle percentile. (Reference of IFVV, CSIL)

Reference intervals may be a general reference interval of adult population defined for the male sample and another reference interval of adult population defined for female sample. Sample considered in this group includes all apparently healthy individuals above 18 years. for adolescent and children <18 years another reference interval may be calculated.

Another method of defining reference interval is the reference interval partitioned in to gender and age groups, where fasting and postprandial samples may be considered separate or together according to availability of sample number. According to this criterion of reference interval calculation there will be two genders and 4 age groups for the study (eight reference intervals)

In this study, we preferred to choose 90 % middle reference interval excluding 5% on either side to exclude the positively skewed regions and outliers with moderately or highly increased lipid profile analytes, respectively.

To calculate the parametric reference interval, the sample distribution should be evaluated for a normal or Gaussian distribution. If the distribution is not Gaussian then log transformation is done to convert the sample to Gaussian distribution.

In the non-parametric method, the 90% central reference interval is calculated from the normal or positively skewed sample using the percentile method for calculating the central 90% reference interval.

To further calculate a parametric base line reference interval, it may be required to exclude the outlier regions and then calculate the parametric reference interval before or after log transformation. If this method does not convert the distribution in to Gaussian distribution due to large number of moderately increased hyperlipoproteinemia leading to positive skewing, then the other methods such as reciprocal and square root methods are used for calculating the reference interval.

15.3. Objectives

To calculate reference interval for lipid profile analytes: Total Cholesterol, Serum triglycerides, LDL Cholesterol and HDL Cholesterol in different age gender groups

15.4. Materials and Methods

15.4.1. Research Design

This is an **Observational Cross-Sectional study** of dyslipidemia conditions presented at Amala Institute of Medical Sciences by **Indirect sampling** method. The lipid profile data was studied by:

- A) Lipid profile data collection from Clinical Biochemistry Laboratory.
- B) Grouping of dyslipidemia samples for reference interval calculation in the various gender-age groups.

15.4.2. Sample size requirement

Sample size was calculated from an earlier report on prevalence of dyslipidaemia in a multicentric study. From the sample size and prevalence data, the relative prevalence of common dyslipidaemia was calculated.

$$n = \frac{Z^2_{(1-\alpha)}pq}{d^2}$$

$d = 20\%$ prevalence; $\alpha = 0.05$; $p = 24\%$; $q = 1 - p$; $Z_{(1-\alpha)}$ value = 1.96; power 80%; $n = 304.12$.

Samples size rounded off to 304 (Qi et al., 2015). Final sample size $n = 15603$ (Refer General Materials and Methods)

15.4.3. Selection of Age Groups for the study

Total samples collected include males and females of all gender – age group. This sample was partitioned according to age into four groups, below 18 years (adolescents), 18 to <25 years (young adults), 25 to <50 years (middle age adults) and 50 and above years (senior adults). (Refer General Materials and Methods)

15.4.5. Sample Collection

The period of sample collection was from September 2018 to March 2022 with a break when hospital was closed due to COVID. For every sample with Lipid profile data collected, its associated other biochemical test 's results values (TSH, glucose, liver function tests, etc.) were also collected from the Clinical Biochemistry Laboratory (Refer General Materials and Methods)

15.4.6. Selection of the Lipid profile data

Lipid profile analytes selected were Total Triglycerides, HDL Cholesterol and LDL Cholesterol. Total Cholesterol, and LDL Cholesterol was assayed by direct assay of LDL cholesterol or by calculation by Friedwald equation. $TC = LDL - C + HDL - C + (Total\ TG/5)$ (Remaley et al., 2012)

15.4.7. Assays of Lipid Profile analytes (Refer General Materials and Method)

The cut-off values selected for normal healthy sample were: Total Cholesterol (<200 mg/dL), Triglycerides (<150 mg/dl), LDL Cholesterol (<130 mg/dl) and HDL Cholesterol (40 mg/dL - 59 mg/dL in males, HDL-C 43 mg/dL -59 mg/dL in females (Joshi et al., 2014; Mach et al., 2020, Vitros FS procedure manual).

15.4.8. Methodology

- a) Collect lipid profile data from clinical biochemistry laboratory
- b) Partition in to different age and gender groups
- c) Remove samples having abnormal values for other diseases
- d) Remove outliers for the lipid profile analytes

Calculate 90% and 95% reference interval for each individual lipid profile analyte in each age/ gender groups.

Calculation of Reference intervals for Lipids in various gender age groups

Excluded samples with abnormal ALT (outside 13 -69 U/L), Creatinine (outside males: 0.6 – 1.4 mg/dL, females: 0.6 – 1.2 mg/dL), TSH (outside 0.4 – 4.5 mIU/L), ALP (outside 38 – 126U/L) FBS (outside 80 – 110 mg/dL), and eliminating Lipid profile analytes outliers detected, using Box-Whisker plot.

15.4.9. Lipid profile reference interval can be calculated using various methods.

3. Direct measurement: measuring lipid parameters directly in a healthy population
4. Statistical analysis.

It involves following steps

6. Data cleaning:
 - c) Check for outliers: identify and handle outliers that may affect the accuracy of reference interval
 - d) Verify data distribution: check if the data follows a normal distribution or if it is skewed
7. Descriptive statistics
 - d) Calculate the mean and median: Determine the central tendency of data

- e) Calculate the standard deviation: determine the variability of data
 - f) Calculate percentiles: determine 2.5th, 5th, 95th, and 97.5th percentile to establish the reference interval
8. Reference interval calculation
- c) Parametric method: if the data follows a normal distribution, calculate the reference interval using $\text{mean} \pm 2\text{SD}$.

If, we assume mean (M) and standard deviation (s) of data from a normal or apparently healthy population, we can estimate reference interval as (m-2s to m+2s)

Note- s x 2 was arrived at by round of 1.96 x s.

1.96 s used to round the reference interval values at 2.5% and 97.5%

- d) Non-parametric method: if the data is skewed, calculate the reference interval using 2.5th and 97.5th percentile.

15.4.10. Steps used for reference interval calculation (Refer General Materials and Methods)

Robust methods in reference interval calculation are statistical techniques used to estimate reference intervals for clinical laboratory tests while minimizing the impact of outliers and non-normality in the data. Robust parametric methods: This method uses robust estimates of location and scale such as median and inters quartile range, to calculate reference interval. Non parametric robust method like boot strap method or rank-based method can be used to estimate reference interval without assuming a specific distribution (Efron and Tibshirani, 1993).

Application of robust methods in reference interval calculation:

1. Clinical laboratory testing: Robust methods are essential for establishing accurate reference interval for laboratory tests, which helps in diagnosing and monitoring diseases (IFCC).

2. Population based studies: these methods are useful in estimating reference intervals for different populations, taking in to account variations in demographics and laboratory measurement (CLSI).

The following reference intervals are calculated

- a) Adult (≥ 18 years of age)
- b) Adolescence and children (<18 years, males and females)
- c) Males and females of the four age groups. There will be eight reference intervals in these groups.
- d) Among all the age groups the most baseline reference interval will be from the physically active and healthy adults of 18 – 25 years partitioned in to male and females.
- e) Male and female reference interval calculated after excluding positive and negative outliers followed by log transformation etc as required.

Parametric reference interval assumes that the data follows a specific distribution (eg. Normal distribution) and use parameters like mean and standard deviation. When sample size is small and are not normally distributed, then non parametric methods are used. Here also percentile methods are used to represent 2.5th and 97.5th percentiles.

Sample size: the CLSI guidelines recommend minimum sample size of 129 for establishing reference interval using a non parametric method this is primarily based on sample size needed to calculate binomial 90% confidence interval.

The purpose of reference interval calculation is to define a method by which an apparently healthy population for a particular test parameter can be differentiated from a unhealthy patient test results. These calculations can use different methods such as normal distribution percentile or robust methods and also calculation of confidence interval. A typical reference interval is middle 95% excluding 2.5% in either side of distribution. But in a positively skewed sample with outliers, the suspicious, increased values may be excluded using percentages greater than 2.5%

Software tools: MedCalc statistical software has tools for calculating reference interval using different methods

15.5. Results

The 90% reference interval calculation was the most acceptable compared to 95% reference interval as data is positively skewed.

15.5.1. Reference intervals for Total Cholesterol (Table 15.1)

In Samples of age group <18 years, Parametric method (Robust method) was used for calculating both 90% and 95% Reference Interval for Total Cholesterol. The lower and upper limit values for 90% Reference interval in males were 113.24 mg/dL and 213.06 mg/dL, respectively. The lower and upper limit values for 90% Reference interval in females were 102.71 mg/dL and 217.90 mg/dL, respectively (Table 15.1).

In Samples of age group 18 - 25 years, Parametric method (Robust method) was used for calculating both 90% and 95% Reference Interval for Total Cholesterol. The lower and upper limit values for 90% Reference interval in males were 112.10 mg/dL and 238.82 mg/dL, respectively. The lower and upper limit values for 90% Reference interval in females were 119.83 mg/dL and 235.55 mg/dL, respectively (Table 15.1).

In Samples of age group 26 – 50 years, Parametric method (Robust method) was used for calculating both 90% and 95% Reference Interval for Total Cholesterol. The lower and upper limit values for 90% Reference interval in males were 113.00 mg/dL and 259.00 mg/dL, respectively. The lower and upper limit values for 90% Reference interval in females were 128.00 mg/dL and 255.00 mg/dL, respectively (Table 15.1).

In Samples of age group >50 years, Parametric method (Robust method) was used for calculating both 90% and 95% Reference Interval for Total Cholesterol. The lower and upper limit values for 90% Reference interval in males were 92.10 mg/dL and 234.00 mg/dL, respectively. The lower and upper limit values for 90% Reference interval in females were 106.00 mg/dL and 252.00 mg/dL, respectively (Table 15.1).

15.5.2. Reference intervals for Serum Triglycerides (Table 15.2)

In Samples of age group <18 years, Parametric method (Robust method) was used for calculating both 90% and 95% Reference Interval for Serum Triglycerides. The lower and upper limit values for 90% Reference interval in males were 26.81 mg/dL and 170.73 mg/dL, respectively. The lower and upper limit values for 90% Reference interval in females were 35.96 mg/dL and 210.66 mg/dL, respectively (Table 15.2).

In Samples of age group 18 – 25 years, Parametric method (Robust method) was used for calculating both 90% and 95% Reference Interval for Serum Triglycerides. The lower and upper limit values for 90% Reference interval in males were 52.88 mg/dL and 234.09 mg/dL, respectively. The lower and upper limit values for 90% Reference interval in females were 32.75 mg/dL and 170.67 mg/dL, respectively (Table 15.2).

In Samples of age group 26 – 50 years, Parametric method (Robust method) was used for calculating both 90% and 95% Reference Interval for Serum Triglycerides. The lower and upper limit values for 90% Reference interval in males were 60.00 mg/dL and 241.85 mg/dL, respectively. The lower and upper limit values for 90% Reference interval in females were 54.00 mg/dL and 182.00 mg/dL, respectively (Table 15.2).

In Samples of age group >50 years, Parametric method (Robust method) was used for calculating both 90% and 95% Reference Interval for Serum Triglycerides. The lower and Upper limit values for 90% Reference interval in males were 53.00 mg/dL and 184.40 mg/dL, respectively. The lower and upper limit values for 90% Reference interval in females were 55.00 mg/dL and 182.00 mg/dL, respectively (Table 15.2).

15.5.3. Reference intervals for LDL Cholesterol (Table 15.3)

In Samples of age group <18 years, Parametric method (Robust method) was used for calculating both 90% and 95% Reference Interval for LDL Cholesterol. The lower and upper limit values for 90% Reference interval in males were 48.05 mg/dL and 154.56 mg/dL, respectively. The lower and upper limit values for 90% Reference interval in females were 47.85 mg/dL and 148.58 mg/dL, respectively (Table 15.3).

In Samples of age group 18 – 25 years, Parametric method (Robust method) was used for calculating both 90% and 95% Reference Interval for LDL Cholesterol. The lower and upper limit values for 90% Reference interval in males were 57.51 mg/dL and 160.66 mg/dL, respectively. The lower and upper limit values for 90% Reference interval in females were 62.08 mg/dL and 149.35 mg/dL, respectively (Table 15.3).

In Samples of age group 26 – 50 years, non-parametric method (Percentile method) was used for calculating both 90% and 95% Reference Interval for LDL Cholesterol. The lower and Upper limit values for 90% Reference interval in males were 52.00 mg/dL and 178.00 mg/dL, respectively. The lower and upper limit values for 90% Reference interval in females were 66.00 mg/dL and 179.00 mg/dL, respectively (Table 15.3).

In Samples of age group >50 years, non-parametric method (Percentile method) was used for calculating both 90% and 95% Reference Interval for LDL Cholesterol. The lower and upper limit values for 90% Reference interval in males were 39.00 mg/dL and 161.00 mg/dL, respectively. The lower and upper limit values for 90% Reference interval in females were 47.00 mg/dL and 180.00 mg/dL, respectively (Table 15.3).

15.5.4. Reference intervals for HDL Cholesterol (Table 15.4)

In Samples of age group <18 years, Parametric method (Robust method) was used for calculating both 90% and 95% Reference Interval for HDL Cholesterol. The lower and upper limit values for 90% Reference interval in males were 16.91 mg/dL and 57.05 mg/dL, respectively. The lower and upper limit values for 90% Reference interval in females were 24.26 mg/dL and 59.81 mg/dL, respectively (Table 15.4).

In Samples of age group 18 – 25 years, non-parametric method (Percentile method) was used for calculating both 90% and 95% Reference Interval for HDL Cholesterol. The lower and upper limit values for 90% Reference interval in males were 24.82 mg/dL and 53.25 mg/dL, respectively. The lower and upper limit values for 90% Reference interval in females were 23.59 mg/dL and 61.23 mg/dL, respectively (Table 15.4).

In Samples of age group 26 – 50 years, non-parametric method (Percentile

method) was used for calculating both 90% and 95% Reference Interval for HDL Cholesterol. The lower and upper limit values for 90% Reference interval in males were 24.00 mg/dL and 54.00 mg/dL, respectively. The lower and upper limit values for 90% Reference interval in females were 26.00 mg/dL and 61.00 mg/dL, respectively (Table 15.4).

In Samples of age group >50 years, non-parametric method (Percentile method) was used for calculating both 90% and 95% Reference Interval for HDL Cholesterol. The lower and upper limit values for 90% Reference interval in males were 23.00 mg/dL and 56.00 mg/dL, respectively. The lower and upper limit values for 90% Reference interval in females were 25.00 mg/dL and 66.00 mg/dL, respectively (Table 15.4).

15.6. Discussions

Reference Interval for Total Cholesterol in India varies based on age, sex, and Geographic location. Studies have reported different ranges for Total Cholesterol levels across various populations in India. A study in Mangaluru, Karnataka, reported a median Total Cholesterol range of 116 – 239 mg/dL for non-fasting samples (Irehanavar et al., 2019). According to NCEP guidelines, a Total Cholesterol level of less than 200 mg/dL is considered desirable for healthy adults. However, studies have shown that Indians have higher reference intervals, with one study reporting range of 132.2 – 262 mg/dL for Total Cholesterol (Malati and Mahesh, 2009).

The reference interval for serum triglycerides in India vary based on age and study population. A study in Mangaluru, Karnataka, reported a median triglyceride range of 26.5 – 156 mg/dL for 18 – 25 years, 40 – 166 mg/dL for 28 – 37 years and, 37 – 204 mg/dL for 38 – 47 years (Ireshanavar et al., 2019).

A study in south Indians reported a reference interval for LDL Cholesterol of 66.2 – 175 mg/dL. The mean LDL Cholesterol levels were significantly higher in men (102.4±29.5 mg/dL) compared to women (99.2±30.6 mg/dL) in a pan-India study (Ireshanavar et al., 2019; Gupta et al., 2017).

Females tend to have higher HDL Cholesterol levels compared to males. A

study reported mean HDL Cholesterol levels of 43.2 ± 11.7 mg/dL, with females having higher levels (47.2 ± 10.4 mg/dL) compared to males (41.9 ± 9.8 mg/dL) (Ireshanavar et al., 2019; Gupta et al., 2017).

In this study, Samples of age group ≥ 18 years, non-parametric method (Percentile method) was used for calculating both 90% and 95% Reference Interval for Total Cholesterol, Serum triglycerides, LDL Cholesterol and HDL Cholesterol. The 90%reference interval range was higher in females for Total Cholesterol; HDL Cholesterol and LDL Cholesterol compared to males of age group ≥ 18 years. The 90%reference interval range was higher in males for serum triglycerides compared to males of age group ≥ 18 years.

15.7. Conclusions

Parametric methods were used for calculating reference interval for all lipid profile analytes of age group < 18 years. For calculating Reference Interval of Total Cholesterol, Serum triglycerides and LDL Cholesterol of age group 18 - 25 years parametric method was used.

Reference interval for Total Cholesterol, Serum triglycerides of age group 26 – 50 and > 50 years was calculated using Parametric method. Non parametric method (Percentile method) was used for calculating reference interval of all lipid profile analytes above 18 yrs. Reference interval for HDL Cholesterol (18 – 25 yrs, 26 – 50 years and, > 50 years) and for LDL Cholesterol (26 – 50 yrs and > 50 years) was calculated using Non parametric method (Percentile method). The 90% reference interval calculation is the most acceptable as data is positively skewed.

Table 15.1. Calculation of Reference intervals for Total Cholesterol (mg/dL) in various gender age groups after excluding samples with abnormal ALT (outside 13 -69 U/L), Creatinine (outside males: 0.6 – 1.4 mg/dL, females: 0.6 – 1.2 mg/dL), TSH (outside 0.4 – 4.5 mIU/L), ALP (outside 38 – 126U/L) FBS (outside 80 – 110 mg/dL), and eliminating Total Cholesterol outliers detected using Box-Whisker plot.

Total Cholesterol	Parametric method (Robust method)		Non parametric method (Percentile method)	
	90%RI		(90% RI)	
	(95%RI)		(95%RI)	
	Lower limit (90%CI)	Upper limit (90%CI)	Lower limit (90%CI)	Upper limit (90%CI)
Samples of age group <18 years				
Male (n = 63)	113.24 104.68 – 122.64	213.66 202.69 – 225.35	-	-
	103.33 (94.27 – 113.91)	223.78 (211.56 – 236.06)	-	-
Female (n=59)	102.71 (91.22 – 113.58)	217.90 (206.60 – 229.10)	-	-
	91.34 (78.69 – 102.66)	229.28 (216.89 – 241.43)	-	-
Samples of age group ≥18 years				
Male (n=5547)	-	-	96.00 (95.00 – 97.00)	246.00 (244.00 – 248.00)
	-	-	85.00 (83.00 – 87.00)	260.00 (258.00 – 263.00)
Female (n= 3593)	-	-	113.00 (110.00 – 115.00)	254.00 (251.00 – 256.00)
	-	-	101.00 (98.00 – 104.00)	267.00 (264.00 – 270.00)

Total Cholesterol	Parametric method (Robust method)		Non parametric method (Percentile method)	
	90%RI		(90% RI)	
	(95%RI)		(95%RI)	
	Lower limit (90%CI)	Upper limit (90%CI)	Lower limit (90%CI)	Upper limit (90%CI)
Samples of age group 18 – 25 years				
Male (n = 142)	112.10 (103.59 – 121.24)	238.82 (231.38 – 247.81)	-	-
	99.81 (90.41 – 109.72)	251.11 (242.87 – 260.80)	-	-
Female (n = 122)	119.83 (113.99 - 125.40)	235.55 (224.64 – 248.88)	-	-
	112.22 (105.92 - 118.38)	251.53 (238.78 – 266.93)	-	-

Total Cholesterol	Parametric method (Robust method)		Non parametric method (Percentile method)	
	90%RI		(90% RI)	
	(95%RI)		(95%RI)	
	Lower limit(90%CI)	Upper limit(90%CI)	Lower limit (90%CI)	Upper limit (90%CI)
Samples of age group 26 – 50 years				
Male (n = 1859)	113.00 (110.00 – 116.00)	259.00 (255.00 – 262.00)	-	-
	99.00 (96.00 – 104.00)	273.00 (268.00 – 278.00)	-	-
Female (n = 1209)	128.00 (125.00 – 131.00)	255.00 (250.00 – 259.00)	-	-
	120.00 (116.00 – 122.00)	266.00 (262.00 – 271.00)	-	-
Samples of age group >50 years				
Male (n = 3201)	92.10 (91.00 – 95.00)	234.00 (231.00 – 237.00)	-	-
	83.00 (81.00 – 85.00)	247.00 (245.00 – 250.00)	-	-
Female (n = 2252)	106.00 (105.00 – 109.00)	252.00 (250.00 – 256.00)	-	-
	96.00 (93.00 – 99.00)	267.00 (262.00 – 272.00)	-	-

Table 15.1 (continuation)

Table 15.2. Calculation of Reference intervals for Serum Triglycerides (mg/dL) in various gender age groups after excluding samples with abnormal ALT (outside 13 -69 U/L), Creatinine (outside males: 0.6 – 1.4 mg/dL, females: 0.6 – 1.2 mg/dL), TSH (outside 0.4 – 4.5 mIU/L), ALP (outside 38 – 126U/L) FBS (outside 80 – 110 mg/dL), and eliminating Serum Triglycerides outliers detected using Box-Whisker plot.

Serum Triglycerides	Parametric method (Robust method)		Non parametric method (Percentile method)	
	90%RI		(90% RI)	
	(95%RI)		(95%RI)	
	Lower limit(90%CI)	Upper limit(90%CI)	Lower limit (90%CI)	Upper limit (90%CI)
Samples of age group <18 years				
Male (n = 59)	26.81 (9.81 – 40.25)	170.73 (155.84 – 187.81)	-	-
	12.60 (5.57 – 27.62))	184.94 (168.32 – 203.29)	-	-
Female (n = 57)	35.96 (31.01 – 43.37)	210.66 (182.64 – 240.60)	-	-
	30.19 (25.57 – 37.05)	250.89 (216.27 – 288.46)	-	-
Samples of age group ≥18 years				
Male (n=5153)	-	-	55.00 (54.00 – 56.00)	209.00 (206.00 – 212.00)
	-	-	48.00 (47.00 – 50.00)	225.00 (223 – 227.00)
Female (n= 3390)	-	-	53.00 (51.00 – 54.00)	182.00 (179.00 – 185.00)
	-	-	47.00 (46.00 – 48.00)	195.00 (193.00 – 199.00)

Serum Triglycerides	Parametric method (Robust method)		Non parametric method (Percentile method)	
	90%RI		(90% RI)	
	(95%RI)		(95%RI)	
	Lower limit(90%CI)	Upper limit(90%CI)	Lower limit (90%CI)	Upper limit (90%CI)
Samples of age group 18 – 25 years				
Male (n = 132)	52.88 (47.03 – 58.66)	234.09 212.91 – 260.30	-	-
	45.76 40.15 – 51.00	270.52 243.94 – 305.98	-	-
Female (n = 119)	32.75 (27.27 – 37.57)	170.67 (159.06 – 185.10)	-	-
	25.09 (19.91 – 29.50)	189.83 (176.67 – 206.21)	-	-

Serum Triglycerides	Parametric method (Robust method)		Non parametric method (Percentile method)	
	90%RI		(90% RI)	
	(95%RI)		(95%RI)	
	Lower limit(90%CI)	Upper limit(90%CI)	Lower limit (90%CI)	Upper limit (90%CI)
Samples of age group 26 – 50 years				
Male (n = 1702)	60.00 (58.00 – 62.00)	241.85 (237.00 – 247.00)	-	-
	53.00 (50.00 – 54.00)	259.42 (255.00– 263.00)	-	-
Female (n = 1133)	51.00 (50.00 – 53.00)	182.00 (178.00 – 189.00)	-	-
	45.00 (42.00 – 48)	197.00 (194.00 – 201.00)	-	-
Samples of age group >50 years				
Male (n = 2991)	53.00 (52.00 – 55.00)	184.40 (182.00 – 188.00)	-	-
	47.80 (46.00 – 49.00)	200.00 (197.00 – 202.00)	-	-
Female (n = 2138)	55.00 (54.00 – 56.00)	182.00 (178.00 – 186.00)	-	-
	49.00 (47.00 – 51.00)	195.00 (191.00 – 199.00)	-	-

Table 15.2 (continuation)

Table 15.3. Calculation of Reference intervals for LDL Cholesterol (mg/dL) in various gender age groups after excluding samples with abnormal ALT (outside 13 -69 U/L), Creatinine (outside males: 0.6 – 1.4 mg/dL, females: 0.6 – 1.2 mg/dL), TSH (outside 0.4 – 4.5 mIU/L), ALP (outside 38 – 126U/L) FBS (outside 80 – 110 mg/dL), and eliminating LDL Cholesterol outliers detected using Box-Whisker plot.

LDL Cholesterol	Parametric method (Robust method)		Non parametric method (Percentile method)	
	90%RI		(90% RI)	
	(95%RI)		(95%RI)	
	Lower limit(90%CI)	Upper limit(90%CI)	Lower limit (90%CI)	Upper limit (90%CI)
Samples of age group <18 years				
Male (n = 63)	48.05 (40.59 – 57.73)	154.56 (141.56 – 165.72)	-	-
	37.55 (29.04 – 48.14)	165.06 (150.54 – 177.31)	-	-
Female (n=59)	47.85 (38.18 – 58.41)	148.58 (139.30 – 158.94)	-	-
	37.90 (27.45 – 49.06)	158.53 (147.44 – 169.71)	-	-
Samples of age group ≥18 years				
Male (n=5526)	-	-	42.00 (41.00 – 43.00)	169.65 (167.00– 171.00)
	-	-	35.00 (33.00 – 36.00)	180.82 (179.00 – 183.00)
Female (n= 3599)	-	-	52.00 (51.00 – 55.00)	180.00 (178.00 – 182.00)
	-	-	43.00 (42.00 – 45.00)	192.00 (189.00 – 195.00)

LDL Cholesterol	Parametric method (Robust method)		Non parametric method (Percentile method)	
	90%RI		(90% RI)	
	(95%RI)		(95%RI)	
	Lower limit(90%CI)	Upper limit(90%CI)	Lower limit (90%CI)	Upper limit (90%CI)
Samples of age group 18 – 25 years				
Male (n = 140)	57.51 (50.59 – 64.85)	160.66 (154.59 – 167.77)	-	-
	47.50 (39.55 – 55.21)	170.67 (164.07 – 178.27)	-	-
Female (n = 119)	62.08 (55.80 – 67.20)	149.35 (143.27 – 157.04)	-	-
	53.59 (46.94 – 59.46)	157.83 (151.25 – 166.19)	-	-

LDL Cholesterol	Parametric method (Robust method)		Non parametric method (Percentile method)	
	90%RI		(90% RI)	
	(95%RI)		(95%RI)	
	Lower limit(90%CI)	Upper limit(90%CI)	Lower limit (90%CI)	Upper limit (90%CI)
Samples of age group 26 – 50 years				
Male (n = 1848)	-	-	52.00 (50.00 – 54.00)	178.00 (176.00 – 181.00)
	-	-	45.00 (42.00 – 47.00)	191.77 (186.00 – 195.00)
Female (n = 1215)	-	-	66.00 (64.00 – 69.00)	179.00 (176 – 183)
	-	-	57.00 (53.00 – 62.00)	192.00 (186.00 - 195.00)
Samples of age group >50 years				
Male (n = 3194)	-	-	39.00 (37.00 – 41.00)	161.00 (159.00 – 163.00)
	-	-	33.00 (31.00 – 35.00)	172.00 (170.00 – 174.00)
Female (n = 2255)	-	-	47.00 (45.00 – 50.00)	180.00 (177.00 – 182.00)
	-	-	40.40 (39.00 – 42.00)	192.00 (188.00 – 196.00)

Table 15.3 (continuation)

Table 15.4. Calculation of Reference intervals for HDL Cholesterol (mg/dL) in various gender age groups after excluding samples with abnormal ALT (outside 13 -69 U/L), Creatinine (outside males: 0.6 – 1.4 mg/dL, females: 0.6 – 1.2 mg/dL), TSH (outside 0.4 – 4.5 mIU/L), ALP (outside 38 – 126U/L) FBS (outside 80 – 110 mg/dL), and eliminating HDL Cholesterol outliers detected using Box-Whisker plot.

HDL Cholesterol	Parametric method (Robust method)		Non parametric method (Percentile method)	
	90%RI		(90% RI)	
	(95%RI)		(95%RI)	
	Lower limit(90%CI)	Upper limit(90%CI)	Lower limit (90%CI)	Upper limit (90%CI)
Samples of age group <18 years				
Male (n = 66)	16.91 (13.96 – 20.63)	57.05 (52.37 – 60.52)	-	-
	12.96 (9.88 – 17.02)	61.00 (55.69 – 64.58)	-	-
Female (n=59)	24.26 (19.96 – 28.03)	59.81 (56.82 – 63.11)	-	-
	20.75 (16.05 – 24.79)	63.33 (60.21 – 66.84)	-	-
Samples of age group ≥18 years				
Male (n=5400)	-	-	24.00 (23.00 – 24.00)	56.00 (55.00 – 56.00)
	-	-	21.00 (20.00 – 21.00)	59.00 (58.00 – 60.00)
Female (n= 3550)	-	-	26.00 (25.00 – 26.00)	64.00 (63.00 – 65.00)
	-	-	23.00 (23.00 – 24.00)	69.00 (63.00 – 70.00)

HDL Cholesterol	Parametric method (Robust method)		Non parametric method (Percentile method)	
	90%RI		(90% RI)	
	(95%RI)		(95%RI)	
	Lower limit(90%CI)	Upper limit(90%CI)	Lower limit (90%CI)	Upper limit (90%CI)
Samples of age group 18 – 25 years				
Male (n = 135)	-	-	24.82 (22.80 – 26.85)	53.25 (51.54 – 55.18)
	-	-	22.06 (19.81 - 24.30)	56.01 (54.05 – 58.17)
Female (n = 123)	-	-	23.59 (20.95 – 26.04)	61.23 (58.99 – 64.03)
	-	-	19.93 (17.10 – 22.60)	64.89 (62.41 – 67.82)

HDL Cholesterol	Parametric method (Robust method)		Non parametric method (Percentile method)	
	90%RI		(90% RI)	
	(95%RI)		(95%RI)	
	Lower limit(90%CI)	Upper limit(90%CI)	Lower limit (90%CI)	Upper limit (90%CI)
Samples of age group 26 – 50 years				
Male (n = 1813)	-	-	24.00 (24.00 – 25.00)	54.00 (53.00 – 55.00)
	-	-	22.00 (21.00 – 23.00)	57.00 (56.00 – 58.00)
Female (n = 1183)	-	-	26.00 (26.00 – 28.00)	61.00 (60.00 – 63.00)
	-	-	24.00 (23.00- 25.00)	64.00 (63.00 – 67.00)
Samples of age group >50 years				
Male (n = 3106)	-	-	23.00 (23.00 – 24.00)	56.00 (55.00 – 57.00)
	-	-	20.00 (20.00 – 21.00)	59.00 (58.00 – 60.00)
Female (n = 2232)	-	-	25.00 (25.00 – 26.00)	66.00 (65.00 – 68.00)
	-	-	22.00 (20.00 – 23.00)	70.00 (70.00 – 72.00)

Table 15.4 (continuation)

CHAPTER 16

DISTRIBUTION ANALYSIS AND COMPARISON OF MEAN VALUES OF FASTING AND POSTPRANDIAL LIPID PROFILE ANALYTES.

16.1. Abstract

Introduction: Family history, metabolic syndrome, carotid and femoral plaque, coronary calcium score are some of the risk factors for development of atherosclerotic cardiovascular disease. There is a need for early intervention and management of dyslipidemia in the Indian population. In this study, fasting and postprandial Lipid profile data was collected from apparently healthy individuals, distribution and mean value comparison of analytes were done.

Objective: To collect Fasting, 2-hour post breakfast, 2-hour post lunch blood samples and analyse for lipid profile data distribution. To Compare the means of Fasting and Postprandial lipid profile analytes

Methods: This study employed an observational cross-sectional design to investigate normal and dyslipidemic conditions in individuals at Amala Institute of Medical Sciences. Chemistry analyses were conducted using the Ortho Clinical Diagnostics (OCD) Vitros 5.1 FS autoanalyzer. Data distribution was analysed by Shapiro-Wilk and Jarque-Bera tests, alongside graphical methods including histograms, Normal Q-Q plots, and Box-Whisker plots. Gender differences and mean comparisons of lipid concentrations were analyzed by Student's t-test for parametric data or Mann-Whitney U test for non-parametric data. SPSS software was used to perform all statistical calculations.

Results: Total of 312 participants were there in the study. Fasting and Postprandial mean Total Cholesterol was higher in males of age group 18 – 25 and 26 – 50 yrs. But > 50 years it was higher in females. Fasting and Postprandial Mean Serum triglycerides were higher in males in all age groups. Fasting and Postprandial Mean HDL-C were higher in females of all age groups. No mean difference between fasting and post prandial Total Cholesterol values in all gender-age groups. Significant mean difference – between fasting and post breakfast TG in all gender - age groups (except female 18 – 25 yrs). There were Significant mean difference – between fasting and post lunch TG in all gender-age groups. No mean difference was observed between fasting and postprandial HDL Cholesterol values in all gender-age groups. There were Significant mean difference between fasting and postprandial LDL Cholesterol values

in both genders of age group 26 – 50 years.

Conclusions: There were significant mean differences between fasting and Postprandial Triglycerides in all gender - age groups.

16.2. INTRODUCTION

Lipoproteins transport lipids in aqueous plasma. **Dyslipidemias** are diseases with abnormal levels of cholesterol and triglycerides in plasma lipoproteins and are commonly caused by lipoprotein metabolism disorders. In the healthy fasting morning plasma sample, most **triglycerides** are in VLDL. In the postprandial state, chylomicrons appear transiently and contribute in a small way to plasma triglyceride. LDL carries about 65 - 70% of total plasma **cholesterol**, 20 – 30% is in HDL and 5 – 10% in VLDL. LDL contains very little triglyceride. A fifth of triglyceride by weight in VLDL is considered as the cholesterol present in VLDL (Remaley et al., 2012). **Apolipoproteins** associated with lipoproteins can be estimated by immunoturbidimetry. **Secondary dyslipidemias** may be due to type 2 diabetes mellitus, obesity, insulin resistance, stress, hypothyroidism, metabolic syndrome, positive energy intake and high fat diet, alcoholism, polycystic ovary, sedentary life style, postmenopausal, stress, renal disease, drugs such as corticosteroids and estrogen or a combination of these conditions.

Fasting lipid profiles are essential for diagnosing dyslipidemia and assessing cardiovascular disease risk. Elevated levels of LDL Cholesterol and Triglycerides are associated with increased disease risk. (Nordestgaard and Varbo, 2014; NCEP)

Post prandial lipid profile assessment is important for evaluating postprandial lipemia, a condition where triglyceride levels increase after a meal. Elevated postprandial triglyceride levels are associated with increased cardiovascular disease risk (Kolovou and Mikhailidis). Non fasting triglyceride concentration increases cardiovascular disease risk and is evident from large, prospective studies (Nordestgaard et al., 2007; Bansal et al., 2007). The lipoproteins responsible for this are postprandial triglyceride rich, IDL and VLDL and may not be due to chylomicrons (Norata et al., 2007, Mamo et al., 1988). Non fasting, postprandial remnant cholesterol

containing lipoproteins also increase cardiovascular risk (Nordestgaard and Freiberg).

There were studies on evaluating the effectiveness of dietary interventions such as different fatty acids on lipid metabolism by measuring postprandial lipid profiles. (Volek and Phinney; 2014)

Fasting and postprandial lipid profiles reflect different metabolic states. Fasting profiles reflect basal metabolic rates, while postprandial profiles reflect the body's responses to dietary fats (Parks and Hellerstein, 2000). postprandial lipid responses can vary greatly between individuals, highlighting the importance of personalized dietary recommendations (Lairon and Lopez, 2017)

16.3. Objectives

- a) To collect Fasting, 2-hour post breakfast, 2-hour post lunch blood samples and analyse for lipid profile data distribution
- b) Comparison of means of Fasting and Postprandial lipid profile analytes

16.4. Materials and Methods

16.4.1 Research Design

This is an **observational cross-sectional study** of normal and dyslipidemic conditions presented at Amala Institute of Medical Sciences by **direct sampling** method.

16.4.2. Sample collection

A) Fasting, 2-hour post breakfast and 2-hour post lunch blood samples were collected from apparently healthy individuals of various age groups after describing the project from patient information sheet and getting their signed consent.

B) Data were also collected from samples using assays for Alanine aminotransferase, vitamin D, hsCRP, Apo a, Apo b, Parathyroid hormone, c peptide, insulin, fasting and postprandial glucose, erythropoietin, ferritin, Total calcium, may also be required to narrow down the differential diagnosis of a particular group of lipid profile.

16.4.2.1 Inclusion Criteria

Male and female adults with dyslipidemia and its complications but otherwise healthy, will be selected for the study. Individuals with defined physiological or pathological conditions such as diabetes mellitus, obesity, metabolic syndrome, hypertension, alcoholism, smoking, varying dietary habits, sedentary life style, fatty liver, thyroid dysfunction, pancreatitis, renal dysfunction and obesity may also be chosen for the initial evaluation.

Participants are instructed to take regular diet, sleep and exercise for 3 days before collecting blood samples. The individual is requested to report fasting to the laboratory between 8 am and 9 am. About 4 – 10 ml of blood sample is collected, centrifuged to separate serum. Assays are done immediately and the remaining serum sample is stored at -20°C. Non-fasting samples were taken 2-3 hours after breakfast and 2-3 hours after lunch.

16.4.2.2. Exclusion criteria

All disease conditions, except those defined above in the inclusion criteria, will be excluded from the study. Individuals with history of serious infections, fracture and injury involving tissue damage for six months are excluded. Individuals with malignancy will be excluded. Participant should not be on any medication or had any minor injury, infection or severe headache for a minimum of 2 weeks. Participants should not be stressed, such as overnight journey, preparing for examination, anxiety and loss of sleep. Participants with defined disease states, as stated in the inclusion criteria will be selected.

16.4.2.3. Sample size requirement

Sample size was calculated from an earlier report on prevalence of dyslipidemia in a multicentric study (Qi et al., 2015). From the sample size and prevalence data, the relative prevalence of common dyslipidemia was calculated.

$$n = \frac{Z^2_{(1-\alpha)}pq}{d^2}$$

d = 20% prevalence; $\alpha = 0.05$; p = 24%; q = 1- p; $Z_{(1-\alpha)}$ value =1.96; power 80%; n = 304.12.

Samples size rounded off to 304.

16.4.3. Clinical Biochemistry Assays

Chemistry tests with Ortho Clinical Diagnostics (OCD) Vitros 5.1 FS autoanalyser

1. Total Triglycerides: Assayed by enzymatic dry chemistry method (Procedure manual, Vitros). Triglycerides were dispersed from lipoprotein by surfactant (Triton X -100); hydrolyzed by lipase; product glycerol is phosphorylated by glycerol kinase and oxidized by oxidase to produce hydrogen peroxide which is estimated by dye-based method. Detection is at 540 nm.
2. Total cholesterol: Cholesterol ester is dispersed from lipoprotein by surfactant; hydrolyzed by hydrolase; Cholesterol oxidised by oxidase to form hydrogen peroxide which is estimated by peroxidase method (Procedure manual, Vitros).
3. LDL cholesterol: LDL cholesterol in all samples with TG<400mg/dl is estimated by Friedwald equation. If the TG value is above 400 mg/dL, direct LDL cholesterol estimation is done. In lipaemic samples cloudiness is cleared by cooling to 4°C and centrifuging at 4°C at 10,000 rpm for 20 minutes (Remaley et al., 2012)
4. HDL cholesterol: HDL is separated from LDL and VLDL by precipitation and the supernatant contains HDL cholesterol. Cholesterol ester is dispersed from lipoprotein by surfactant; hydrolyzed by hydrolase; cholesterol is oxidized by oxidase to form hydrogen peroxide which is estimated by peroxidase method.
5. Estimations of serum Alanine aminotransferase, amylase is by enzymatic assay.
6. Serum glucose will be estimated by glucose oxidase – peroxidase method.

7. High sensitivity CRP. By Dry Chemistry/immunoturbidimetry ((Procedure manual, Vitros).
8. Apo B, apo A by immunoturbidimetry in OCD Vitros 5.1 FS chemistry autoanalyser.

Immunochemistry Tests in Beckman Coulter DXI 600 autoanalyser using enhanced chemiluminescence detection (Procedure manual, Beckmann Coulter) **or Liaison** from Diasorin by chemiluminescence (Procedure manual Liason):

1. Serum insulin, C peptide, and TSH by immunometric sandwich immunoassay.
2. vitamin D by competitive immunoassay,

Body Mass Index = Weight in kg / Height in m²,

Waist circumference is taken in centimetres (Refer General Materials and Methods)

Hip circumference is highest circumference around the buttocks.

Electrophoresis: Evaluation of the level of Lipoproteins by 1% agarose gel or cellulose acetate membrane alkaline electrophoresis will be done. Lipids stained with Oil red–O/Sudan black. Relative quantification will be done visually (Refer General Materials and Methods).

16.4.4. Statistical Methods

Samples and their analytes will be evaluated for their **distribution** visually by histogram, Quantile-Quantile plot and Box-Whisker plot. Aspects of distribution will be estimated by Shapiro-Wilk. Relationships will be evaluated by **correlation coefficient**. **Mean differences between groups** will be tested by Student t test, Mann Whitney U test (Altman, 1999; Gao and Smith, 2003)

16.5. RESULTS

Total of 312 participants were there in the study. There were 16 participants with severe hypertriglyceridemia with Triglyceride >885 mg/dL. There were 20 participants with triglyceride between 400 – 885 mg/dL with other disease conditions. There were 3 samples in age group below 18 years. These above samples were not considered for study.

Out of 273 participants above 18 years of age, there were 101 males and 172 female samples from which fasting and postprandial samples were collected. samples were partitioned based on gender and age groups as male and females between 18 – 25 years, 26 – 50 years and >50 years.

16.5.1. Distribution of Total Cholesterol

16.5.1.1. Fasting Total Cholesterol

In 18 – 25 years there was no significant gender differences between mean fasting Total Cholesterol values in male and female samples. There was significant gender difference between mean values of fasting Total Cholesterol ($P=0.026$) in age group 26 – 50 years, with higher mean values in males (202.89 ± 34.09). In >50 years, females (206.18 ± 48.03) had higher mean fasting Total Cholesterol values than males (176.60 ± 48.43) with $P=0.025$ (Table 16.1). Normal Q-Q plot shows slight deviation away from normal deviation in Fig 16.1 A, 1 D, 1 E. Histogram showed positive skewed distribution in Fig. 16.13. Box – Whisker plot showed skewing in Fig 16.25 A, 16.25 B, 16.25C, and 16.25E.

16.5.1.2. Post breakfast Total Cholesterol

In 18 – 25 years there was significant gender differences in mean post breakfast Total Cholesterol values between male and female samples with higher mean values in males (179.60 ± 37.51) compared to females of same age group. There was significant gender difference between mean values of post breakfast Total Cholesterol ($P=0.052$) in age group 26 – 50 years, with higher mean values in males (197.56 ± 32.58). In >50 years of age group, females (203.31 ± 46.86) have higher mean

post breakfast Total Cholesterol values than males (174.85 ± 46.83) with $P = 0.054$ (Table 16.2). Normal Q-Q plot showed deviations from normal straight-line distribution in Fig. 16.2 A, 2 D, and 2F. Histogram showed positive skewed distribution in Fig. 16.14 A, 16.14 D. Box – Whisker plot showed high skewing in Fig. 16.26 A

16.5.1.3. Post Lunch Total Cholesterol

In 18 – 25 years there was significant gender differences in mean post lunch Total Cholesterol values ($P = 0.006$) between male and female samples with higher mean values in males (179.15 ± 35.45) compared to females of same age group. There was no significant gender difference between mean values of post lunch Total Cholesterol in age group 26 – 50 years. In > 50 years of age group, females (200.36 ± 46.33) have higher mean post lunch Total Cholesterol values than males (173.23 ± 46.33) with $P = 0.035$ (Table 16.3). Normal Q-Q plot showed slight deviations from normal straight-line distribution in Fig. 16.3. Histogram showed positive skewed distribution in Fig. 16.15 A, 15 D, 15 E. Box – Whisker plot showed high skewing in Fig 16.27A

16.5.2. Distribution of Serum triglycerides

16.5.2.1. Fasting Serum triglycerides

There were significant gender differences in mean fasting serum triglyceride values in both 18 – 25 years and 26 – 50 years age group with $P = < 0.001$. In both these age groups males had increased mean fasting serum triglyceride values compared to females. There were no significant gender differences in mean fasting serum triglyceride values in age group > 50 years (Table 16.4). Normal Q-Q plot showed larger deviations from normal straight-line distribution in Fig. 16.4. Histogram showed positive skewed distribution in Fig. 16.16 C, and 16.16 D. Box – Whisker plot showed high skewing in Fig 16.28.

16.5.2.2. Post breakfast Serum triglycerides

There were significant gender differences in mean Post breakfast serum

triglyceride values (higher in males) in both 18 – 25 years and 26 – 50 years age group with $P = <0.001$. There was no significant gender differences in mean post breakfast serum triglyceride values in age group >50 years (Table 16.5). Normal Q-Q plot showed larger deviations from normal straight-line distribution in Fig. 16.5C, 5D, 5E. Histogram showed positive skewed distribution in Fig. 16.17 D. Box – Whisker plot showed high skewing in Fig 16.29 C, 6.29D, and 16.29E.

16.5.2.3. Post lunch Serum triglycerides

There were significant gender differences in mean Post lunch serum triglyceride values - in both 18 – 25 years and 26 – 50 years age group with $P = <0.001$. In 18 – 25 years, mean post breakfast serum triglyceride values were higher in males (194.30 ± 121.64) compared to females. The mean post lunch serum triglyceride values were higher in males (341.88 ± 188.91). There were no significant gender differences in mean post lunch serum triglyceride values in age group >50 years (Table 16.6). Normal Q-Q plot showed larger deviations from normal straight-line distribution in Fig. 16.6 Histogram showed positive skewed distribution in Fig. 16.18 A, and 16.18 D. Box – Whisker plot showed high skewing in Fig. 16.30 B, 16.30 D, and 16.30 F.

16.5.3. Distribution of HDL Cholesterol

16.5.3.1. Fasting HDL Cholesterol

In age groups 18 – 25 years and >50 years there were no significant gender differences in mean fasting HDL Cholesterol values. In 26 – 50 years age group, females had higher mean fasting HDL Cholesterol values than males with $P = 0.002$ (Table 16.7). Normal Q-Q plot showed slight deviations from normal straight-line distribution in Fig. 16.7. Histogram showed positive skewed distribution in Fig. 16.19 B. Box – Whisker plot showed skewing in Fig 16.31

16.5.3.2. Post breakfast HDL Cholesterol

There is significant gender difference in mean post breakfast HDL Cholesterol values in age group 26 – 50 years, with higher mean values in females compared to males ($P = 0.001$) (Table 16.8). Normal Q-Q plot showed moderate deviations from

normal straight-line distribution in Fig. 16.8. Histogram showed positive skewed distribution in Fig. 16.20.A, E. Box – Whisker plot showed skewing in Fig. 16. 32

16.5.3.3. Post Lunch HDL Cholesterol

There is significant gender difference in mean post lunch HDL Cholesterol values in age group 26 – 50 years, with higher mean values in females (44.75 ± 10.24) compared to males (39.00 ± 9.16) ($P = 0.001$) (Table 16.9). Normal Q-Q plot showed slight deviations from normal straight-line distribution in Fig. 16.9. Histogram showed positive skewed distribution in Fig. 16.21. Box – Whisker plot showed skewing in Fig 16.33.

16.5.4. Distribution of LDL Cholesterol

16.5.4.1. Fasting LDL Cholesterol

There were significant gender differences in fasting LDL Cholesterol mean values with higher values in females compared to males in the age group >50 years. There were no significant gender differences in the age group 18 - 25 years and 26 – 50 years (Table 16.10). Normal Q-Q plot showed large deviations from normal straight-line distribution in Fig. 16.10A, 10D. Histogram showed positive skewed distribution in Fig. 16.22 A, D, Box – Whisker plot showed skewing in Fig 16.34.

16.5.4.2. Post breakfast LDL Cholesterol

There were significant gender differences in post breakfast LDL Cholesterol mean values with higher values in females compared to males in the age group >50 years. There were no significant gender differences in the age group 18 – 25 years and 26 – 50 years (Table 16.11). Normal Q-Q plot showed large deviations from normal straight-line distribution in Fig. 16.11A, 11B, 11D. Histogram showed positive skewed distribution in Fig. 16.23 A, D. Box – Whisker showed positive skewing (Fig.16.35)

16.5.4.3. Post lunch LDL Cholesterol

There were significant gender differences in post lunch LDL Cholesterol mean values with higher values in females compared to males in the age groups 26 – 50 and >50 years. There were no significant gender differences in the age group 18 - 25 years (Table 16.12). Normal Q-Q plot showed slight deviations from normal straight-line distribution in Fig. 16.12 Histogram showed positive skewed distribution in Fig. 16.24.D. Box Whisker showed skewing in all gender-age groups (Fig. 16.36)

16.5.5. Comparing means of fasting and postprandial lipid profile analytes.

There was no mean difference between fasting and post prandial Total Cholesterol values in gender-age groups. Significant mean difference between fasting and post breakfast serum triglyceride was observed in all gender - age groups (except female 18 – 25 yrs). Significant mean difference between fasting and post lunch triglyceride was observed in all gender-age groups (Table 16.14). There was no mean difference between fasting and post prandial HDL Cholesterol values in all gender-age groups (Table 16.15). Significant mean difference between fasting and post prandial LDL Cholesterol values was observed in both genders of age group 26 – 50 years (Table 16.16)

16.6. Discussion

Males (18–25, 26–50) had higher Total Cholesterol, while females over 50 surpassed males. Most studies focus on fasting Total Cholesterol rather than postprandial. However, gender and age differences are well-documented. Notably, pre-menopausal women typically have lower Total Cholesterol compared to men, but post-menopause, Total Cholesterol rises, narrowing or reversing this gap due to estrogen decline. Estrogen enhances LDL clearance, and its reduction after menopause leads to higher LDL Cholesterol and Total Cholesterol in women (Lopez-miranda and Martin, 2010).

Males had higher TG (fasting and postprandial) across all age groups. Significant post-breakfast Triglycerides changes in all groups except females 18–25; significant post-lunch changes in all. Men consistently exhibit a stronger postprandial

TG response than women; this is largely attributed to visceral adiposity and slower TG clearance (Lopez-miranda and Martin, 2010). The Tromsø Study (ages 40+) confirms that men have higher non-fasting TG values, and that postprandial TG correlates inversely with age in men but rises in post-menopausal women. Physiologically, women, especially pre-menopausal clear postprandial TG more efficiently due to better lipoprotein lipase (LPL) activity in subcutaneous fat, influenced by estradiol (Couillard et al., 1999). The post-lunch Triglycerides rises significantly in all groups aligns with literature showing cumulative serum triglycerides burden over successive meals, and gender-age differences in metabolic handling.

Females had higher HDL-C across all age groups; no significant fasting vs postprandial difference. Women generally have higher HDL-C than men due to estrogen's positive effects on HDL metabolism, including promoting reverse cholesterol transport (Loh et al., 2022). Postprandial changes in HDL-C are minimal, as HDL is relatively stable throughout the day.

Significant fasting vs postprandial LDL differences only in ages 26–50 (both genders). LDL-Cholesterol is generally regarded as minimally affected by meals, though minor fluctuations can occur due to haemodilution and changes in VLDL/TG affecting calculated LDL (Shetty et al., 2025). However, in metabolic conditions (e.g. diabetes), postprandial LDL-C can be more pronounced. Finding in 26–50 years could suggest early metabolic changes in that cohort, potentially related to insulin sensitivity shifts or diet composition during meals.

Postprandial Triglyceride tolerance worsens with age in both genders; visceral fat strongly predicts Triglyceride response (Lopez-miranda and Martin, 2010).

In pre-menopausal women, estradiol upregulates LPL, aids chylomicron remnant clearance, and maintains higher HDL-C; these effects diminish post-menopause leading to increased TC and TG (Lopez-miranda and Martin, 2010). Men have less efficient TG clearance, greater visceral fat, and lower baseline HDL-Cholesterol amplifying their higher TG response across meals.

16.7. Conclusion

Fasting and Postprandial mean Total Cholesterol was higher in males of age group 18 – 25 and 26 – 50 yrs. But > 50 years, it was higher in females. Fasting and Postprandial mean Serum triglycerides was higher in males in all age groups. Fasting and postprandial mean HDL-C was higher in females of all age groups. There was no mean difference between fasting and post prandial Total Cholesterol values in gender-age groups. Significant mean difference between fasting and post breakfast serum triglyceride was observed in all gender - age groups (except female 18 – 25 yrs). Significant mean difference between fasting and post lunch triglyceride was observed in all gender-age groups. There was no mean difference between fasting and post prandial HDL Cholesterol values in all gender-age groups. Significant mean difference between fasting and post prandial LDL Cholesterol values was observed in both genders of age group 26 – 50 years

This study supports and elaborates on established patterns: males have consistently higher fasting and postprandial Triglycerides. Females maintain higher HDL. LDL shifts in mid-age groups may signal emerging metabolic risk. These insights align well with hormonal physiology, fat distribution, and growing evidence emphasizing non-fasting lipid profiling in risk assessment.

Table 16.1. Distribution and mean differences of Fasting Total cholesterol (mg/dL) in samples of age group 18 – 25 years, 26 – 50 years and >50 years

Samples	Sample number (n)	Mean±SD 95%CI for mean Range: Min –Max	Median Skewness kurtosis	Shapiro – Wilk Jarque Bera (Log transformation)		Student’s t test/Mann-Whitney U Test (P)
				Before	After	
Samples: Age group 18 – 25 years						
Male	20	177.00±33.12 161.49 – 192.50 131 – 252	166.50 0.950 0.531	0.061 0.275	0.296 0.650	0.162
Female	42	166.02±26.12 157.88 – 174.16 114 - 231	165.00 0.213 0.139	0.646 0.862	0.586 0.813	
Samples: Age group 26 – 50 years						
Male	48	202.89±34.09 192.99 – 212.79 134 - 282	197.00 0.237 -0.175	0.784 0.749	0.825 0.814	0.026
Female	97	189.52±35.43 182.38 – 196.66 117 – 321	186.00 0.798 1.561	0.010 (<0.001)	0.840 0.626	
Samples: Age group > 50 years						
Male	33	176.60±48.43 159.43 – 193.78 105 - 262	188.00 -0.090 -1.516	0.008 0.220	0.004 0.186	0.025
Female	33	206.18±48.03 189.15 – 223.21 113 – 309	206.00 -0.092 -0.206	0.841 0.898	0.143 0.307	

Table 16.2. Distribution and mean differences of Post breakfast Total cholesterol (mg/dL) in samples of age group 18 – 25 years, 26 – 50 years and >50 years

Samples	Sample number (n)	Mean±SD 95%CI for mean Range: Min –Max	Median Skewness kurtosis	Shapiro – Wilk Jarque Bera (Log transformation)		Student’s t test/Mann- Whitney U Test (P)
				Before	After	
Samples: Age group 18 – 25 years						
Male	20	179.60±37.51 162.04 – 197.15 126 – 275	166.00 1.103 1.228	0.044 0.148	0.284 0.630	0.019
Female	42	160.78±23.72 153.39 – 168.18 114 - 229	158.50 0.444 0.640	0.662 0.448	0.952 0.996	
Samples: Age group 26 – 50 years						
Male	44	197.56±32.58 187.66 – 207.47 138.00 – 270.00	197.00 0.171 -0.375	0.806 0.741	0.735 0.695	0.052
Female	92	186.09±33.17 179.22 – 192.96 120 - 309	182.50 0.986 2.122	0.001 (<0.001)	0.517 0.253	
Samples: Age group > 50 years						
Male	27	174.85±46.83 156.32 – 193.37 97 – 252	196.00 -0.235 -1.372	0.030 0.323	0.011 0.262	0.054
Female	32	203.31±46.86 186.41 – 220.20 116 – 313	198.00 0.181 0.076	0.605 0.914	0.294 0.590	

Table 16.3. Distribution and mean differences of Post lunch Total cholesterol (mg/dL) in samples of age group 18 – 25 years, 26 – 50 years and >50 years

Samples	Sample number (n)	Mean±SD 95%CI for mean Range: Min –Max	Median Skewness kurtosis	Shapiro – Wilk Jarque Bera (Log transformation)		Student’s t test/Mann- Whitney U Test (P)
				Before	After	
Samples: Age group 18 – 25 years						
Male	20	179.15±35.45 162.55 – 195.74 125 – 261	170.00 0.826 0.601	0.136 0.374	0.469 0.870	0.006
Female	42	157.97±22.62 150.92 – 165.02 119 – 221	155.00 0.617 0.501	0.289 0.267	0.854 0.844	
Samples: Age group 26 – 50 years						
Male	43	196.16±34.68 185.48 – 206.83 132.00 – 286.00	198.00 0.369 0.083	0.805 0.632	0.968 0.881	0.101
Female	96	186.02±33.59 179.21 – 192.82 125 - 304	179.50 0.936 1.744	0.001 (<0.001)	0.533 0.388	
Samples: Age group > 50 years						
Male	30	173.23±46.51 155.86 – 190.60 100 – 256	186.50 -0.112 -1.377	0.037 0.310	0.015 0.252	0.035
Female	33	200.36±46.33 183.93 – 216.79 116 – 315	200.00 0.240 -0.016	0.708 0.844	0.593 0.674	

Table 16.4. Distribution and mean differences of Fasting Serum triglycerides (mg/dL) in samples of age group 18 – 25 years, 26 – 50 years and >50 years

Samples	Sample number (n)	Mean±SD 95%CI for mean Range: Min –Max	Median Skewness kurtosis	Shapiro – Wilk Jarque Bera (Log transformation)		Student’s t test/Mann- Whitney U Test (P)
				Before	After	
Samples: Age group 18 – 25 years						
Male	20	125.25±54.79 99.60 – 150.89 49 – 243	116.50 0.605 -0.428	0.281 0.508	0.688 0.751	<0.001
Female	42	71.28±25.37 63.37 – 79.19 36 – 145	66.00 1.422 1.621	<0.001 (<0.001)	0.067 0.231	
Samples: Age group 26 – 50 years						
Male	48	204.66±111.68 172.23 – 237.09 63 - 651	169.50 1.640 4.122	<0.001 (<0.001)	0.742 0.774	<0.001
Female	97	112.17±54.64 101.16 – 123.18 42 – 317	105.00 1.475 2.538	<0.001 (<0.001)	0.191 0.451	
Samples: Age group > 50 years						
Male	33	137.84±65.88 114.48 – 161.21 55 – 340	125.00 1.768 3.198	<0.001 (<0.001)	0.235 0.355	0.632
Female	33	129.78±50.19 111.98 – 147.58 59 - 230	126.00 0.340 -0.997	0.115 0.637	0.167 0.363	

Table 16.5. Distribution and mean differences of Post breakfast Serum triglycerides (mg/dL) in samples of age group 18 – 25 years, 26 – 50 years and >50 years

Samples	Sample number (n)	Mean±SD 95%CI for mean Range: Min –Max	Median Skewness kurtosis	Shapiro – Wilk Jarque Bera (Log transformation)		Student’s t test/Mann-Whitney U Test (P)
				Before	After	
Samples: Age group 18 – 25 years						
Male	20	171.70±79.40 134.53 – 208.86 78 – 349	158.00 0.745 -0.418	0.056 0.390	0.318 0.594	<0.001
Female	42	84.21±35.59 73.12 – 95.30 41 – 173	69.50 1.085 0.241	<0.001 0.021	0.049 0.247	
Samples: Age group 26 – 50 years						
Male	44	272.43±116.33 237.06 – 307.80 102 - 670	216.00 1.145 1.645	0.001 0.002	0.150 0.644	<0.001
Female	92	152.53±76.59 136.66 – 168.39 49 – 492	143.50 1.539 3.903	<0.001 <0.001	0.536 0.791	
Samples: Age group > 50 years						
Male	27	194.55±71.08 166.43 – 222.67 69 – 357	171.00 0.802 0.265	0.069 0.275	0.487 0.818	0.250
Female	32	173..09±70.27 147.75 – 198.43 68–315	169.00 0.326 -0.887	0.263 0.430	0.293 0.458	

Table 16.6. Distribution and mean differences of Post lunch Serum triglycerides (mg/dL) in samples of age group 18 – 25 years, 26 – 50 years and >50 years

Samples	Sample number (n)	Mean±SD 95%CI for mean Range: Min –Max	Median Skewness kurtosis	Shapiro – Wilk Jarque Bera (Log transformation)		Student’s t test/Mann- Whitney U Test (P)
				Before	After	
Samples: Age group 18 – 25 years						
Male	20	194.30±121.64 137.36 – 251.23 69 – 586	165.50 1.920 4.827	0.002 <0.001	0.852 0.785	<0.001
Female	42	90.33±38.92 78.20 – 102.46 45 – 222	72.00 1.570 2.546	<0.001 (<0.001)	0.013 0.171	
Samples: Age group 26 – 50 years						
Male	43	341.88±188.91 283.74 – 400.02 120–831	261.00 1.467 1.359	<0.001 (<0.001)	0.044 0.258	<0.001
Female	96	180.69±92.30 161.99 – 199.40 56 – 473	157.00 1.240 1.218	<0.001 (<0.001)	0.397 0.396	
Samples: Age group > 50 years						
Male	30	224.13±95.72 188.38 – 259.87 50 – 417	213.00 0.360 -0.532	0.477 0.577	0.186 0.186	0.250
Female	33	196.24±94.65 162.67 – 229.80 48 – 441	199.00 0.708 0.282	0.153 0.284	0.466 0.524	

Table 16.7. Distribution and mean differences of Fasting HDL Cholesterol (mg/dL) in samples of age group 18 – 25 years, 26 – 50 years and >50 years

Samples	Sample number (n)	Mean±SD 95%CI for mean Range: Min –Max	Median Skewness kurtosis	Shapiro – Wilk Jarque Bera (Log transformation)		Student’s t test/Mann- Whitney U Test (P)
				Before	After	
Samples: Age group 18 – 25 years						
Male	20	44.60±11.53 39.20 – 49.99 25 – 69	45.50 0.101 -0.278	0.947 0.888	0.627 0.644	0.087
Female	42	50.11±11.75 46.45 – 53.78 29 – 85	50.00 0.599 0.461	0.291 0.292	0.955 0.897	
Samples: Age group 26 – 50 years						
Male	48	41.83±9.94 38.94 – 44.72 25 - 67	42.00 0.551 -0.129	0.190 0.302	0.829 0.750	0.002
Female	97	46.95±10.15 44.91 – 49.00 28 – 80	45.00 0.871 0.818	0.001 (<0.001)	0.669 0.568	
Samples: Age group > 50 years						
Male	33	45.78±12.70 41.28 – 50.29 27 - 78	43.00 0.850 0.128	0.049 0.163	0.740 0.597	0.093
Female	33	50.51±12.31 46.14 – 54.88 34 – 78	48.00 0.621 -0.660	0.037 0.261	0.189 0.403	

Table 16.8. Distribution and mean differences of Post breakfast HDL Cholesterol (mg/dL) in samples of age group 18 – 25 years, 26 – 50 years and >50 years

Samples	Sample number (n)	Mean±SD 95%CI for mean Range: Min –Max	Median Skewness kurtosis	Shapiro – Wilk Jarque Bera (Log transformation)		Student’s t test/Mann- Whitney U Test (P)
				Before	After	
Samples: Age group 18 – 25 years						
Male	20	43.85±10.76 38.81 – 48.88 25 – 62	46.50 -0.171 -0.734	0.623 0.710	0.225 0.492	0.109
Female	42	48.85±11.58 45.24 – 52.46 29 – 84	48.50 0.668 0.647	0.212 0.198	0.936 0.885	
Samples: Age group 26 – 50 years						
Male	44	39.79±9.11 37.02 – 42.56 25 - 63	40.50 0.654 0.043	0.051 0.230	0.416 0.654	0.001
Female	92	45.52±9.84 43.48 – 47.56 26 – 77	44.00 0.787 0.599	0.005 0.006	0.802 0.745	
Samples: Age group > 50 years						
Male	27	44.29±12.39 39.39 – 49.19 28 - 76	43.00 0.834 0.257	0.086 0.248	0.567 0.610	0.141
Female	32	48.50±11.82 44.23 – 52.76 32 – 72	46.00 0.636 -0.525	0.033 0.288	0.232 0.538	

Table 16.9. Distribution and mean differences of Post lunch HDL Cholesterol (mg/dL) in samples of age group 18 – 25 years, 26 – 50 years and >50 years

Samples	Sample number (n)	Mean±SD 95%CI for mean Range: Min –Max	Median Skewness kurtosis	Shapiro – Wilk Jarque Bera (Log transformation)		Student’s t test/Mann- Whitney U Test (P)
				Before	After	
Samples: Age group 18 – 25 years						
Male	20	44.15±10.44 39.26 – 49.03 25 – 60	45.50 -0.310 -0.767	0.520 0.634	0.146 0.420	0.156
Female	42	48.26±10.57 44.96 – 51.55 29 – 74	47.50 0.303 -0.387	0.606 0.605	0.723 0.700	
Samples: Age group 26 – 50 years						
Male	43	39.00±9.16 36.17 – 41.82 26 - 68	37.00 1.091 1.343	0.005 0.006	0.210 0.392	0.001
Female	96	44.75±10.24 42.67 – 46.82 25– 74	44.00 0.738 0.475	0.005 0.010	0.769 0.832	
Samples: Age group > 50 years						
Male	30	42.50±11.86 38.07 – 46.92 27 - 74	41.00 0.780 0.258	0.096 0.253	0.508 0.599	0.137
Female	33	47.00±11.82 42.80 – 51.19 30– 72	46.00 0.577 -0.313	0.097 0.379	0.427 0.691	

Table 16.10. Distribution and mean differences of Fasting LDL Cholesterol (mg/dL) in samples of age group 18 – 25 years, 26 – 50 years and >50 years

Samples	Sample number (n)	Mean±SD 95%CI for mean Range: Min –Max	Median Skewness kurtosis	Shapiro – Wilk Jarque Bera (Log transformation)		Student’s t test/Mann- Whitney U Test (P)
				Before	After	
Samples: Age group 18 – 25 years						
Male	20	107.30±36.70 90.12 – 124.47 63 – 208	96.50 1.313 1.605	0.019 0.059	0.422 0.500	0.810
Female	42	101.80±23.15 94.59 – 109.02 49 – 156	101.00 0.229 0.830	0.049 0.619	0.009 0.028	
Samples: Age group 26 – 50 years						
Male	48	120.66±27.32 112.73 – 128.60 60 - 179	120.00 -0.019 0.158	0.616 0.998	0.021 0.025	0.677
Female	97	120.70±32.20 114.20 – 127.19 57 – 256	118.00 1.021 2.582	0.001 <0.001	0.846 0.543	
Samples: Age group > 50 years						
Male	33	103.27±46.43 86.80 – 119.73 22 - 180	110.00 -0.065 -1.245	0.137 0.343	0.012 0.148	0.010
Female	33	132.39±42.27 117.40 – 147.38 48 – 226	136.00 -0.006 0.031	0.596 0.984	0.022 0.116	

Table 16.11. Distribution and mean differences of Post breakfast LDL Cholesterol (mg/dL) in samples of age group 18 – 25 years, 26 – 50 years and >50 years

Samples	Sample number (n)	Mean±SD 95%CI for mean Range: Min –Max	Median Skewness kurtosis	Shapiro – Wilk Jarque Bera (Log transformation)		Student’s t test/Mann- Whitney U Test (P)
				Before	After	
Samples: Age group 18 – 25 years						
Male	20	101.35±43.19 81.13 – 121.56 54 – 223	92.00 1.255 1.807	0.021 0.064	0.394 0.613	0.880
Female	42	95.02±21.08 88.45 – 101.59 53 – 146	94.50 0.418 0.485	0.796 0.526	0.107 0.636	
Samples: Age group 26 – 50 years						
Male	44	103.22±27.09 94.98 – 111.46 47 - 163	102.00 0.246 -0.272	0.776 0.715	0.519 0.463	0.229
Female	92	110.22±32.31 103.53 – 116.92 39 – 245	111.00 0.883 2.696	0.002 (<0.001)	0.029 0.008	
Samples: Age group > 50 years						
Male	27	91.59±44.85 73.87–109.37 5– 163	98.00 -0.266 -0.817	0.408 0.557	<0.001 (<0.001)	0.017
Female	32	120.28±44.31 104.30 – 136.25 41– 228	124.50 0.168 0.306	0.484 0.930	0.011 0.115	

Table 16.12. Distribution and mean differences of Post lunch LDL Cholesterol (mg/dL) in samples of age group 18 – 25 years, 26 – 50 years and >50 years

Samples	Sample number (n)	Mean±SD 95%CI for mean Range: Min –Max	Median Skewness kurtosis	Shapiro – Wilk Jarque Bera (Log transformation)		Student’s t test/Mann- Whitney U Test (P)
				Before	After	
Samples: Age group 18 – 25 years						
Male	20	92.25±42.30 75.45 – 115.04 31 – 204	93.00 0.812 0.889	0.370 0.367	0.779 0.772	0.940
Female	42	91.57±20.28 85.24 – 97.89 45– 140	89.00 0.480 1.050	0.008 0.273	0.009 0.088	
Samples: Age group 26 – 50 years						
Male	41	89.70±32.33 79.50 – 99.91 17 - 157	93.00 -0.025 -0.103	0.906 0.952	0.001 (<0.001)	0.011
Female	96	105.28±32.23 98.74 – 111.81 30 – 233	104.50 0.574 2.061	0.019 (<0.001)	<0.001 <0.001	
Samples: Age group > 50 years						
Male	30	85.70±42.45 69.84–101.55 7 – 158	89.50 -0.156 -0.889	0.570 0.539	<0.001 (<0.001)	0.012
Female	33	113.93±43.57 98.48 – 129.39 33 – 225	120.00 0.250 0.388	0.663 0.840	0.028 0.090	

Figure. 16.1 Comparison of sample distribution of Fasting Total Cholesterol (mg/dL) in 18 – 25 years of age (Fig 1A and Fig 1B), samples of age 26 - 50 years (Fig 1C and Fig 1D), samples of age >50 years (Fig 1E and Fig 1F) visualized by Normal Q-Q plot.

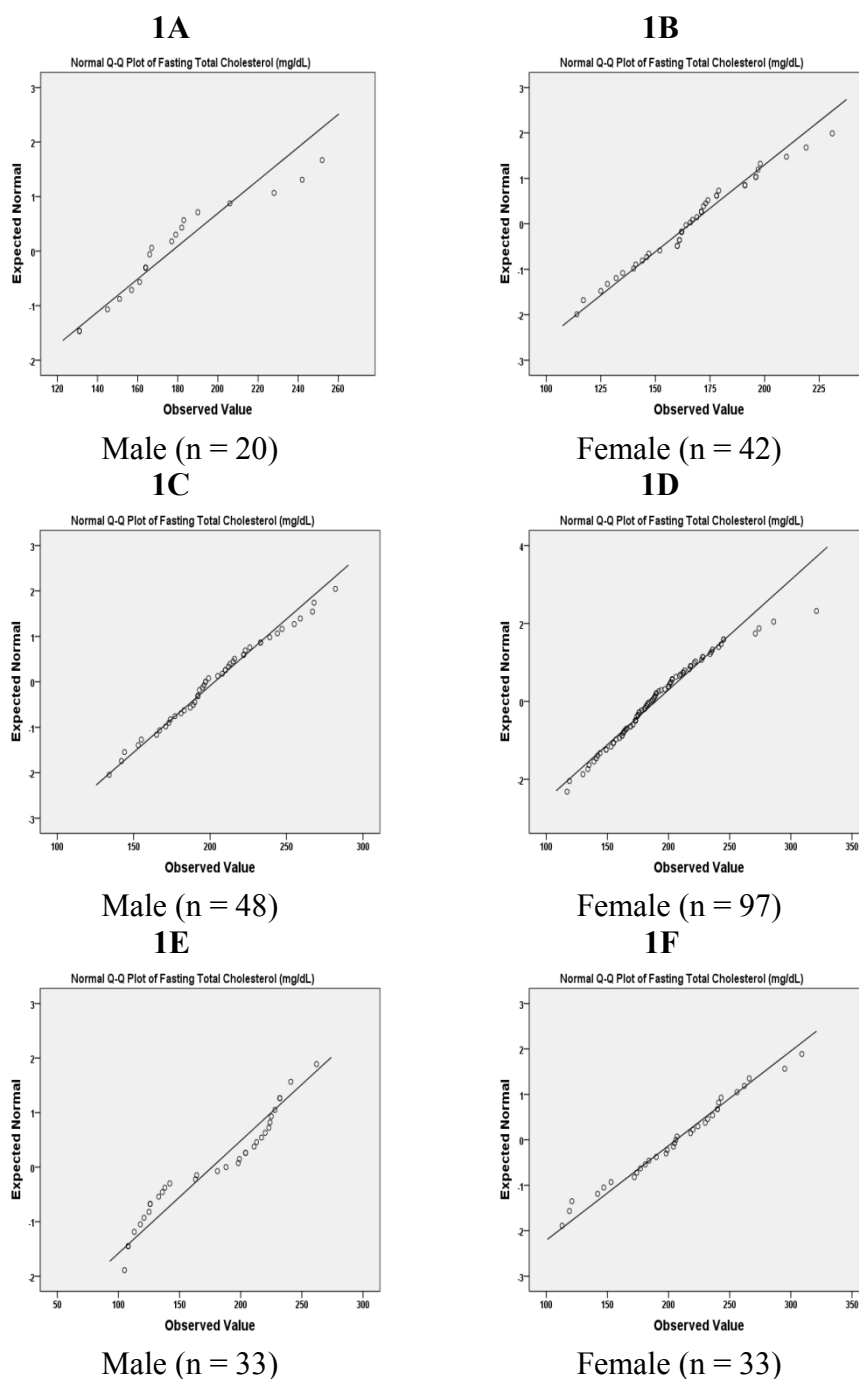


Figure. 16.2 Comparison of sample distribution Post Breakfast Total Cholesterol (mg/dL) in 18 – 25 years of age (Fig 2A and Fig 2B), samples of age 26 - 50 years (Fig 2C and Fig 2D); samples of age >50 years (Fig 2E and Fig 2F) visualized by Normal Q-Q plot

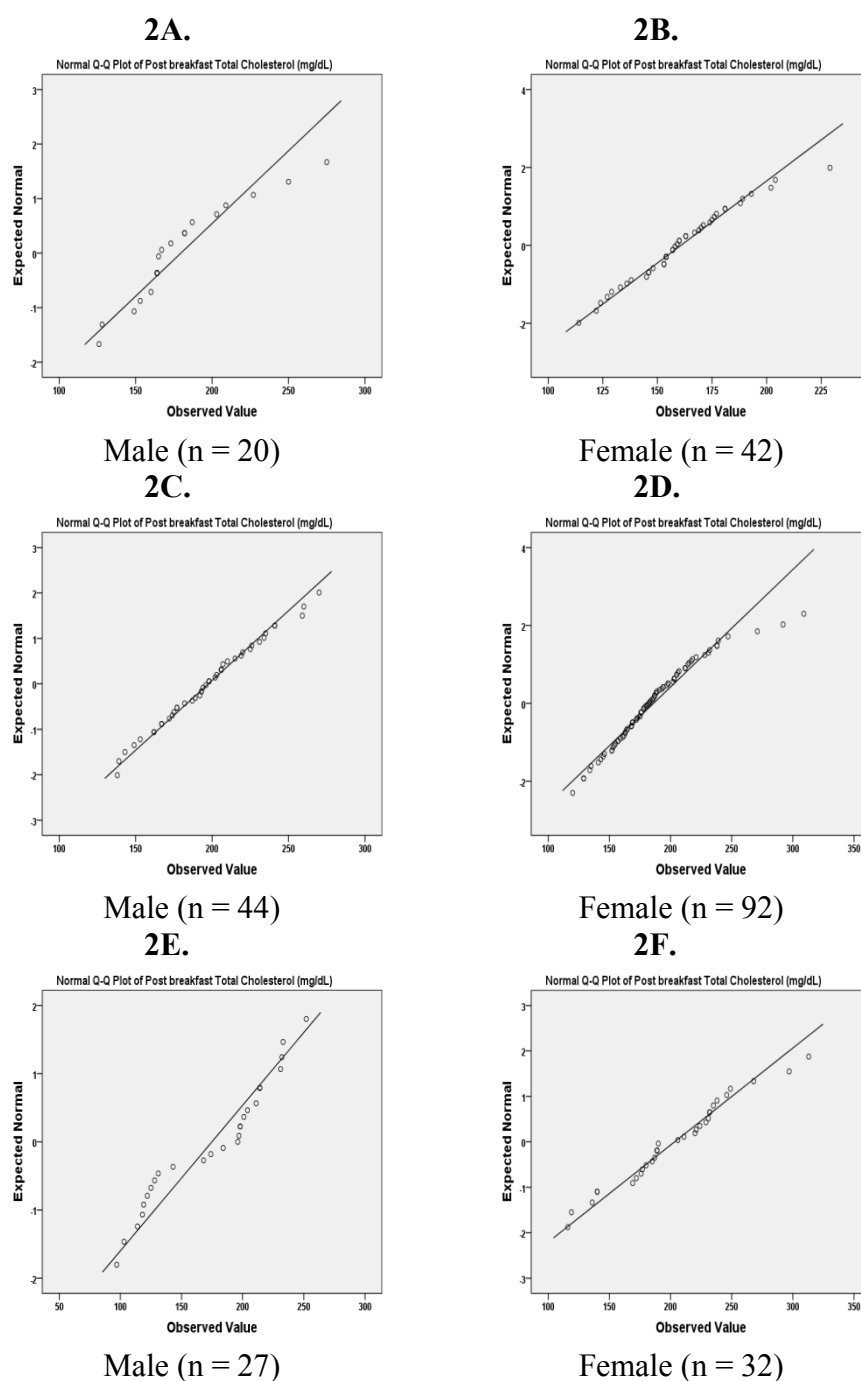


Figure.16.3 Comparison of sample distribution Post Lunch Total Cholesterol (mg/dL) in 18 – 25 years of age (Fig 3A and Fig 3B), samples of age 26 - 50 years (Fig 3C and Fig 3D); samples of age >50 years (Fig 3E and Fig 3F) visualized by Normal Q-Q plot

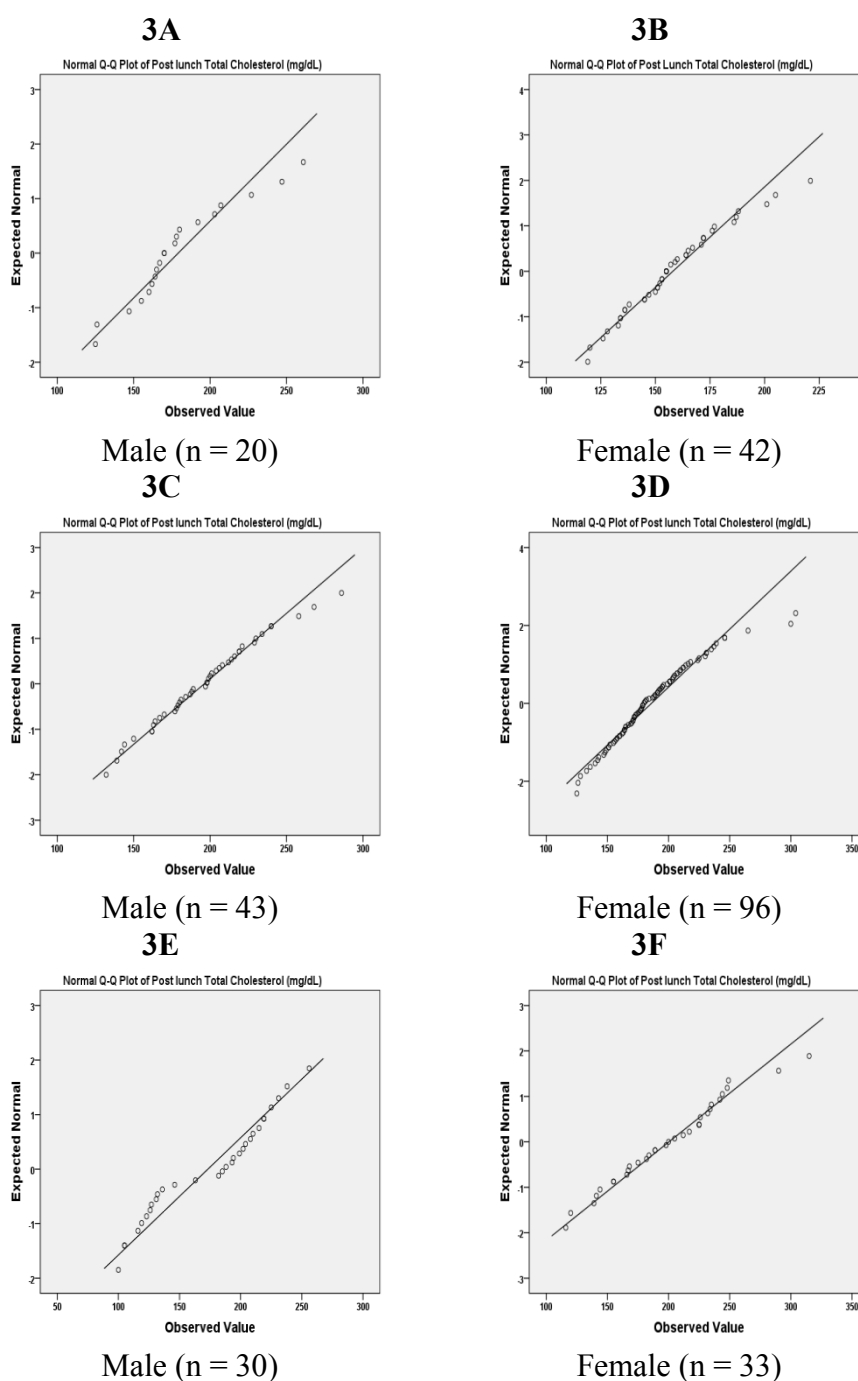


Figure. 16.4 Comparison of sample distribution of Fasting serum Triglycerides (mg/dL) in 18 – 25 years of age (Fig 4A and Fig 4B), samples of age 26 - 50 years (Fig 4C and Fig 4D); samples of age >50 years (Fig 4E and Fig 4F) visualized by Normal Q-Q plot

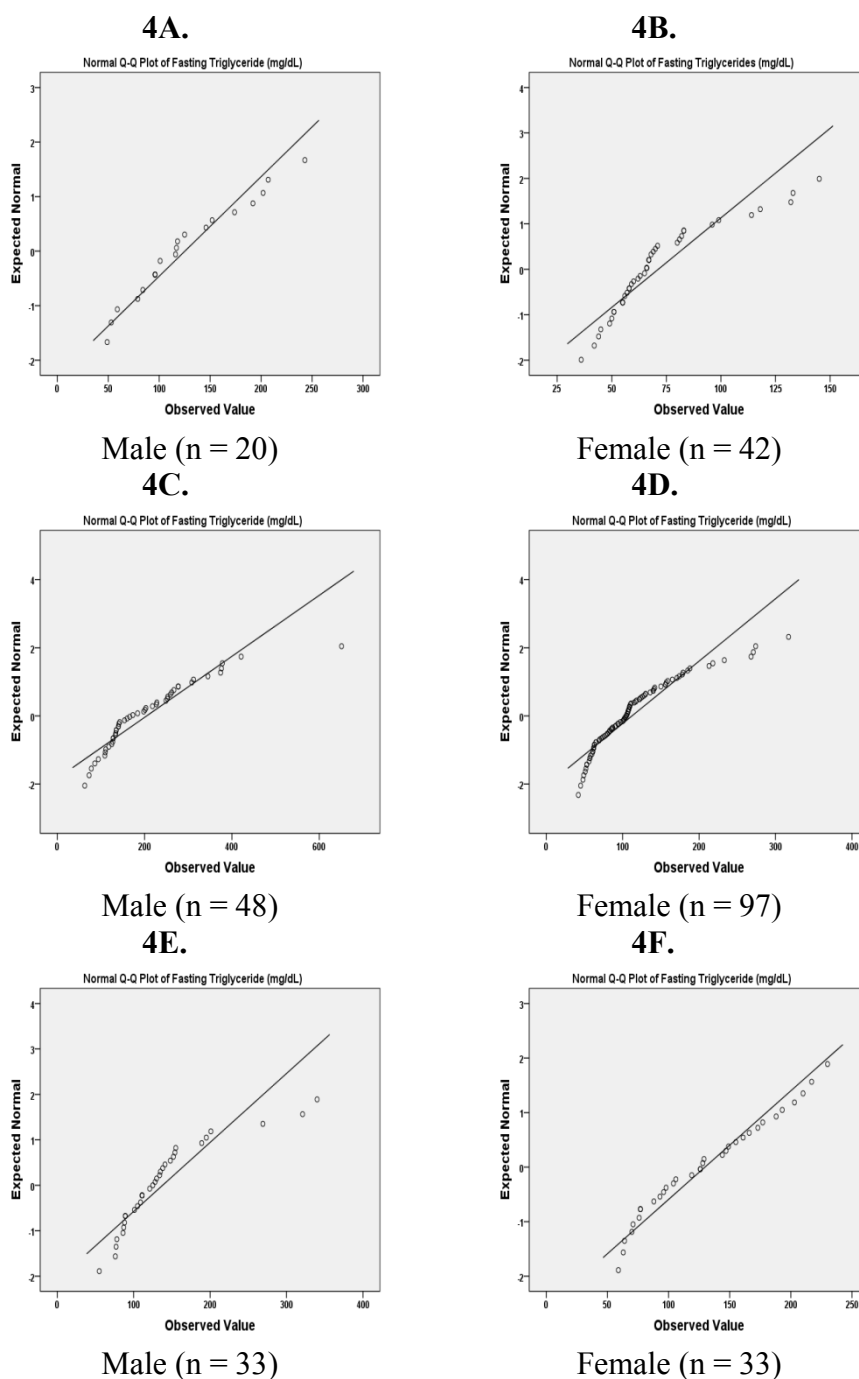


Figure. 16.5 Comparison of sample distribution of Post Breakfast serum Triglycerides (mg/dL) in 18 – 25 years of age (Fig 5A and Fig 5B), samples of age 26 - 50 years (Fig 5C and Fig 5D); samples of age >50 years (Fig 5E and Fig 5F) visualized by Normal Q-Q plot

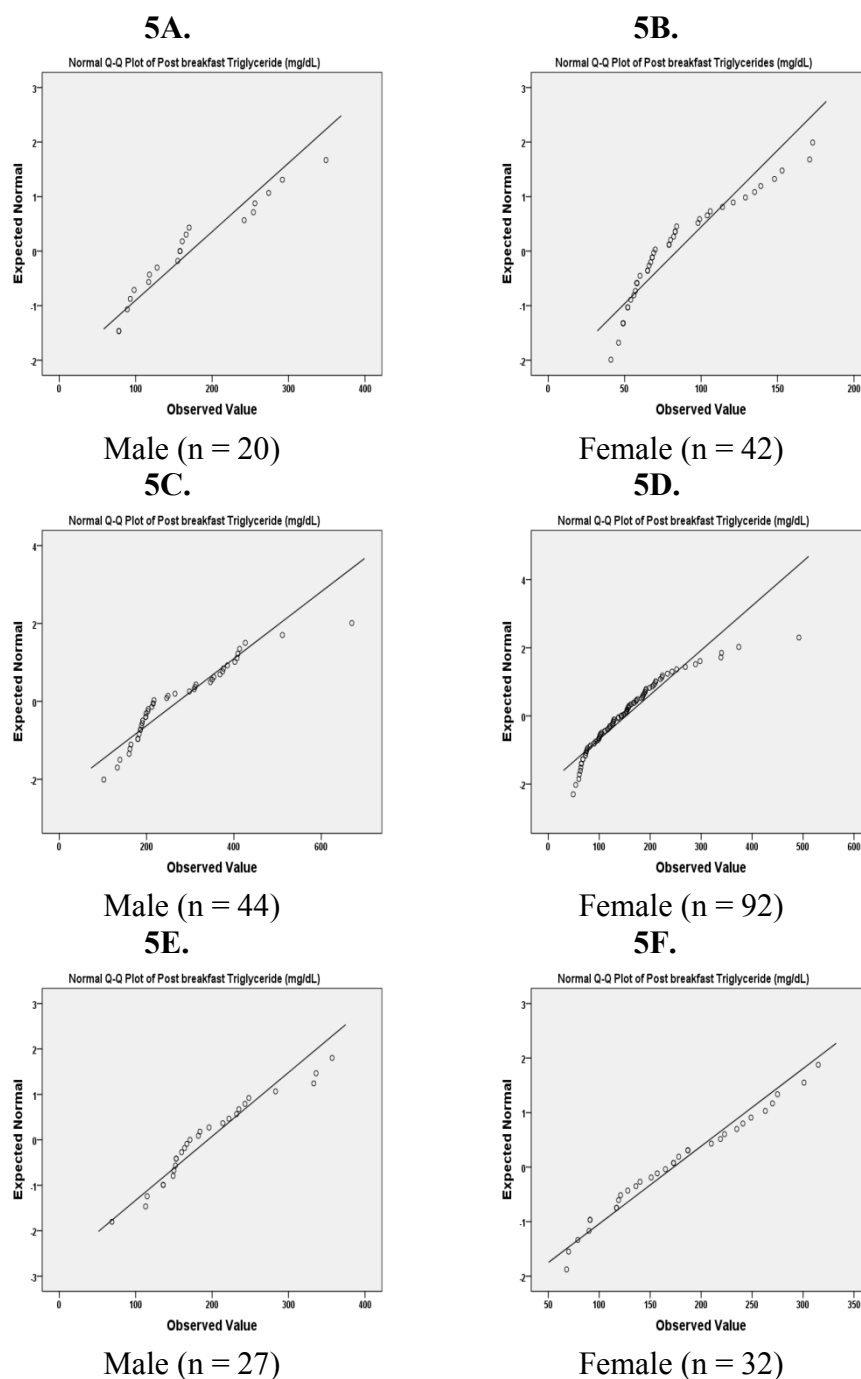


Figure. 16.6 Comparison of sample distribution of Post Lunch serum Triglycerides (mg/dL) in 18 – 25 years of age (Fig 6A and Fig 6B), samples of age 26 - 50 years (Fig 6C and Fig 6D); samples of age >50 years (Fig 6E and Fig 6F) visualized by Normal Q-Q plot

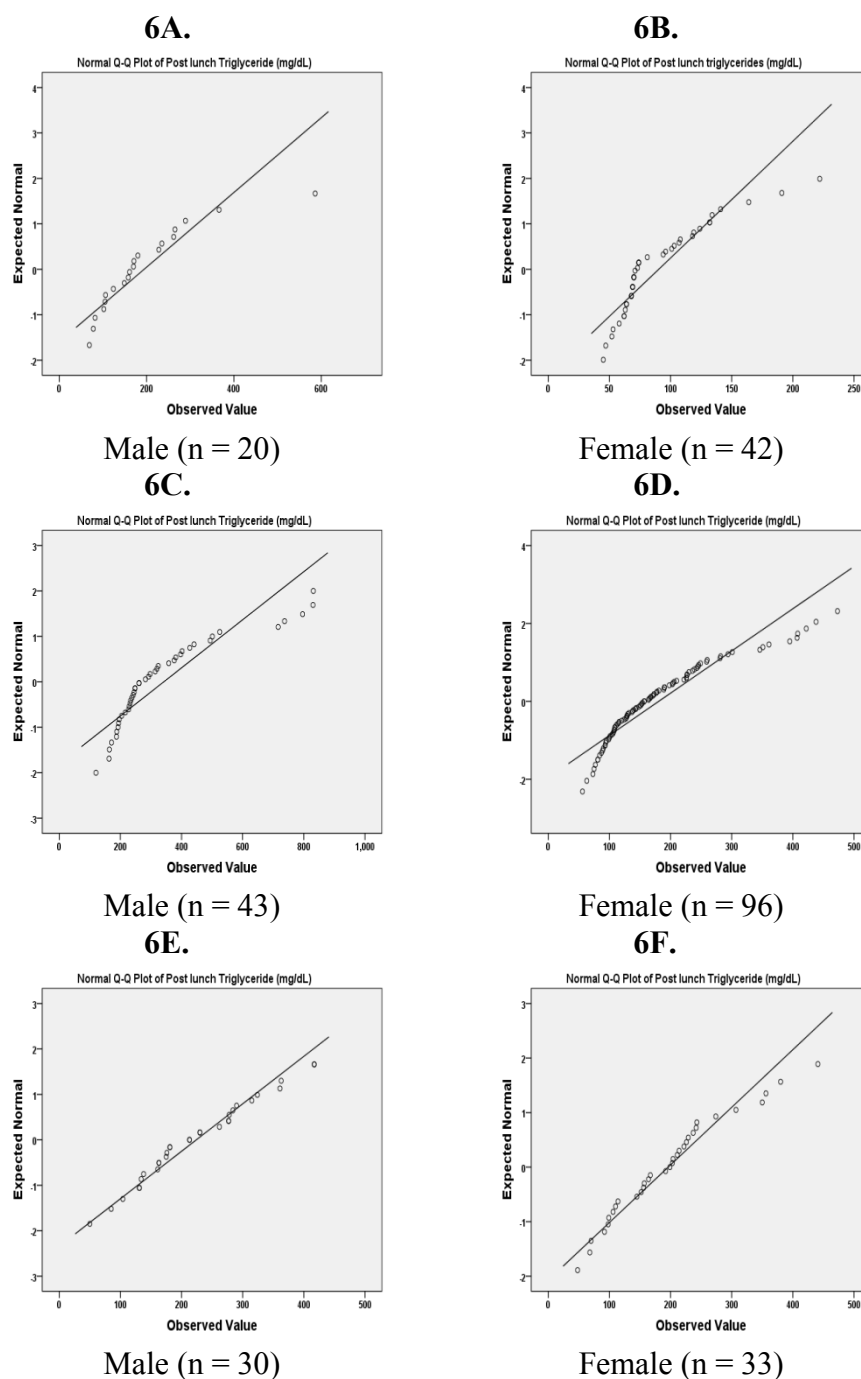


Figure 16.7. Comparison of sample distribution of Fasting HDL Cholesterol(mg/dL) in 18 – 25 years of age (Fig 7A and Fig 7B), samples of age 26 - 50 years (Fig 7C and Fig 7D); samples of age >50 years (Fig 7E and Fig 7F) visualized by Normal Q-Q plot

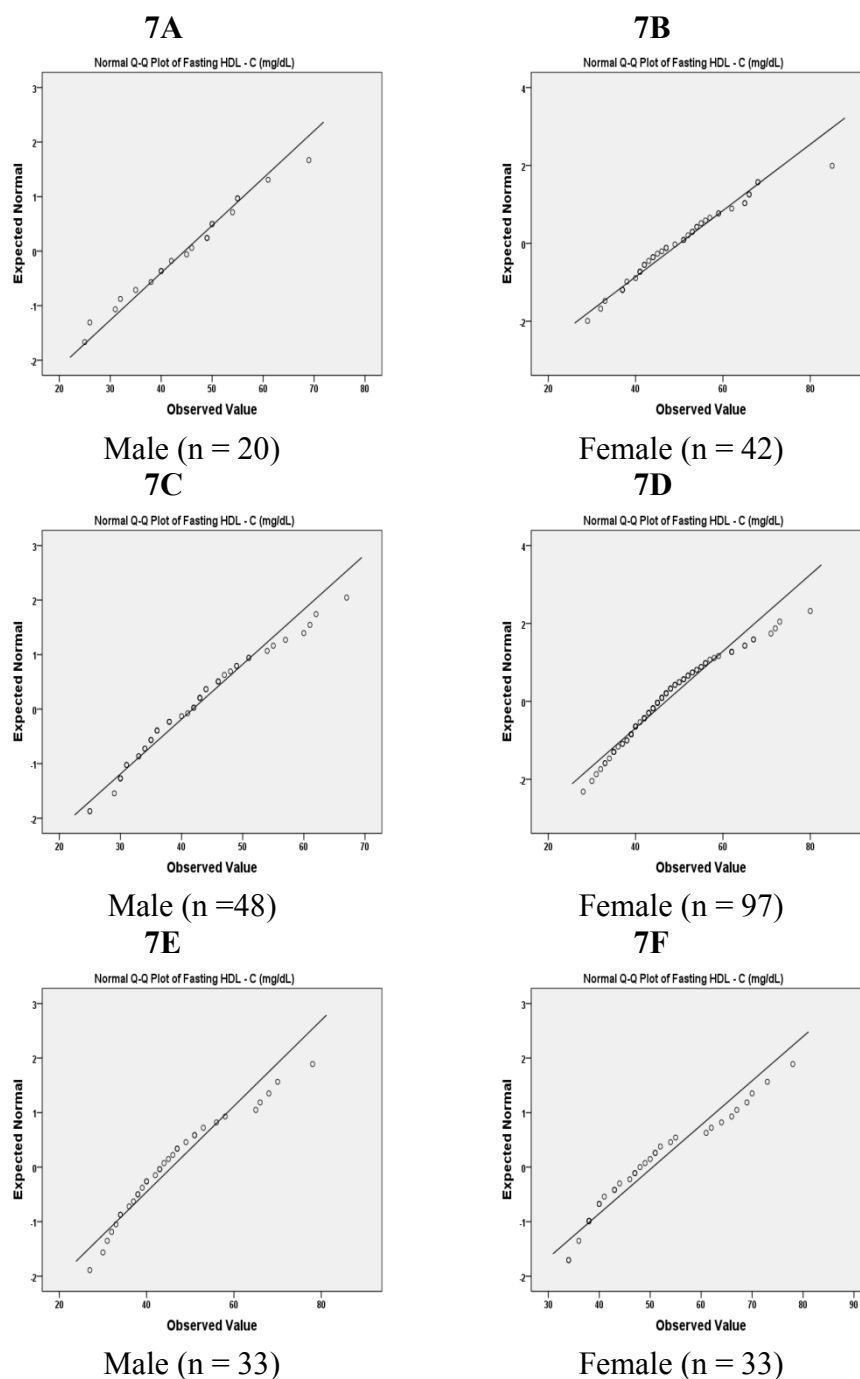


Figure. 16.8. Comparison of sample distribution of Post Breakfast HDL Cholesterol (mg/dL) in 18 – 25 years of age (Fig 8A and Fig 8B), samples of age 26 - 50 years (Fig 8C and Fig 8D); samples of age >50 years (Fig 8E and Fig 8F) visualized by Normal Q-Q plot

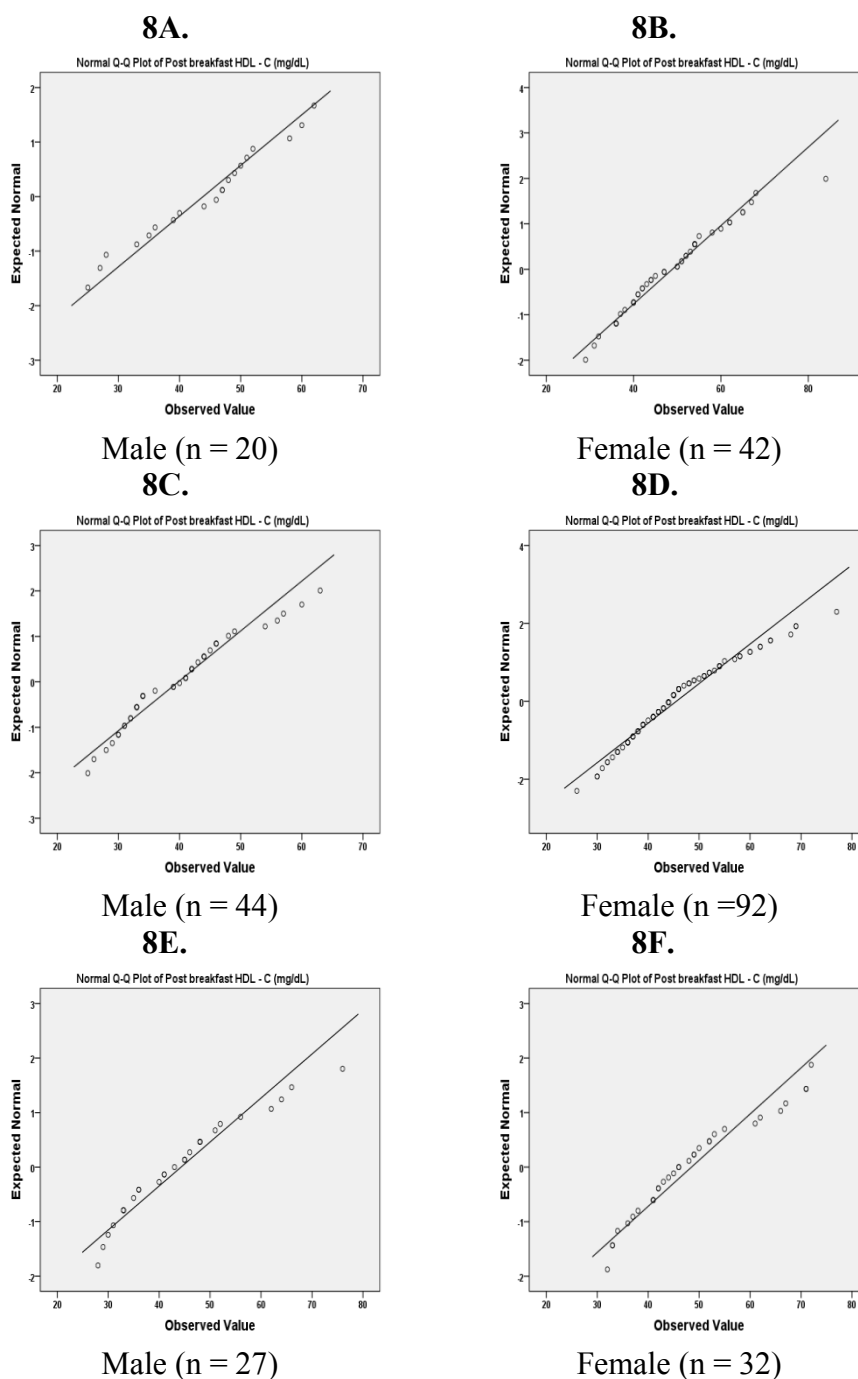


Figure. 16.9. Comparison of sample distribution of Post Lunch HDL Cholesterol (mg/dL) in 18 – 25 years of age (Fig 9A and Fig 9B), samples of age 26 - 50 years (Fig 9C and Fig 9D); samples of age >50 years (Fig 9E and Fig 9F) visualized by Normal Q-Q plot

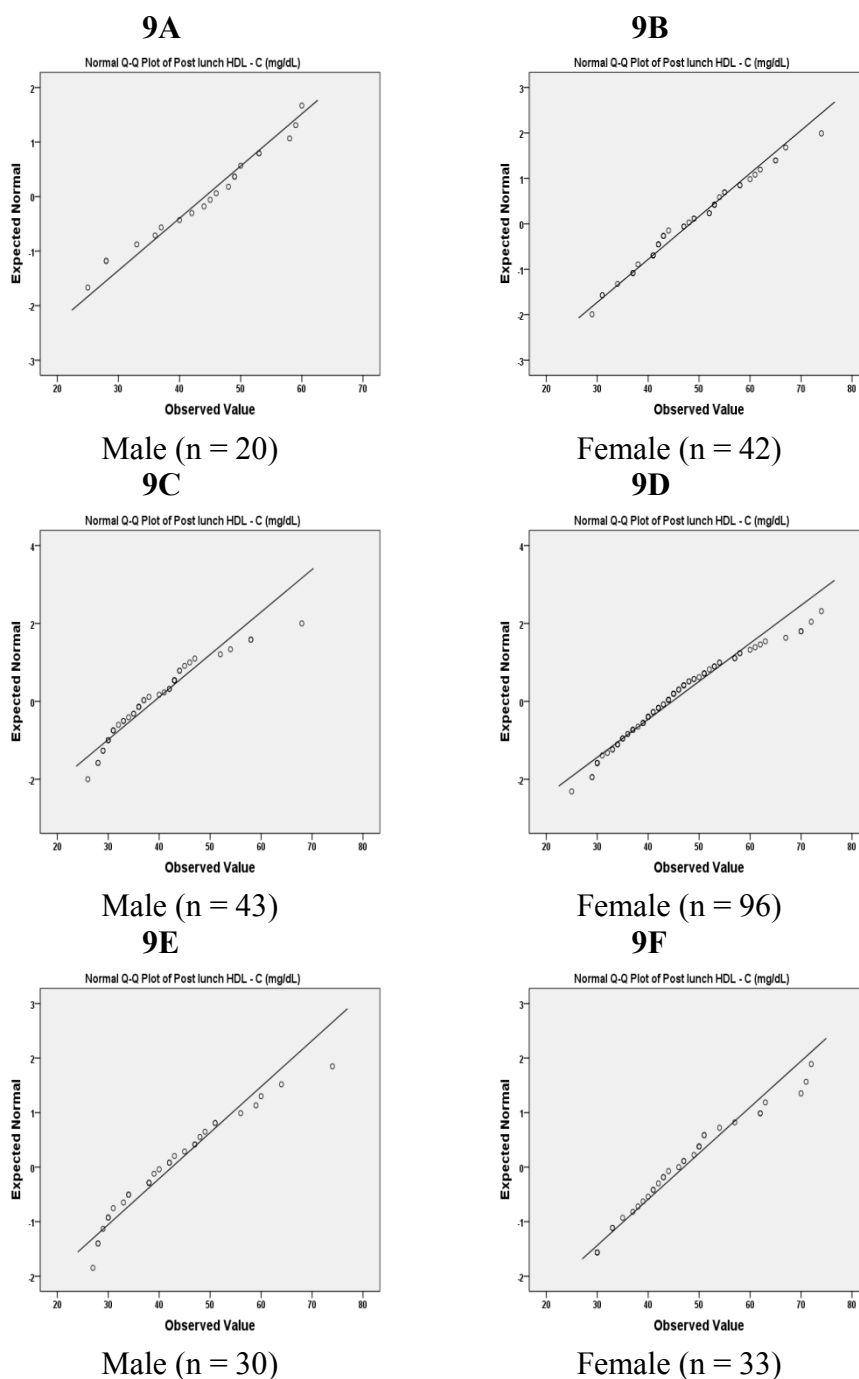


Figure. 16.10. Comparison of sample distribution of Fasting LDL Cholesterol (mg/dL) in 18 – 25 years of age (Fig 10A and Fig 10B), samples of age 26 - 50 years (Fig 10C and Fig 10D); samples of age >50 years (Fig 7E and Fig 7F) visualized by Normal Q-Q plot

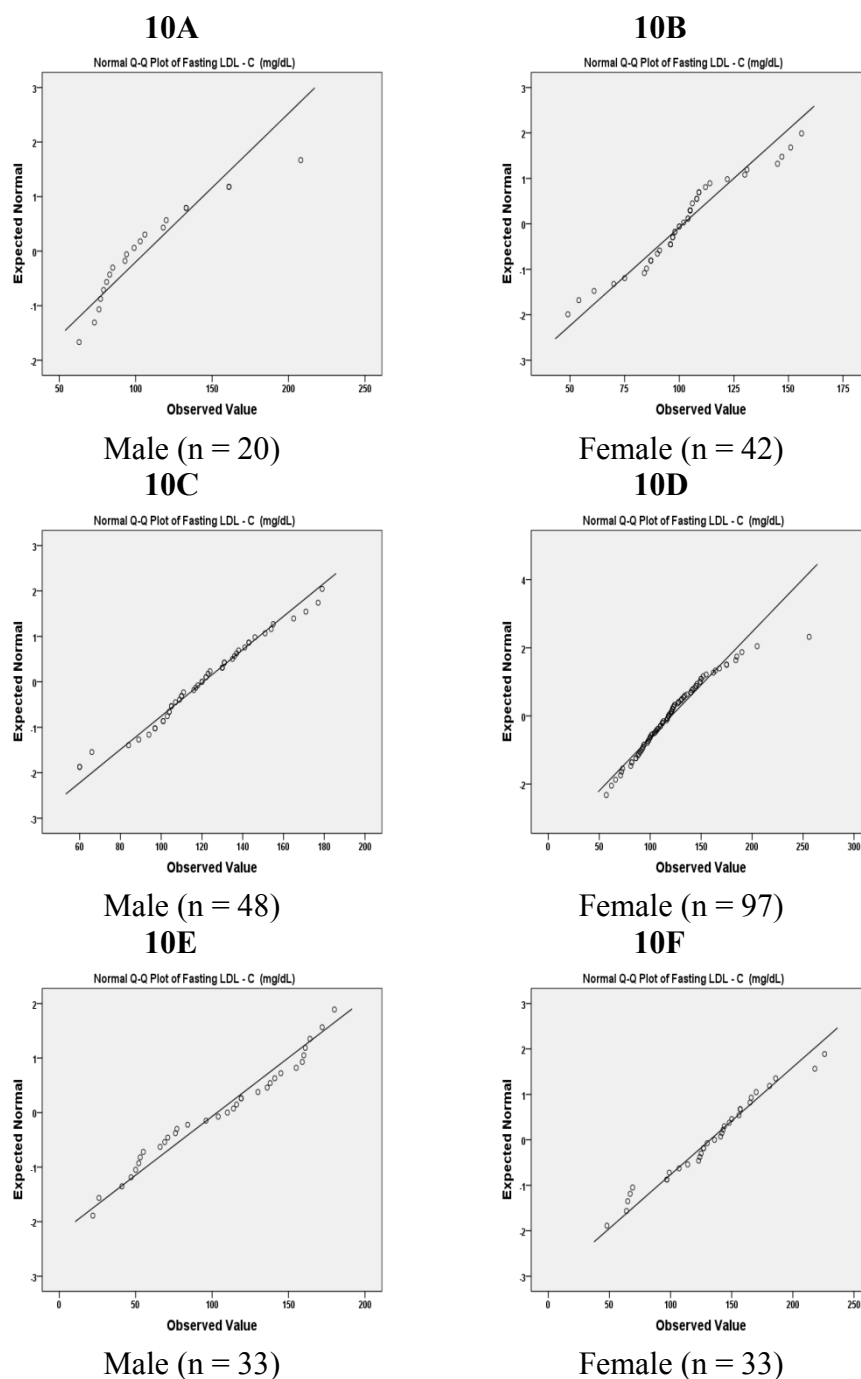


Figure. 16.11. Comparison of sample distribution of Post Breakfast LDL Cholesterol (mg/dL) in 18 – 25 years of age (Fig 11A and Fig 11B), samples of age 26 - 50 years (Fig 11C and Fig 11D); samples of age >50 years (Fig 11E and Fig 11F) visualized by Normal Q-Q plot

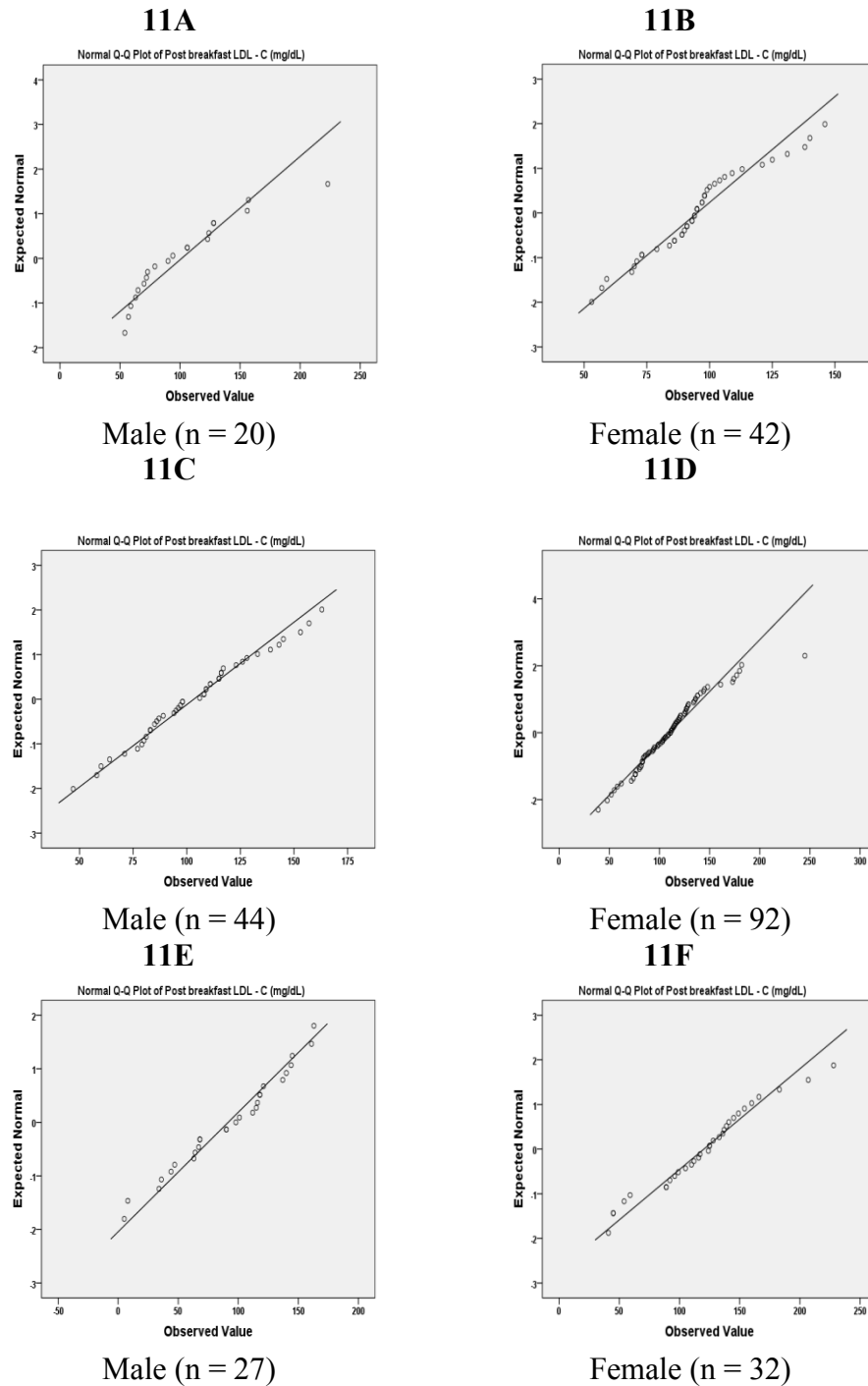


Figure. 16.12. Comparison of sample distribution of Post Lunch LDL Cholesterol (mg/dL) in 18 – 25 years of age (Fig 12A and Fig 12B), samples of age 26 - 50 years (Fig 12C and Fig 12D); samples of age >50 years (Fig 9E and Fig 9F) visualized by Normal Q-Q plot

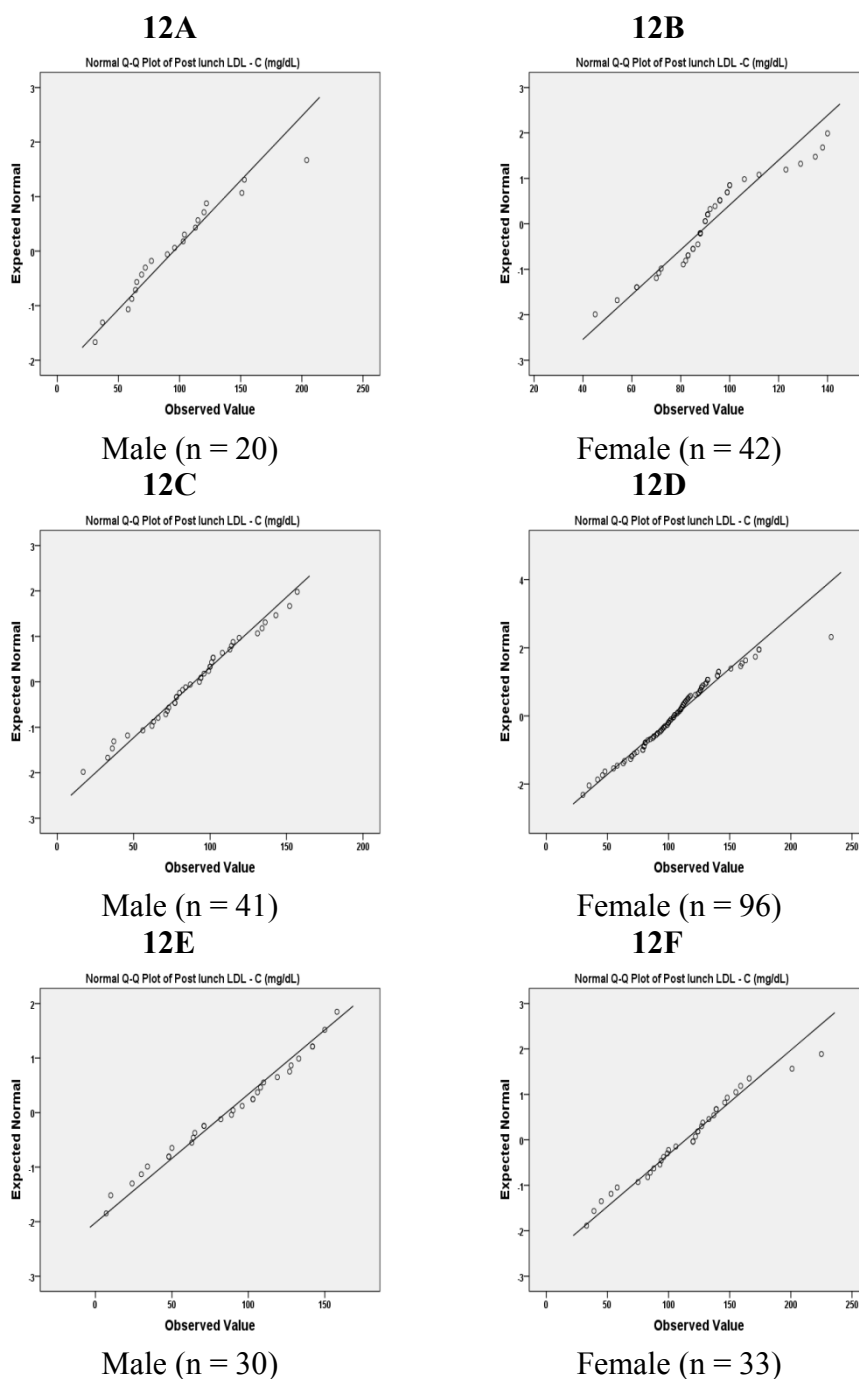


Figure. 16.13 Comparison of sample distribution of Fasting Total Cholesterol (mg/dL) in 18 – 25 years of age (Fig 13A and Fig 13B), samples of age 26 - 50 years (Fig 13C and Fig 13D); samples of age >50 years (Fig 1E and Fig 1F) visualized by Histogram

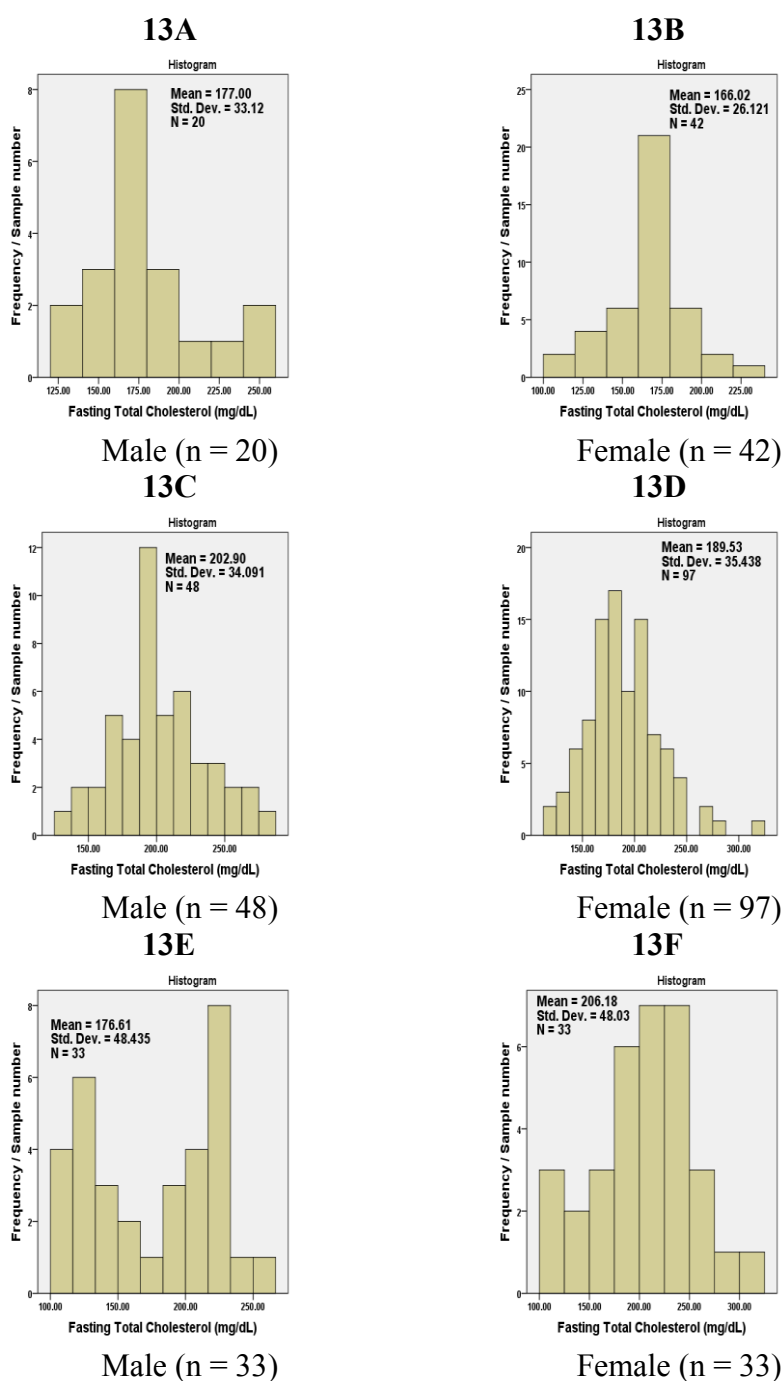


Figure. 16.14. Comparison of sample distribution Post Breakfast Total Cholesterol (mg/dL) in 18 – 25 years of age (Fig 14A and Fig 14B), samples of age 26 - 50 years (Fig 14C and Fig 14D); samples of age >50 years (Fig 14E and Fig 14F) visualized by Histogram

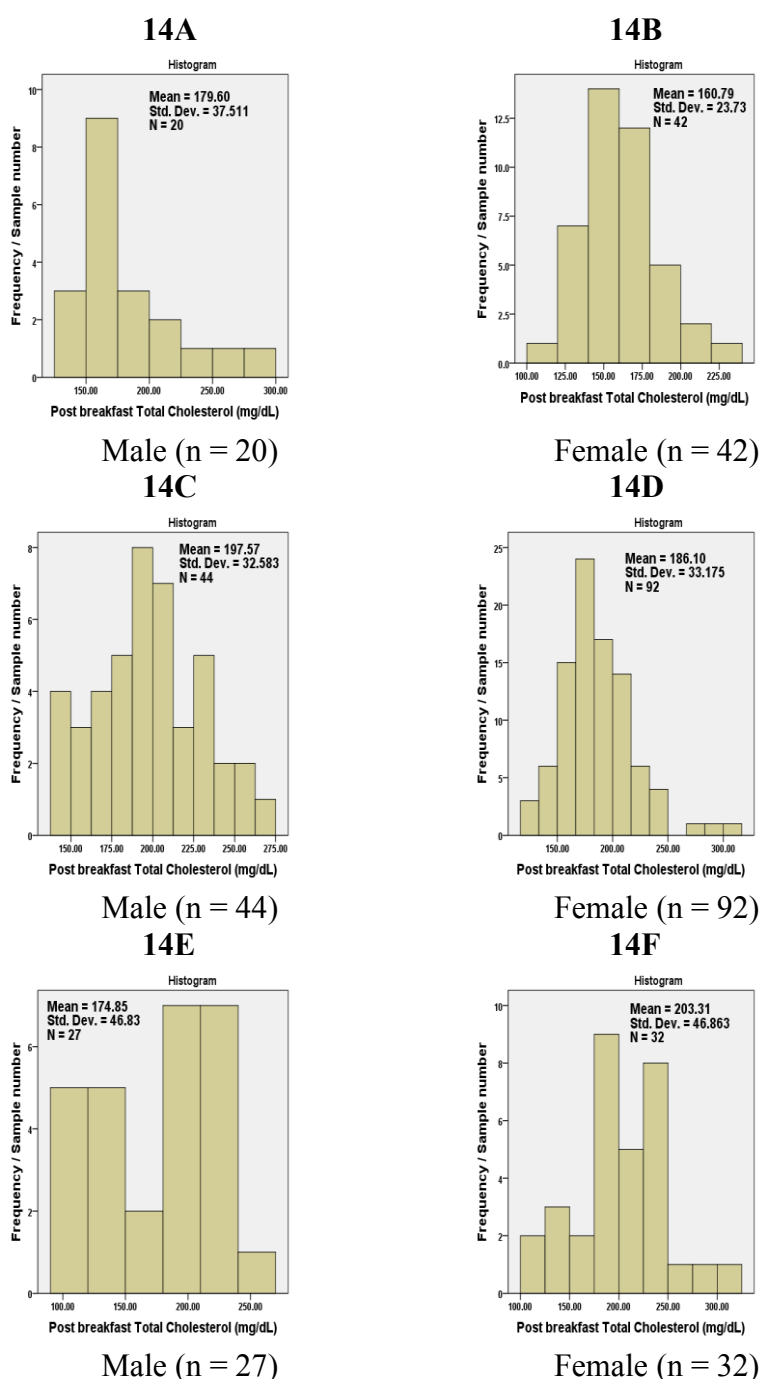


Figure 16.15. Comparison of sample distribution Post Lunch Total Cholesterol (mg/dL) in 18 – 25 years of age (Fig 15A and Fig 15B), samples of age 26 - 50 years (Fig 15C and Fig 15D); samples of age >50 years (Fig 15E and Fig 15F) visualized by Histogram

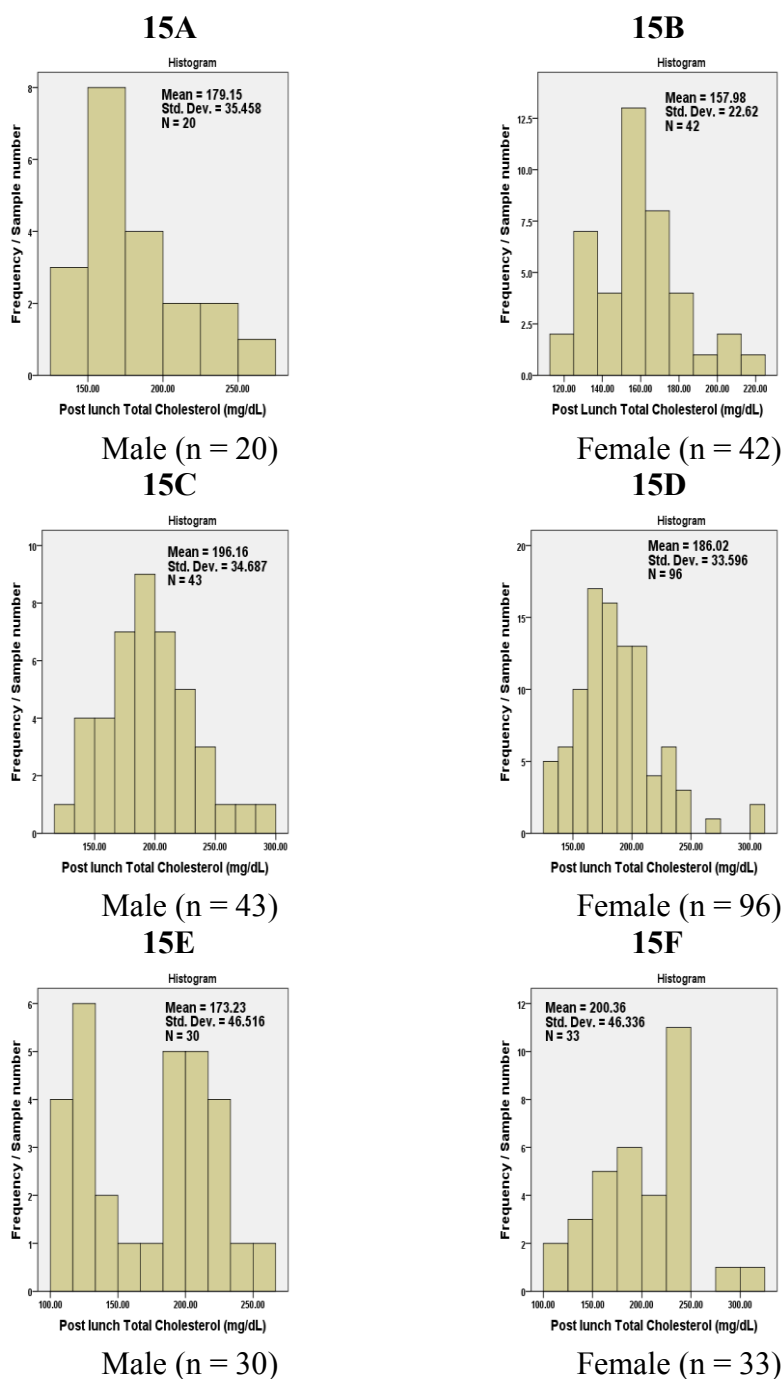


Figure 16.16 Comparison of sample distribution of Fasting serum Triglycerides (mg/dL) in 18 – 25 years of age (Fig 16A and Fig 16B), samples of age 26 - 50 years (Fig 16C and Fig 16D); samples of age >50 years (Fig 16E and Fig 16F) visualized by Histogram

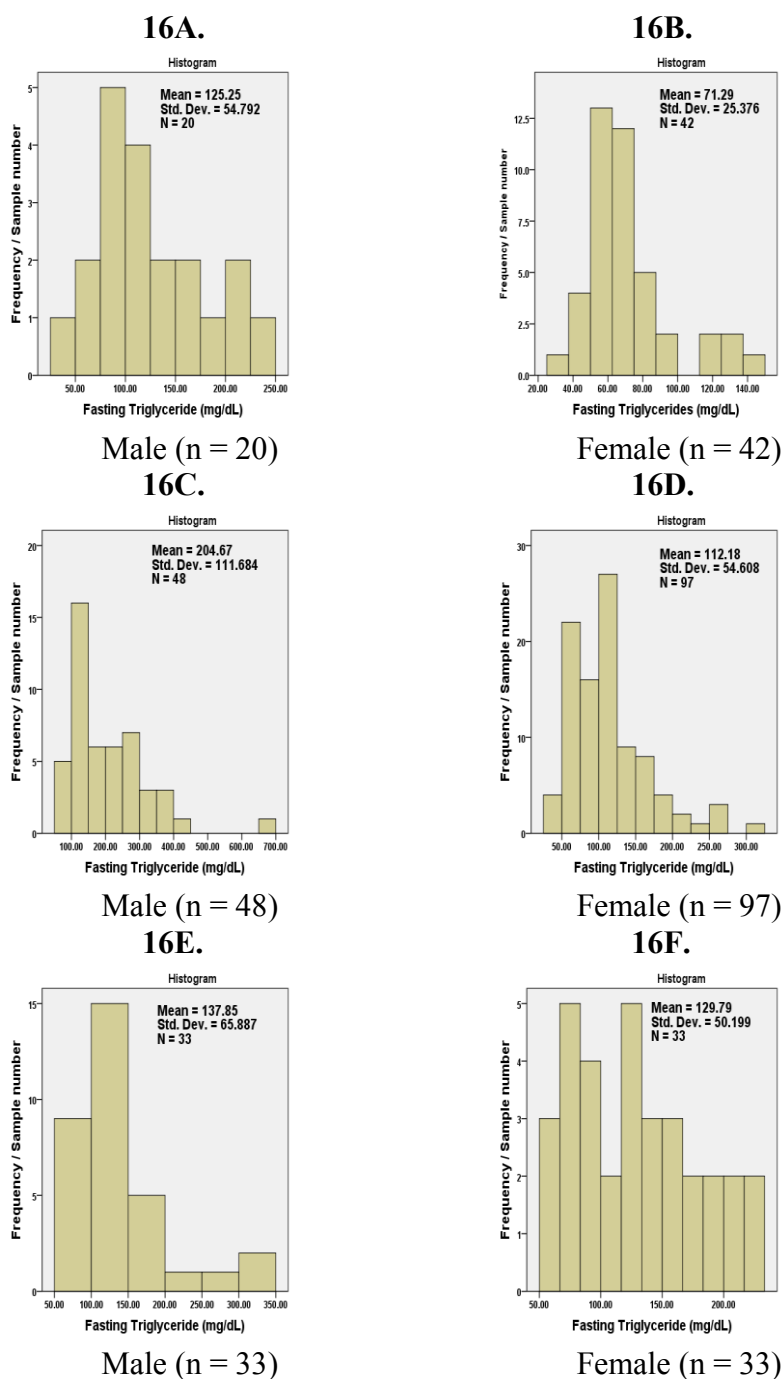


Figure 16.17. Comparison of sample distribution of Post Breakfast serum Triglycerides (mg/dL) in 18 – 25 years of age (Fig 17A and Fig 17B), samples of age 26 - 50 years (Fig 18C and Fig 18D); samples of age >50 years (Fig 18E and Fig 18F) visualized by Histogram

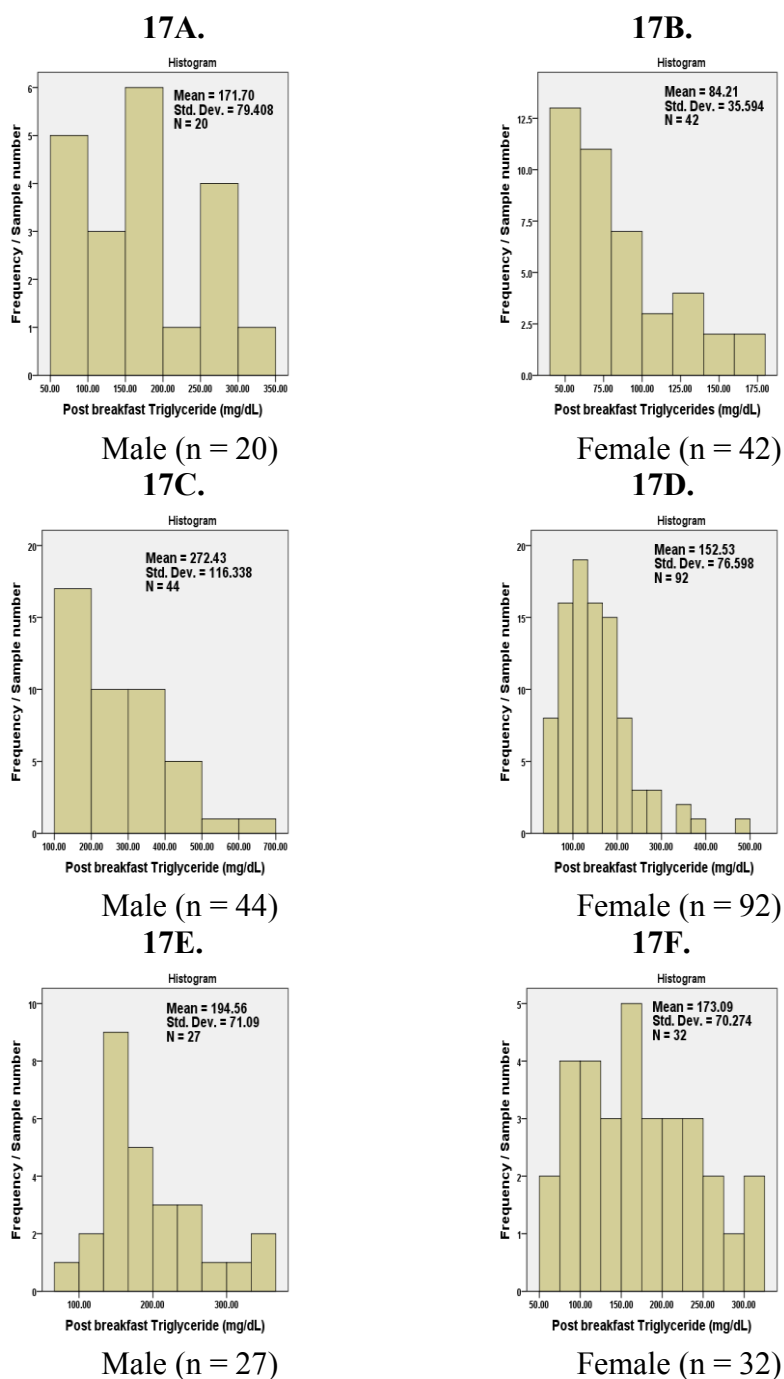


Figure 16.18. Comparison of sample distribution of Post Lunch serum Triglycerides (mg/dL) in 18 – 25 years of age (Fig 18A and Fig 18B), samples of age 26 - 50 years (Fig 18C and Fig 18D); samples of age >50 years (Fig 18E and Fig 18F) visualized by Histogram

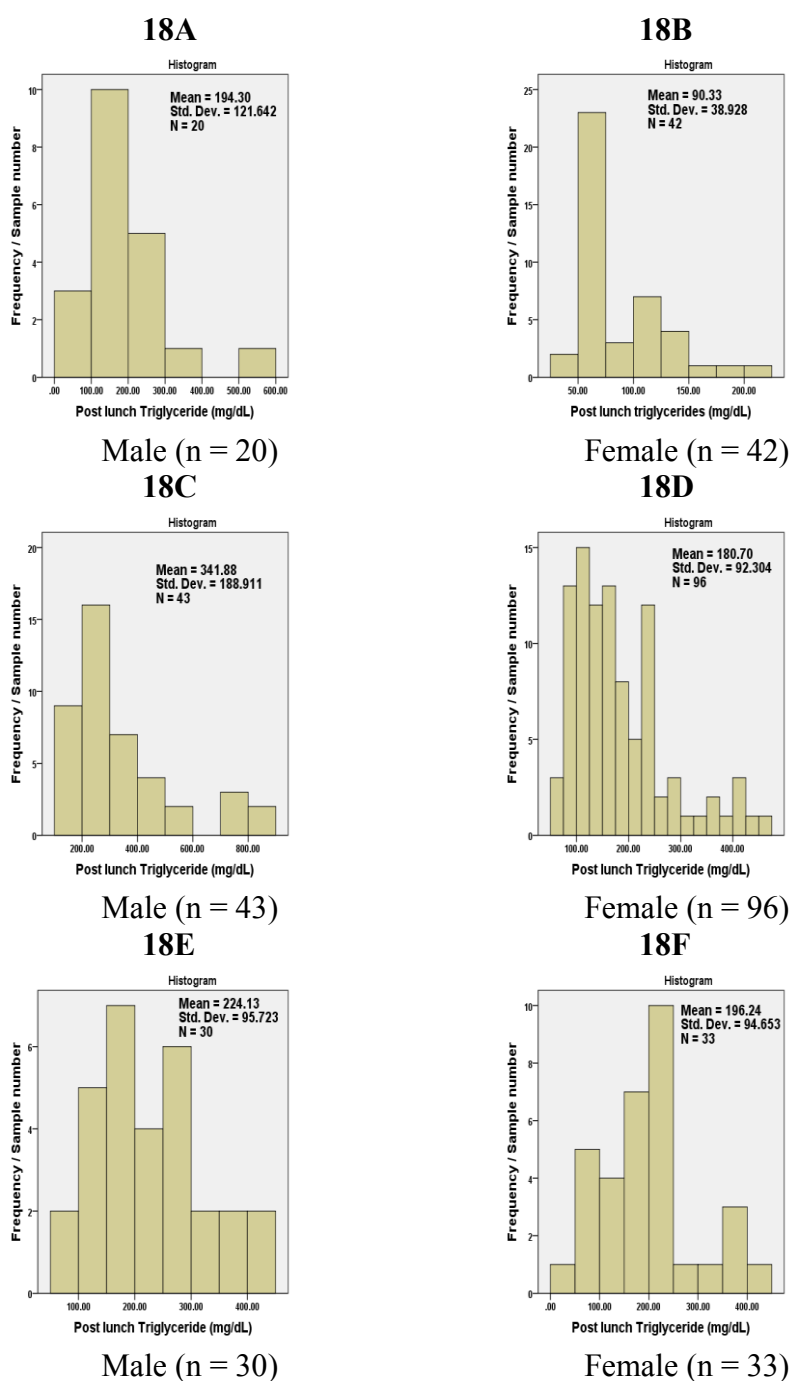


Figure 16.19. Comparison of sample distribution of Fasting HDL Cholesterol (mg/dL) in 18 – 25 years of age (Fig 19A and Fig 19B), samples of age 26 - 50 years (Fig 19C and Fig 19D); samples of age >50 years (Fig 19E and Fig 19F) visualized by Histogram

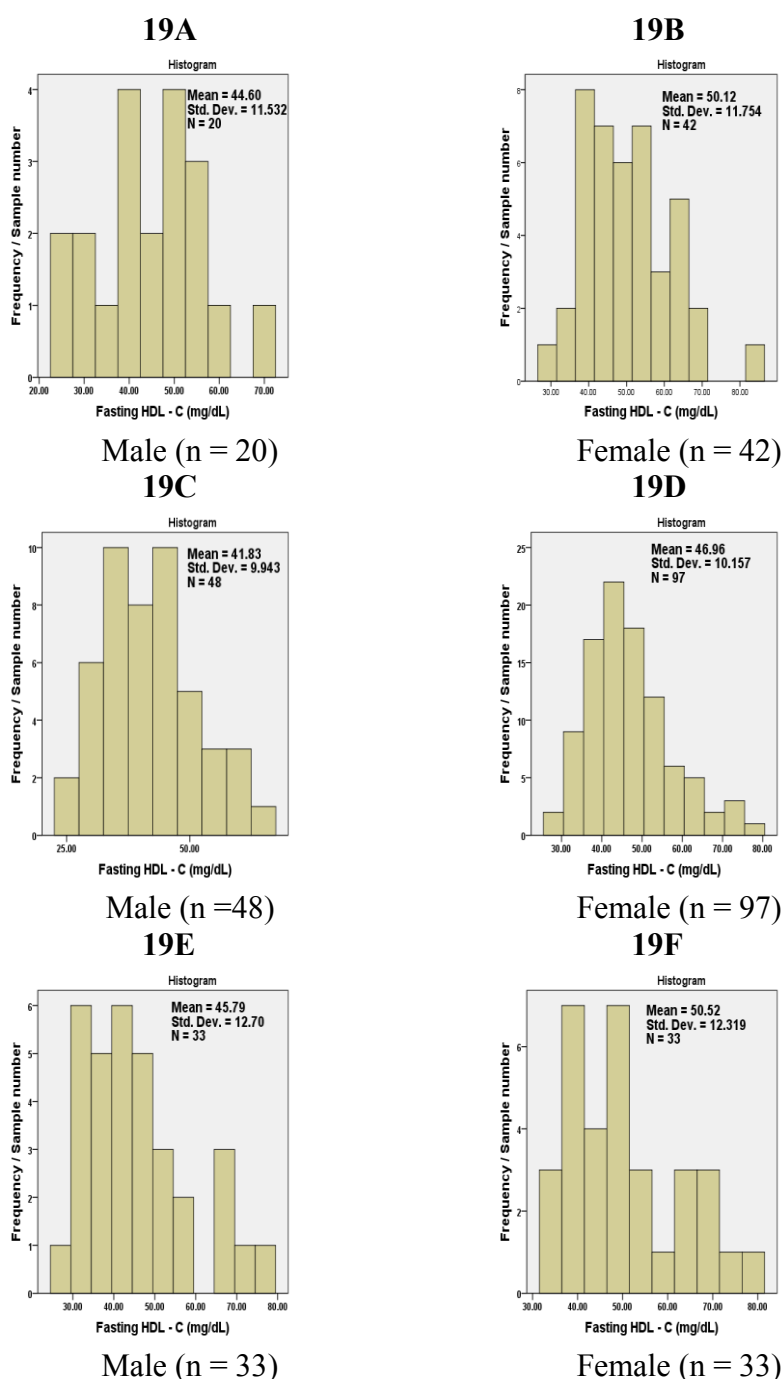


Figure. 16.20. Comparison of sample distribution of Post Breakfast HDL Cholesterol (mg/dL) in 18 – 25 years of age (Fig 20A and Fig 20B), samples of age 26 - 50 years (Fig 20C and Fig 20D); samples of age >50 years (Fig 20E and Fig 20F) visualized by Histogram

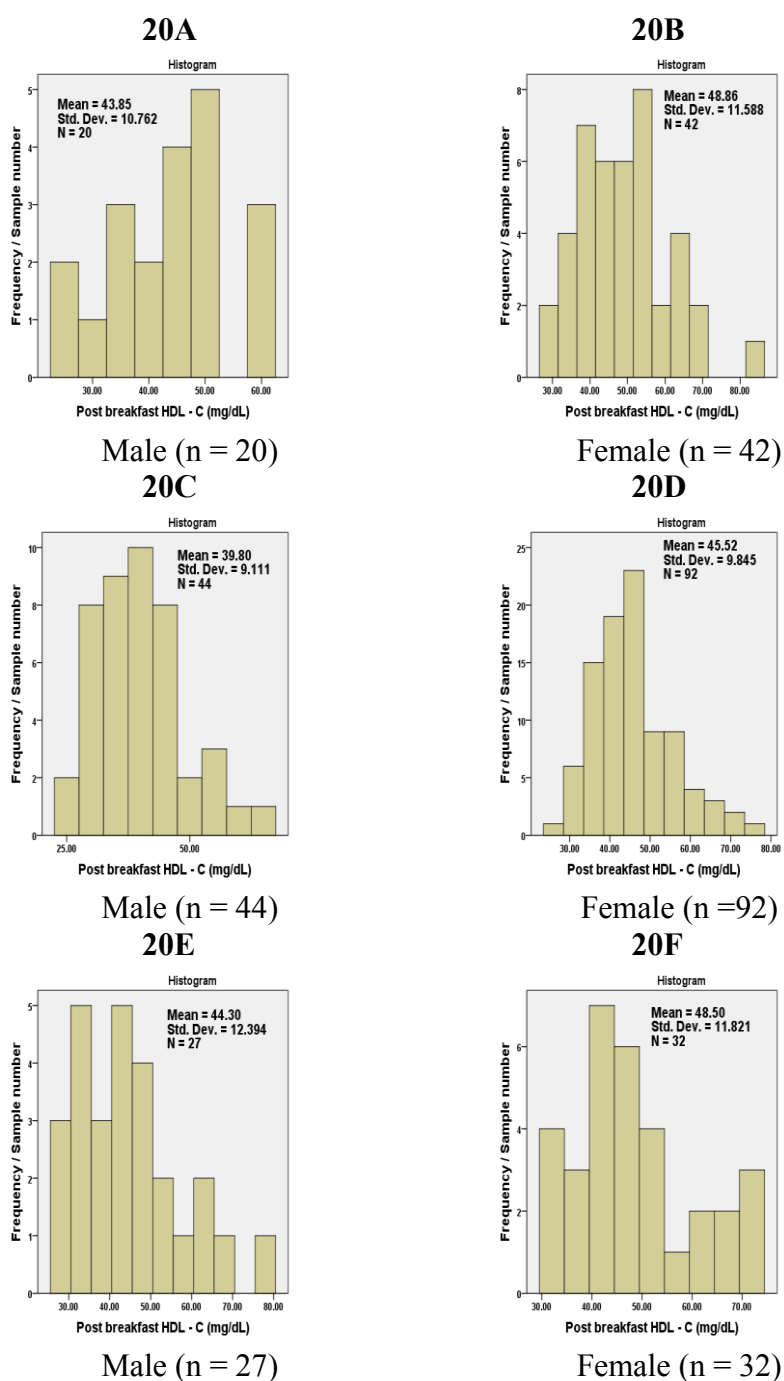


Figure 16.21. Comparison of sample distribution of Post Lunch HDL Cholesterol (mg/dL) in 18 – 25 years of age (Fig 21A and Fig 21B), samples of age 26 - 50 years (Fig 21C and Fig 21D); samples of age >50 years (Fig 21E and Fig 21F) visualized by Histogram

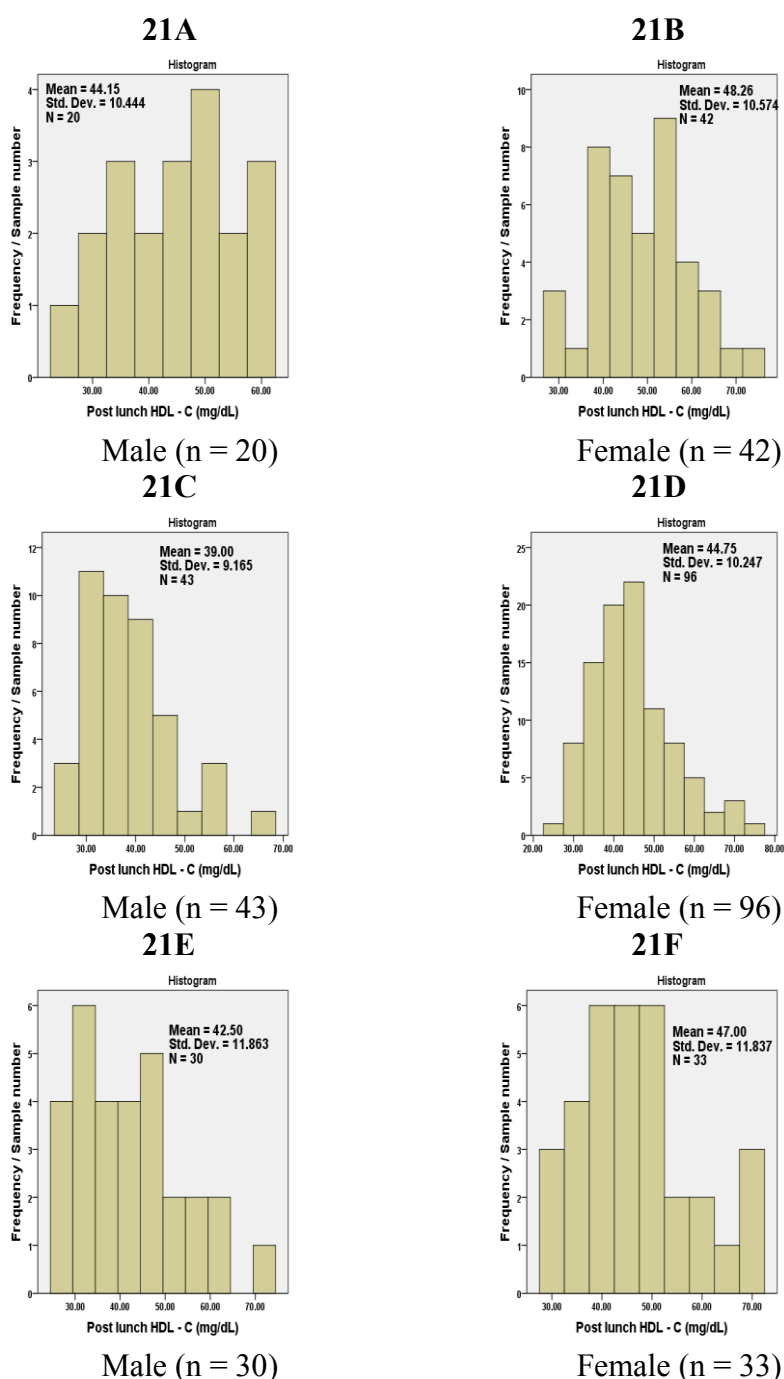


Figure 16.22. Comparison of sample distribution of Fasting LDL Cholesterol (mg/dL) in 18 – 25 years of age (Fig 22A and Fig 22B), samples of age 26 - 50 years (Fig 22C and Fig 22D); samples of age >50 years (Fig 22E and Fig 22F) visualized by Histogram

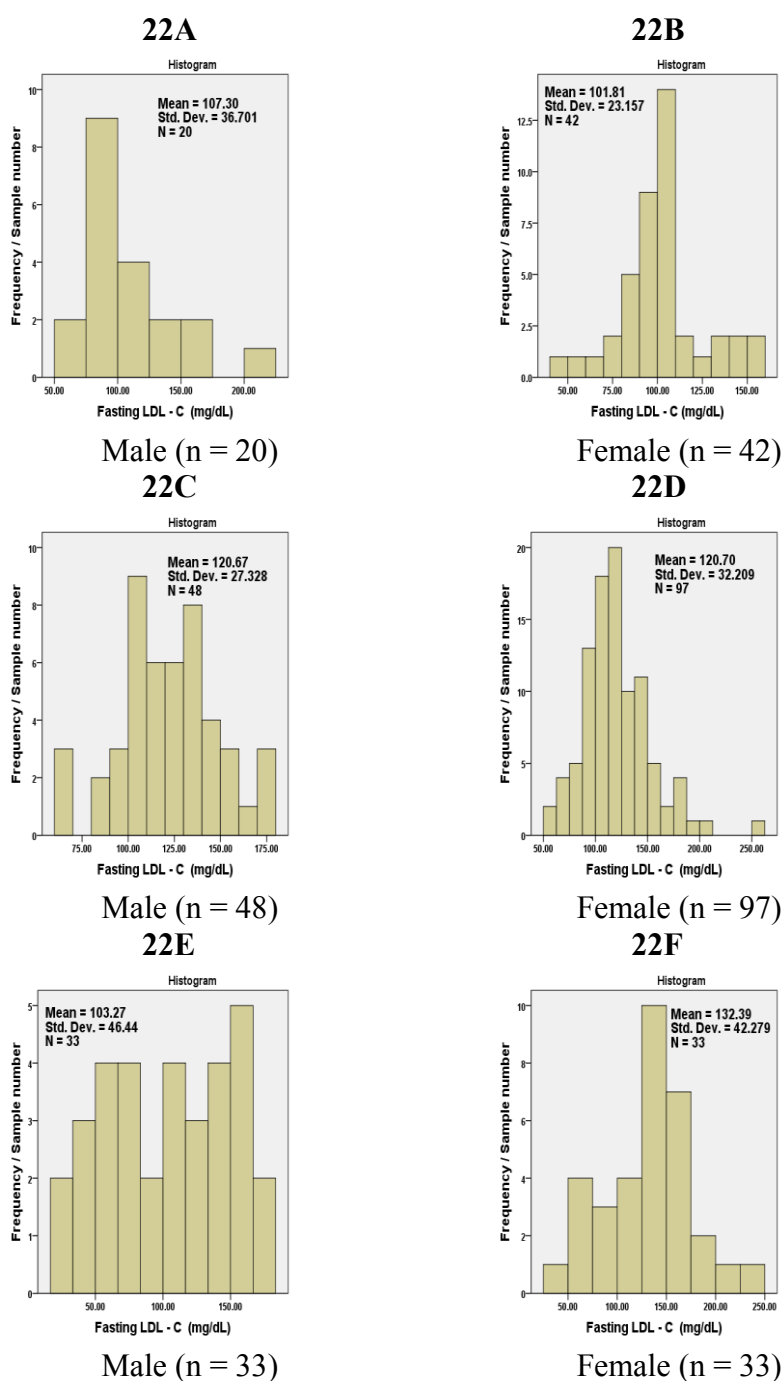


Figure 16.23. Comparison of sample distribution of Post Breakfast LDL Cholesterol (mg/dL) in 18 – 25 years of age (Fig 23A and Fig 23B), samples of age 26 - 50 years (Fig 23C and Fig 23D); samples of age >50 years (Fig 23E and Fig 23F) visualized by Histogram

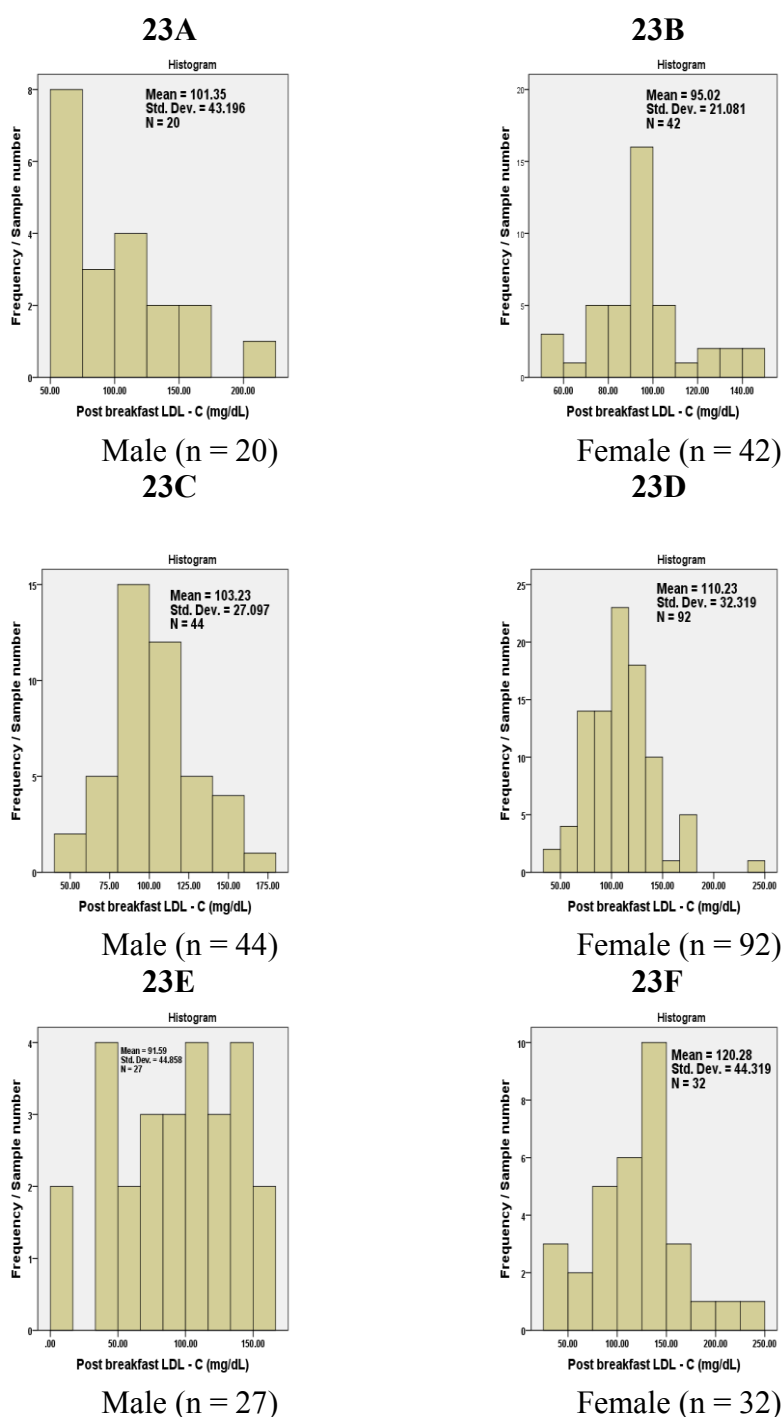


Figure 16.24. Comparison of sample distribution of Post Lunch LDL Cholesterol (mg/dL) in 18 – 25 years of age (Fig 24A and Fig 24B), samples of age 26 - 50 years (Fig 24C and Fig 24D); samples of age >50 years (Fig 24E and Fig 24F) visualized by Histogram

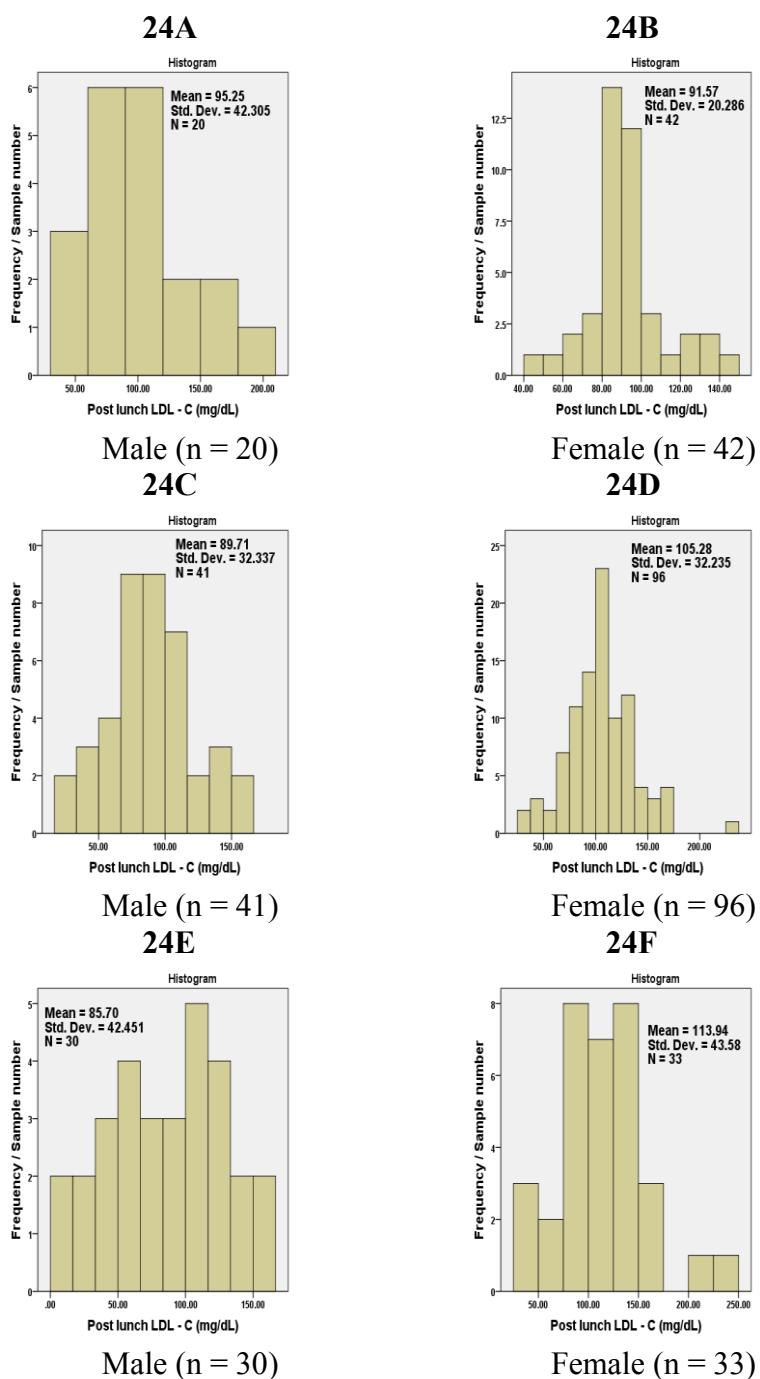


Figure 16.25 Comparison of sample distribution of Fasting Total Cholesterol (mg/dL) in 18 – 25 years of age (Fig 25A and Fig 25B), samples of age 26 - 50 years (Fig 25C and Fig 25D); samples of age >50 years (Fig 25E and Fig 25F) visualized by Box-Whisker plot

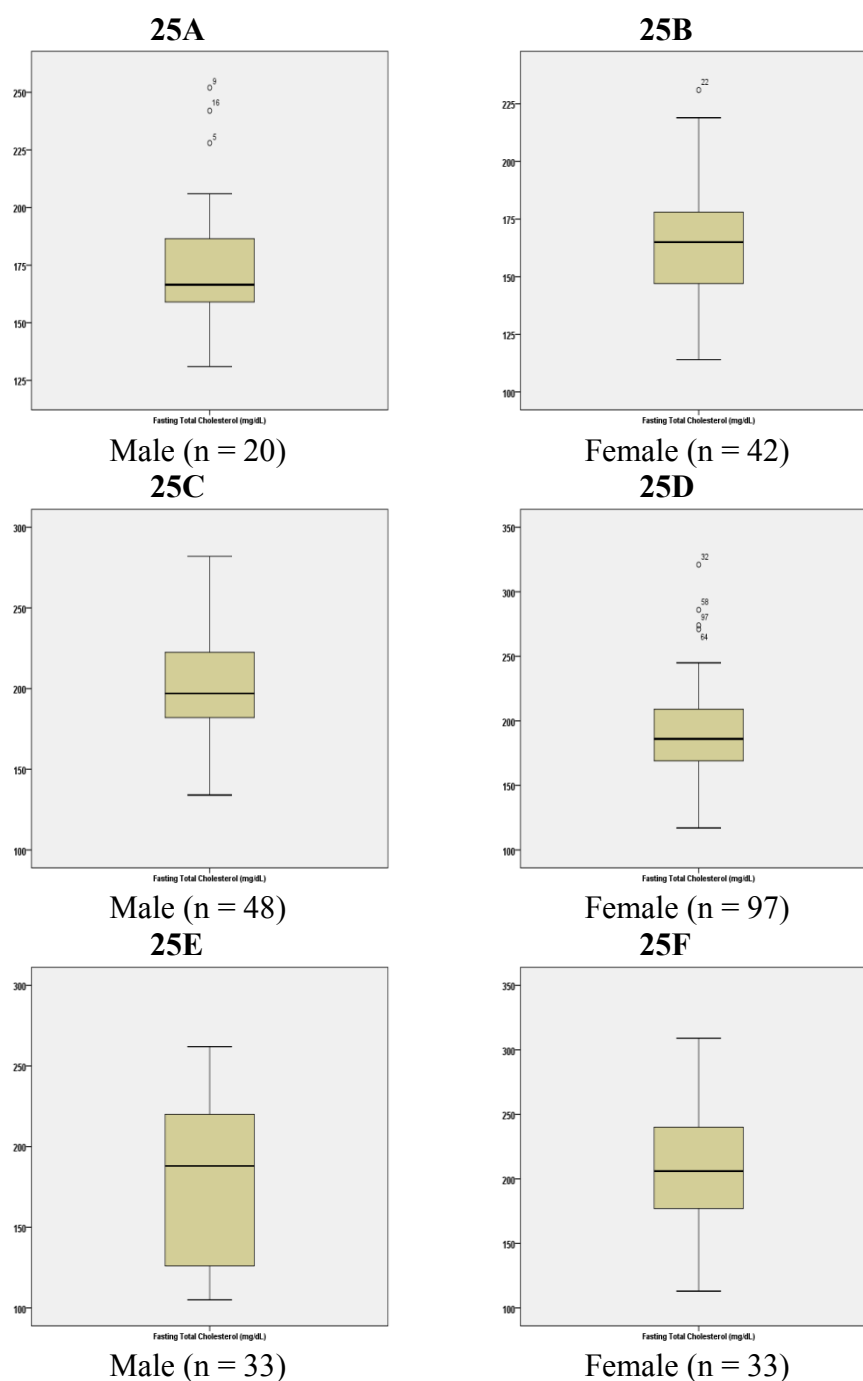


Figure 16.26 Comparison of sample distribution of Post Breakfast Total Cholesterol (mg/dL) in 18 – 25 years of age (Fig 26A and Fig 26B), samples of age 26 - 50 years (Fig 26C and Fig 26D); samples of age >50 years (Fig 26E and Fig 26F) visualized by Box-Whisker plot

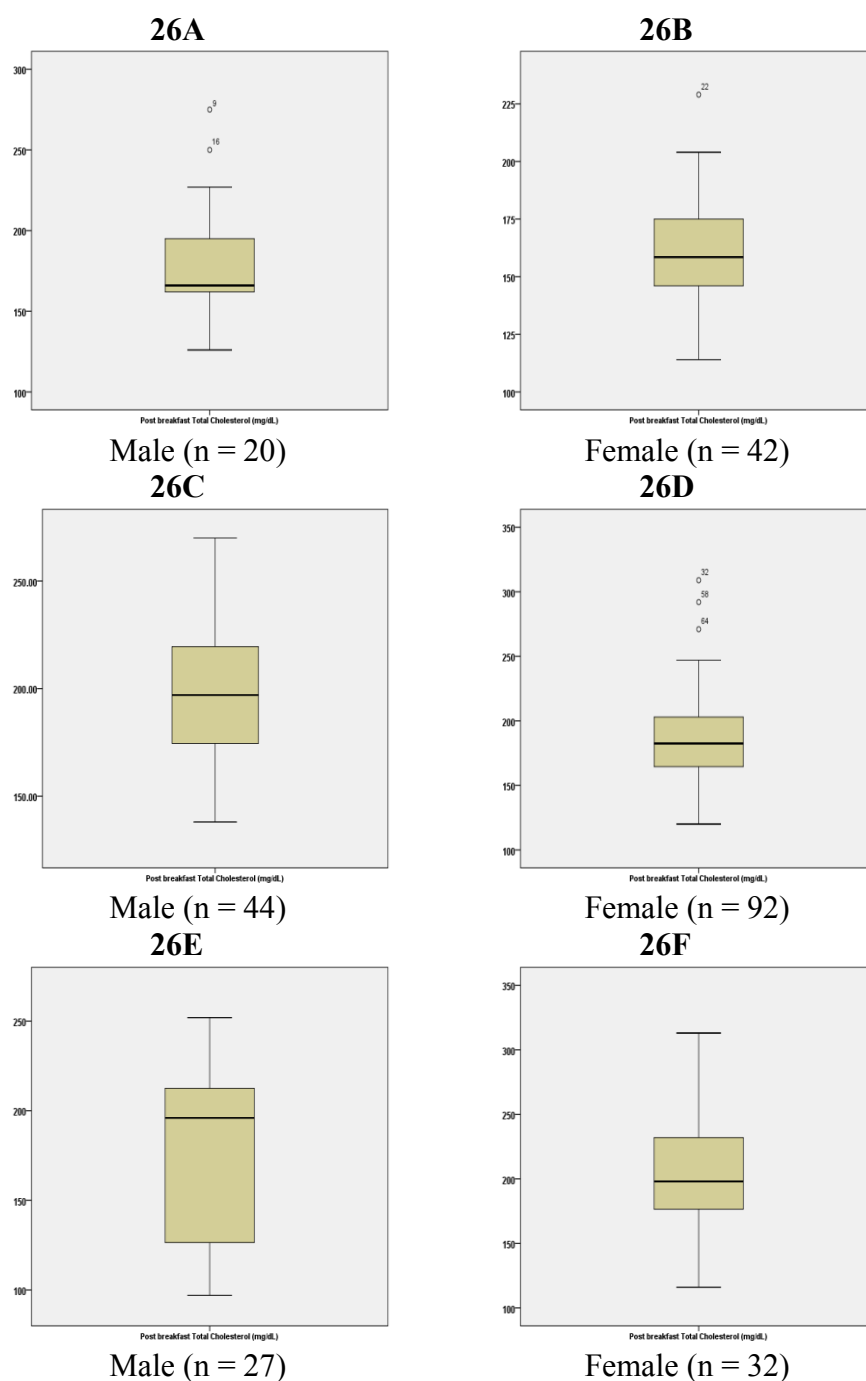


Figure 16.27. Comparison of sample distribution of Post Lunch Total Cholesterol (mg/dL) in 18 – 25 years of age (Fig 27A and Fig 27B), samples of age 26 - 50 years (Fig 27C and Fig 27D); samples of age >50 years (Fig 27E and Fig 27F) visualized by Box-Whisker plot

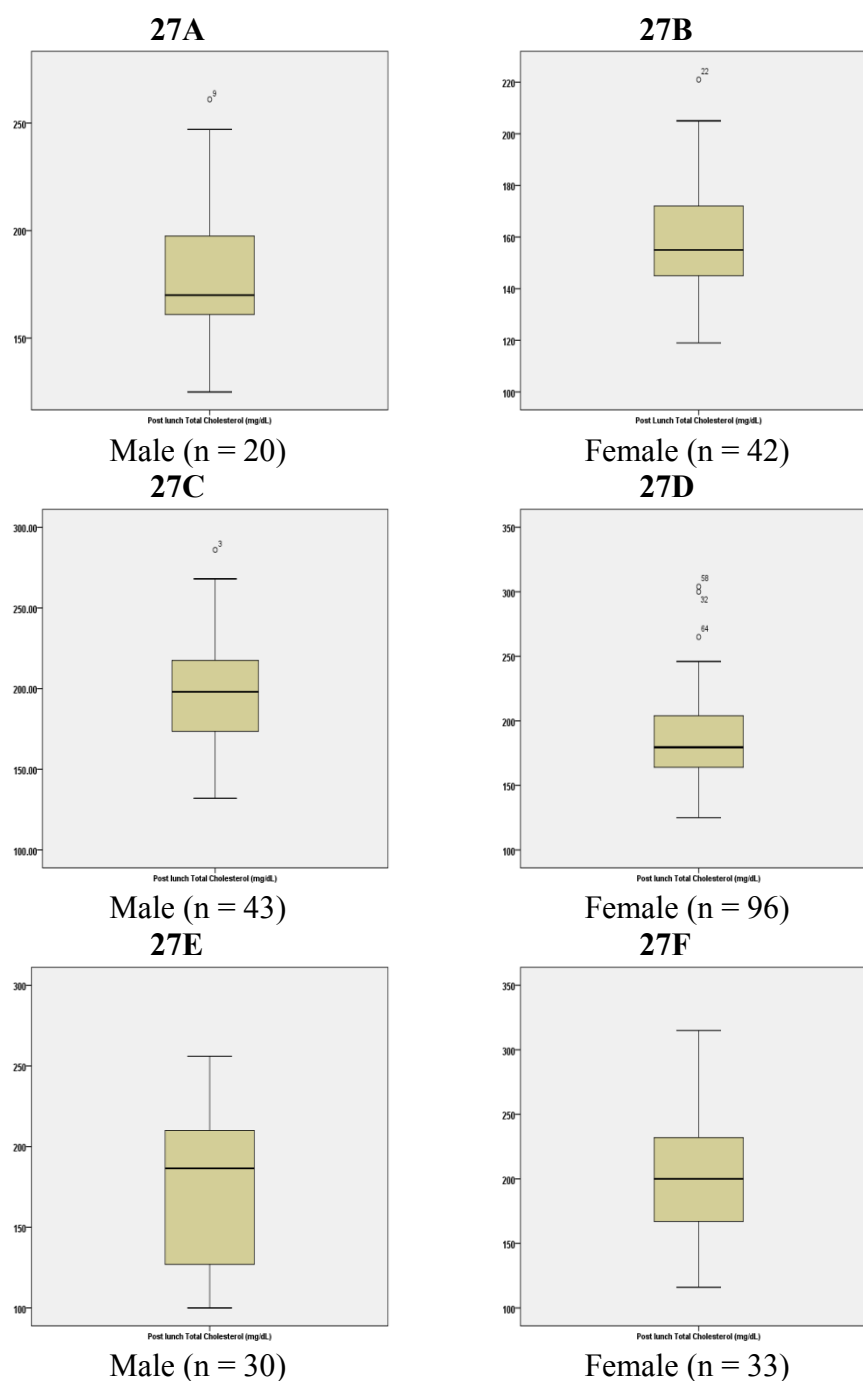


Figure. 16.28. Comparison of sample distribution of Fasting serum Triglycerides (mg/dL) in 18 – 25 years of age (Fig 28A and Fig 28B), samples of age 26 - 50 years (Fig 28C and Fig 28D); samples of age >50 years (Fig 28E and Fig 28F) visualized by Box-Whisker plot

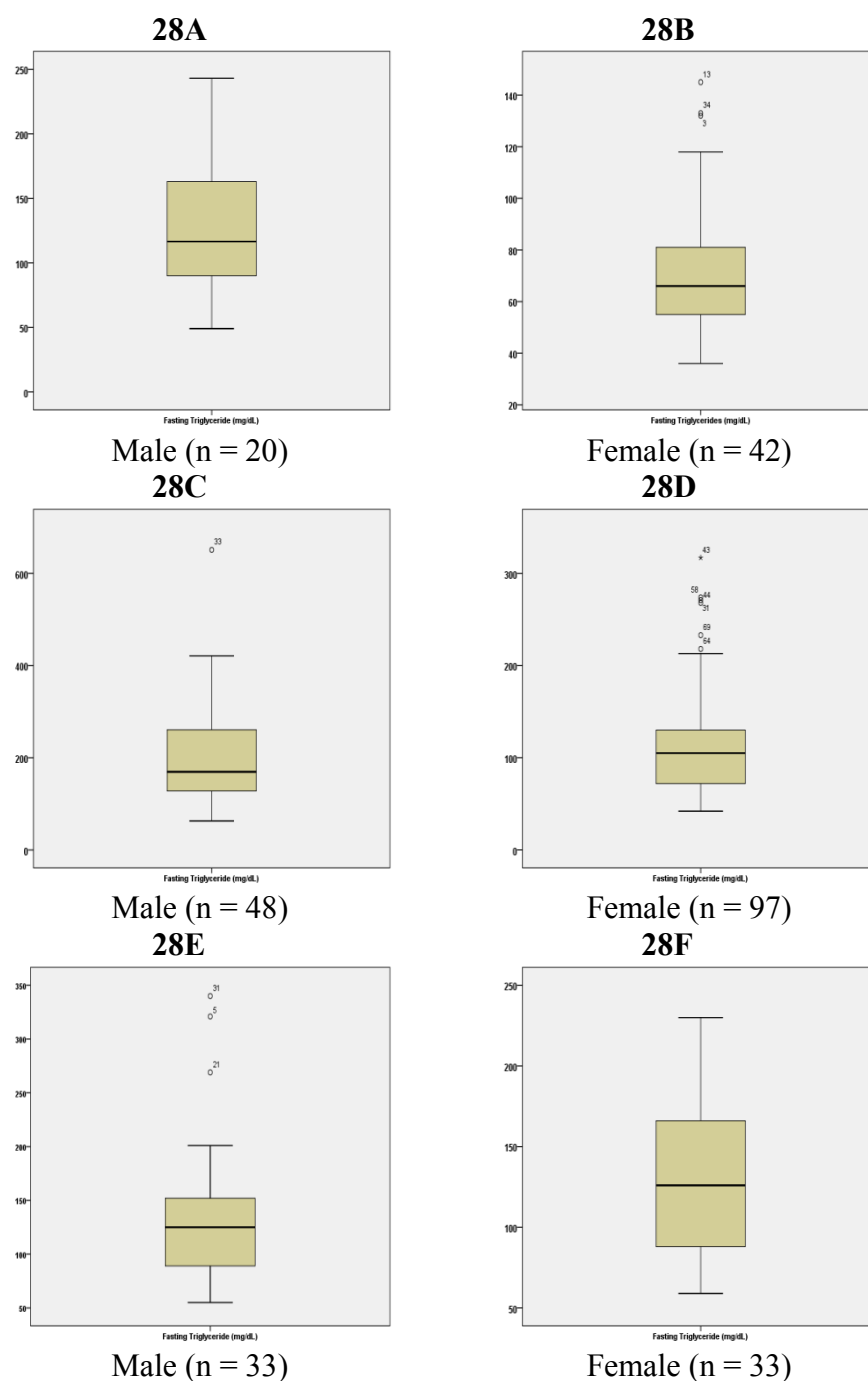


Figure. 16.29. Comparison of sample distribution of Post Breakfast serum Triglycerides (mg/dL) in 18 – 25 years of age (Fig 29A and Fig 29B), samples of age 26 - 50 years (Fig 29C and Fig 29D); samples of age >50 years (Fig 29E and Fig 29F) visualized by Box-Whisker plot

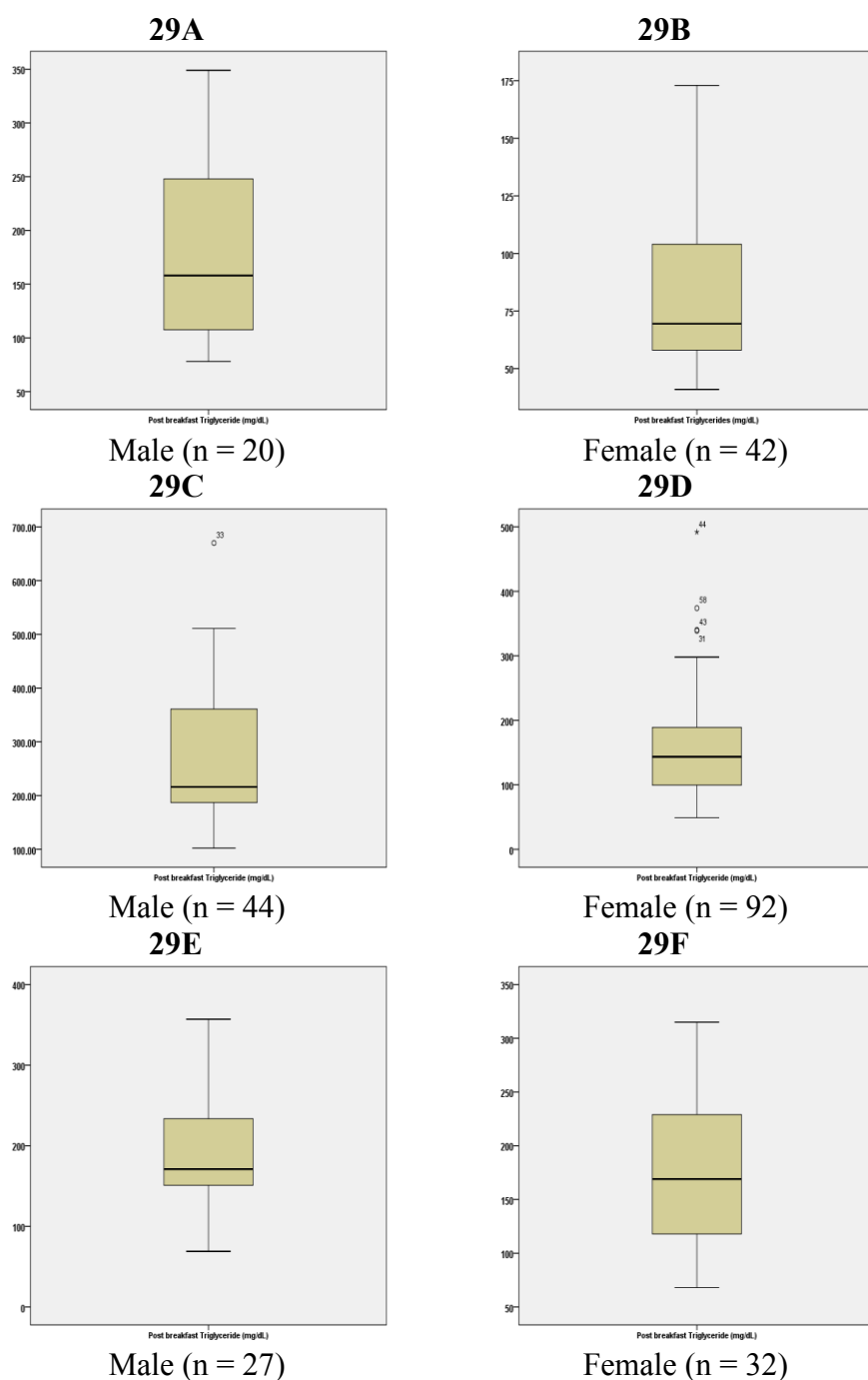


Figure. 16.30 Comparison of sample distribution of Post Lunch serum Triglycerides (mg/dL) in 18 – 25 years of age (Fig 30A and Fig 30B), samples of age 26 - 50 years (Fig 30C and Fig 30D); samples of age >50 years (Fig 30E and Fig 30F) visualized by Box-Whisker plot

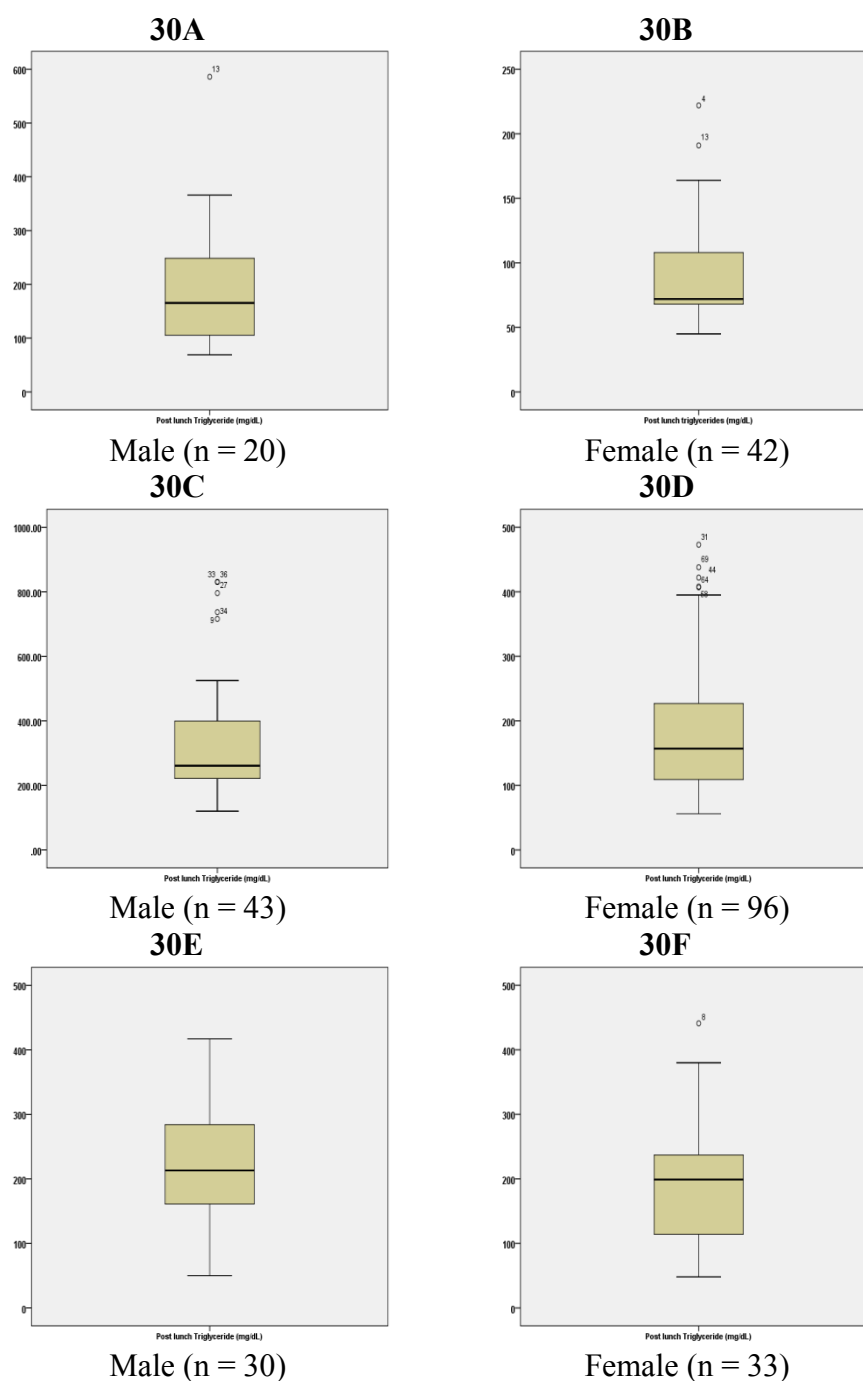


Figure. 16.31. Comparison of sample distribution of Fasting HDL Cholesterol (mg/dL) in 18 – 25 years of age (Fig 31A and Fig 31B), samples of age 26 - 50 years (Fig 31C and Fig 31D); samples of age >50 years (Fig 31E and Fig 31F) visualized by Box-Whisker plot

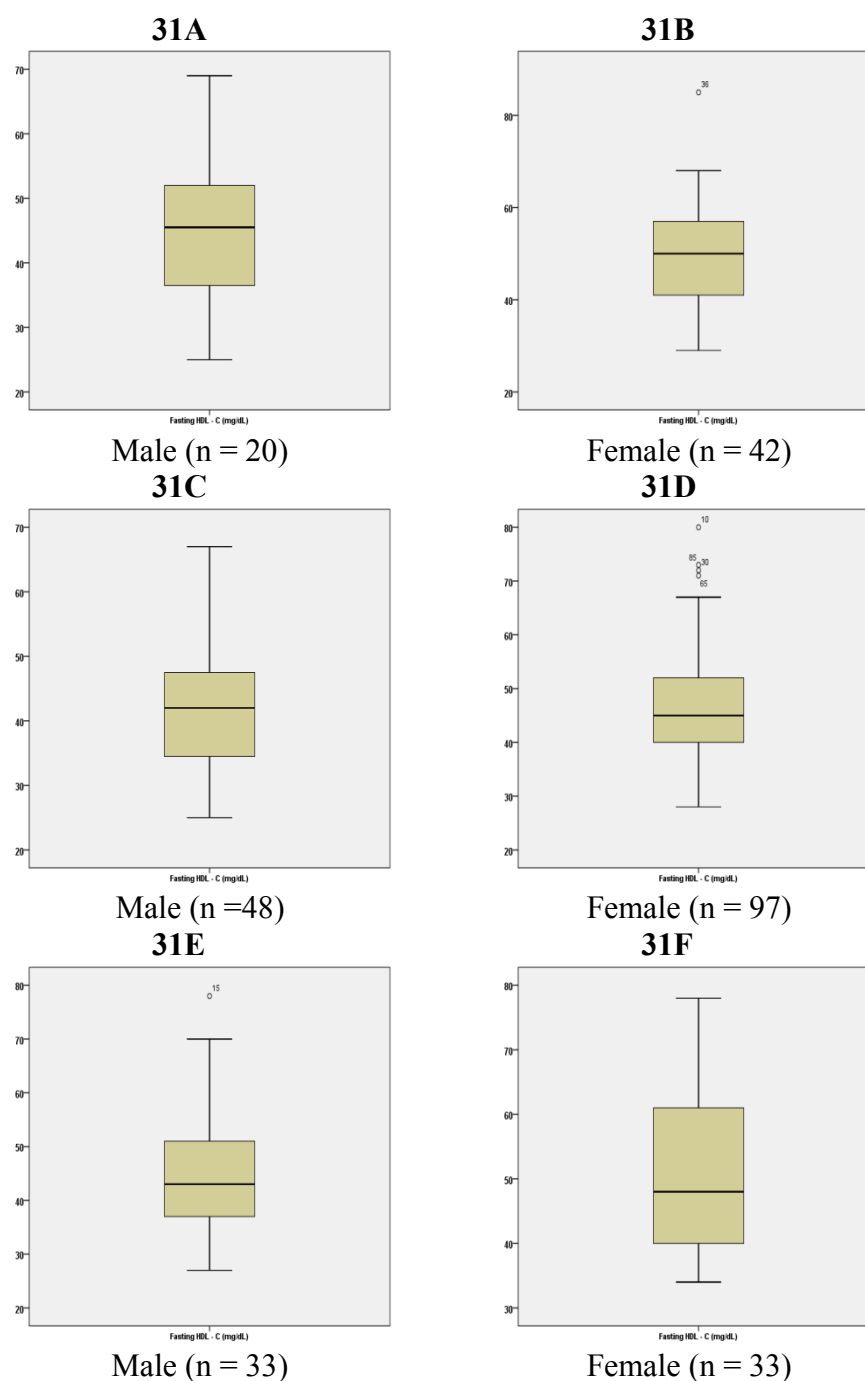


Figure. 16.32. Comparison of sample distribution of Post Breakfast HDL Cholesterol (mg/dL) in 18 – 25 years of age (Fig 32A and Fig 32B), samples of age 26 - 50 years (Fig 32C and Fig 32D); samples of age >50 years (Fig 32E and Fig 32F) visualized by Box-Whisker plot

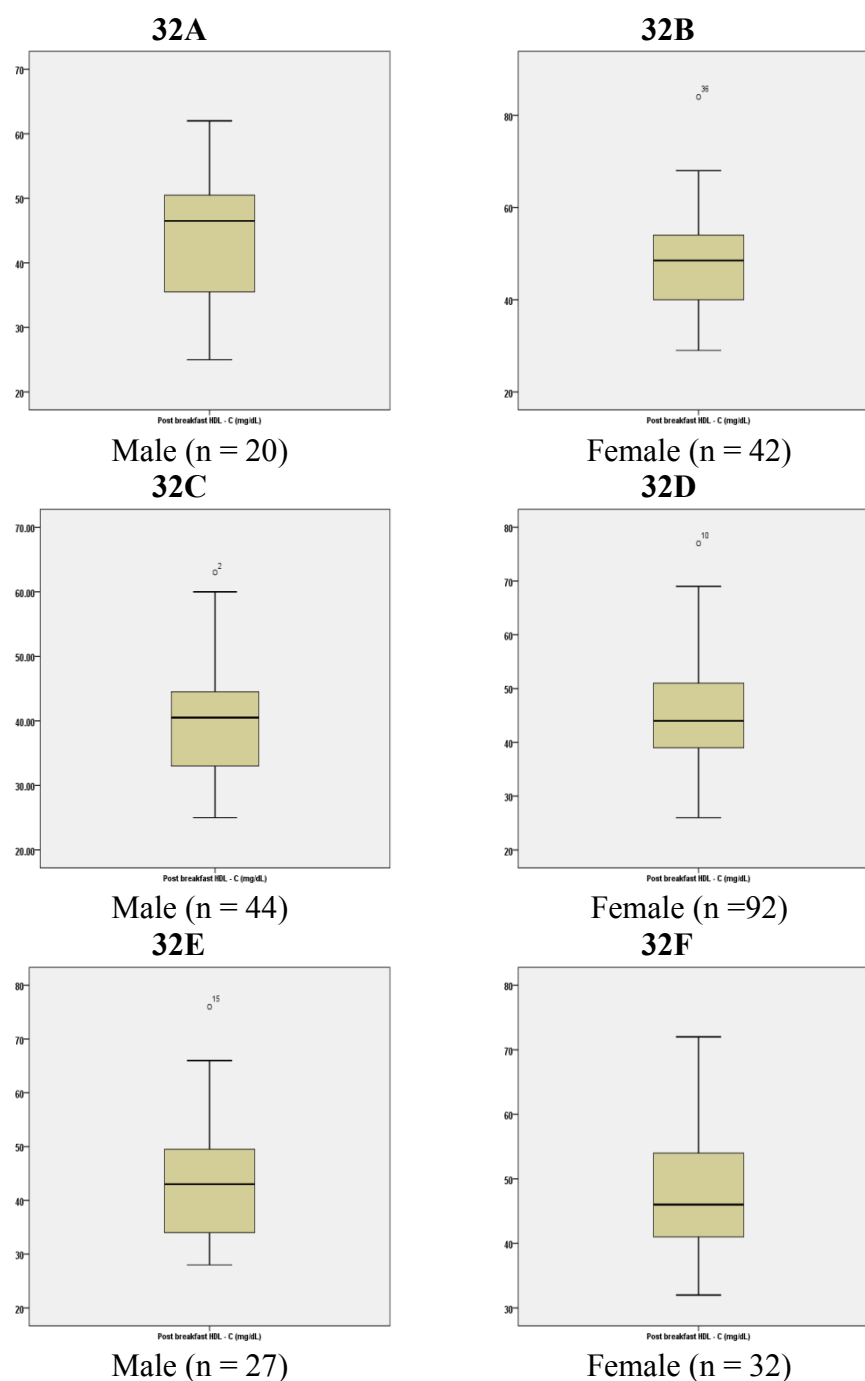


Figure. 16.33. Comparison of sample distribution of Post Lunch HDL Cholesterol (mg/dL) in 18 – 25 years of age (Fig 33A and Fig 33B), samples of age 26 - 50 years (Fig 33C and Fig 33D); samples of age >50 years (Fig 33E and Fig 33F) visualized by Box-Whisker plot

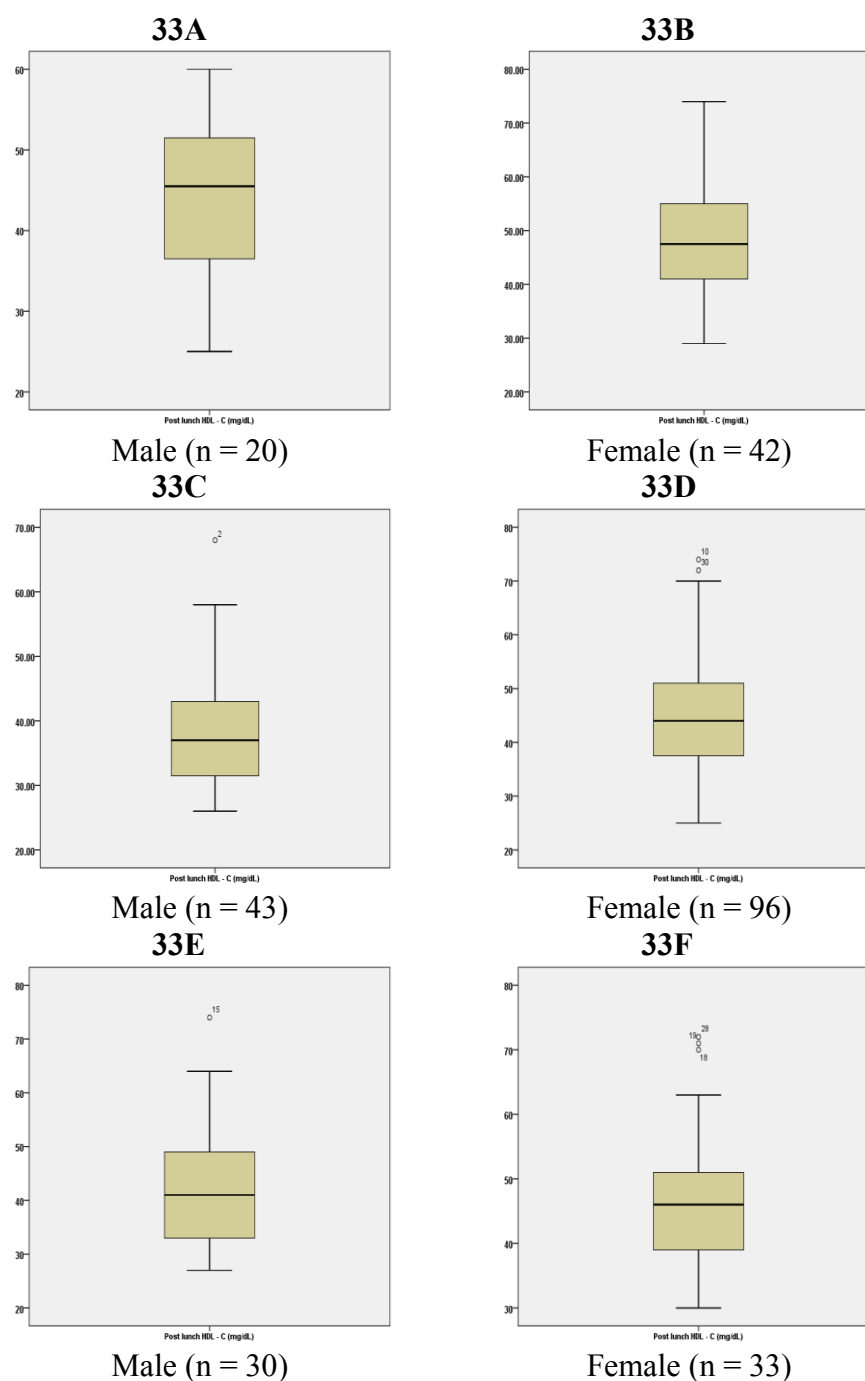


Figure. 16.34. Comparison of sample distribution of Fasting LDL Cholesterol (mg/dL) in 18 – 25 years of age (Fig 34A and Fig 34B), samples of age 26 - 50 years (Fig 34C and Fig 34D); samples of age >50 years (Fig 34E and Fig 34F) visualized by Box-Whisker plot

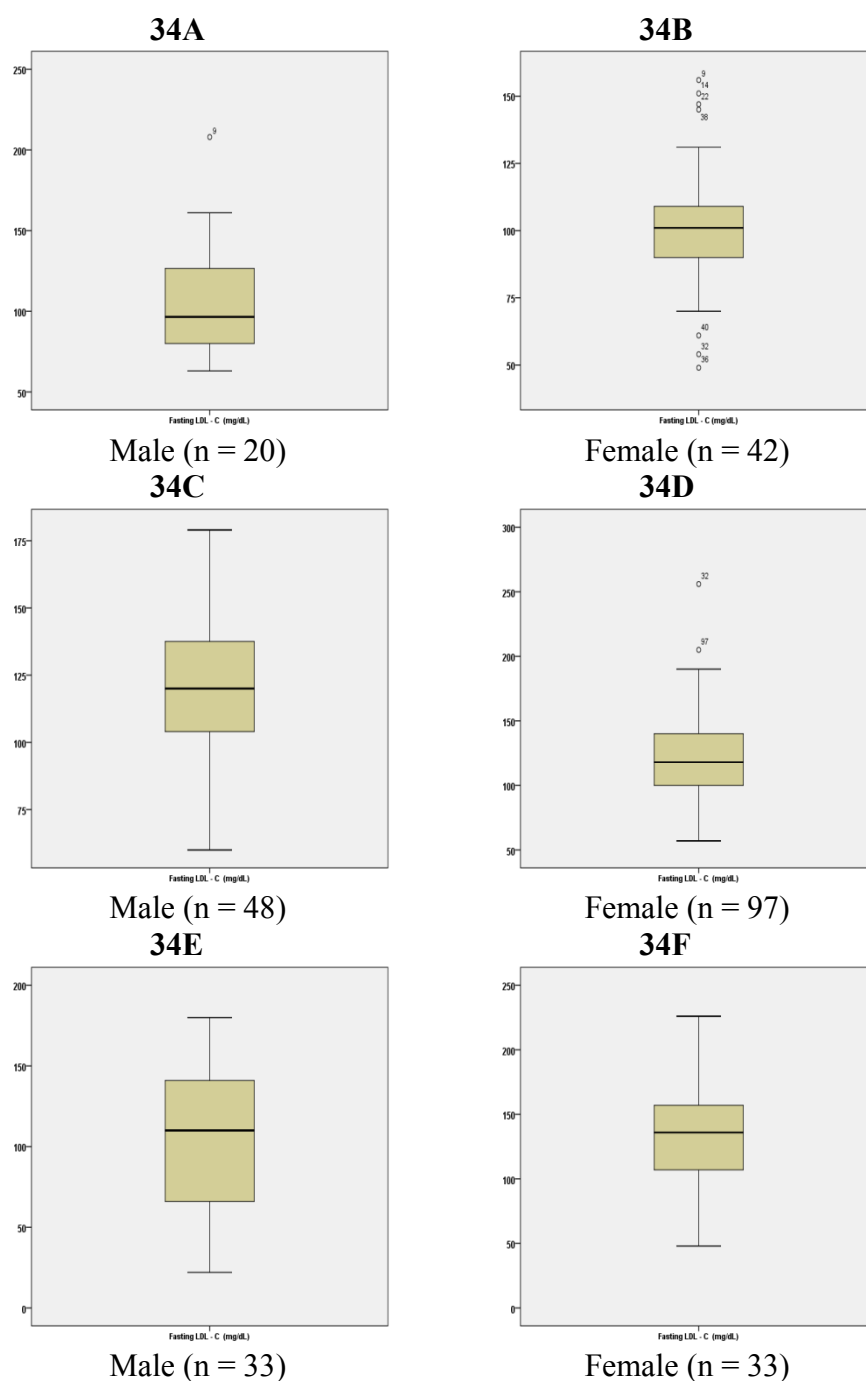


Figure. 16.35. Comparison of sample distribution of Post Breakfast LDL Cholesterol (mg/dL) in 18 – 25 years of age (Fig 35A and Fig 35B), samples of age 26 - 50 years (Fig 35C and Fig 35D); samples of age >50 years (Fig 35E and Fig 35F) visualized by Box-Whisker plot

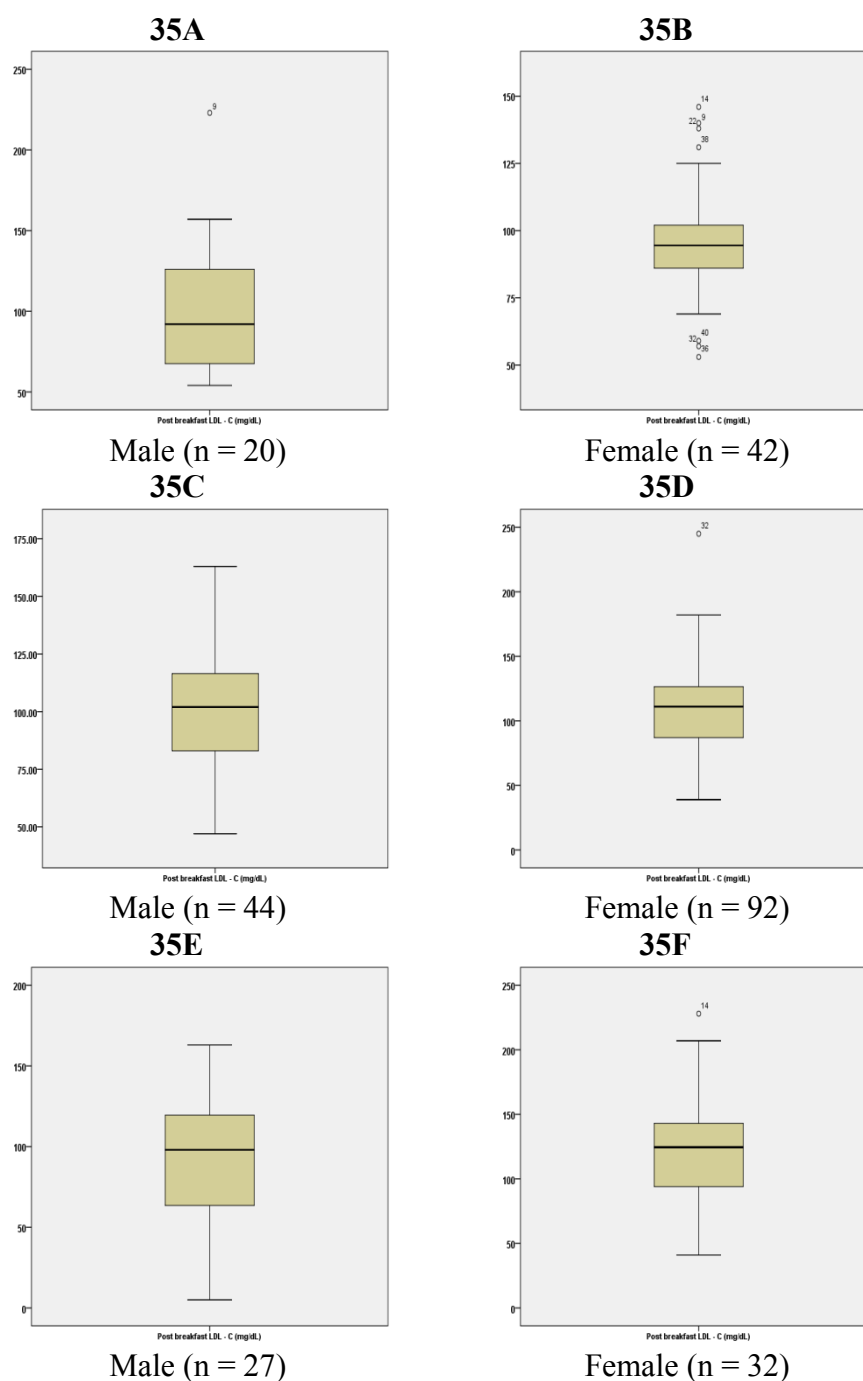


Figure. 16.36. Comparison of sample distribution of Post Lunch LDL Cholesterol (mg/dL) in 18 – 25 years of age (Fig 36A and Fig 36B), samples of age 26 - 50 years (Fig 36C and Fig 36D); samples of age >50 years (Fig 36E and Fig 36F) visualized by Box-Whisker plot

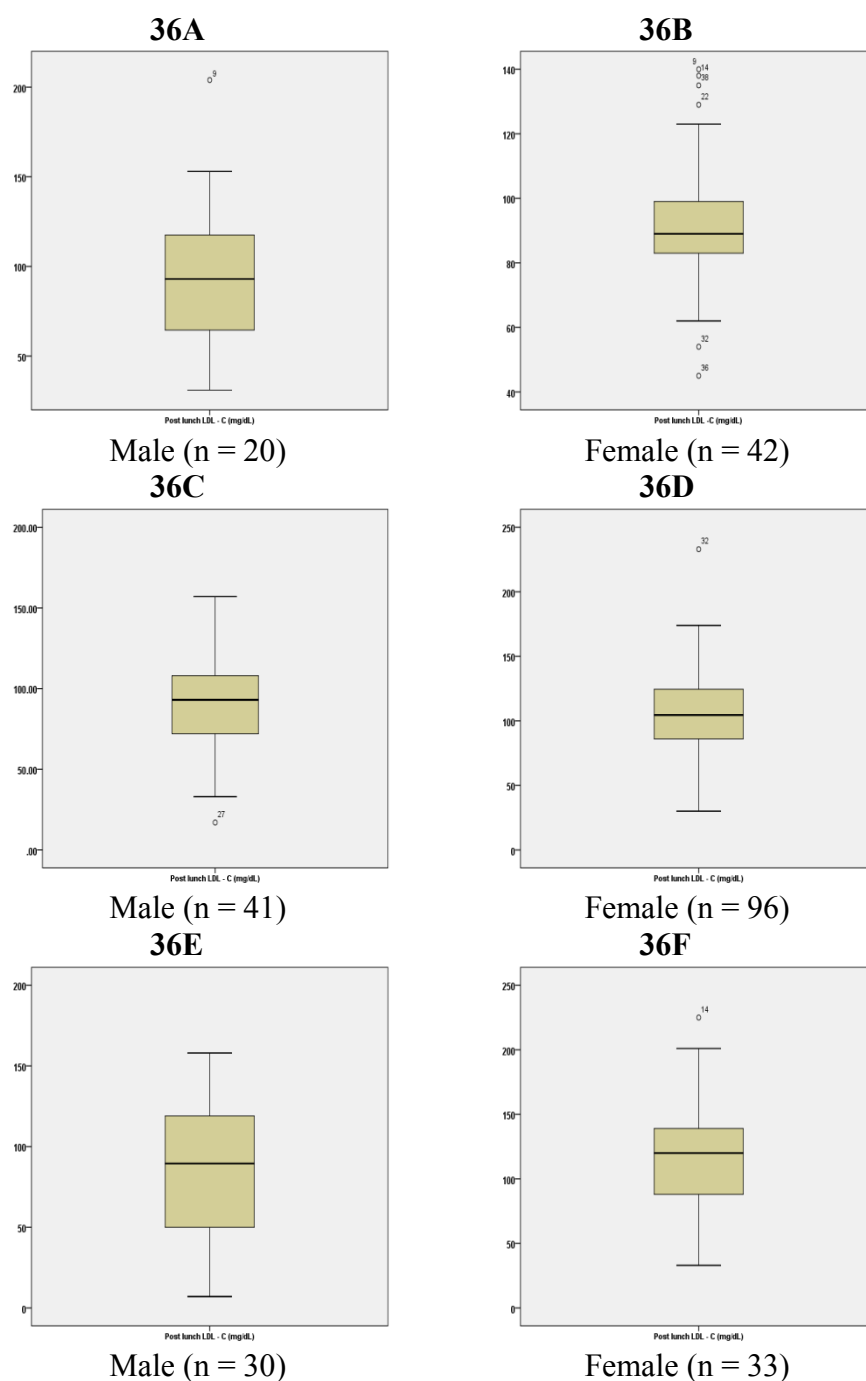


Table 16.13. Comparing means of fasting, post breakfast and post lunch Total Cholesterol (mg/dL) in age and gender groups. Fasting Total Cholesterol (A); 2 hr. Post Breakfast Total Cholesterol (B); 2 hr. Post Lunch Total Cholesterol (C)

Age groups	Groups:		Sample number (n)	Shapiro-Wilk (Log transformation)		Student t test or Mann Whitney U test (P value)
				Before	After	
Males						
18 – 25 years	A and B	Fasting	20	0.061	0.296	0.854
		2 hr Post Breakfast	20	0.044	0.584	
	A and C	Fasting	20	0.061	0.296	0.844
		2 hr Post Lunch	20	0.136	0.469	
	B and C	2 hr Post Breakfast	20	0.044	0.584	0.985
		2 hr Post Lunch	20	0.136	0.496	
25 – 60 years	A and B	Fasting	48	0.784	0.825	0.765
		2 hr Post Breakfast	44	0.806	0.735	
	A and C	Fasting	48	0.784	0.825	0.933
		2 hr Post Lunch	43	0.805	0.968	
	B and C	2 hr Post Breakfast	44	0.806	0.735	0.195
		2 hr Post Lunch	43	0.805	0.968	
>50 years	A and B	Fasting	33	0.008	0.004	0.744
		2 hr Post Breakfast	27	0.030	0.011	
	A and C	Fasting	33	0.008	0.004	0.635
		2 hr Post Lunch	30	0.037	0.015	
	B and C	2 hr Post Breakfast	27	0.030	0.011	0.974
		2 hr Post Lunch	30	0.037	0.015	

Age groups	Groups:		Sample number (n)	Shapiro-Wilk (Log transformation)		Student t test or Mann Whitney U test (P value)
				Before	After	
Females						
18 – 25 years	A and B	Fasting	42	0.646	0.586	0.339
		2 hr Post Breakfast	42	0.662	0.952	
	A and C	Fasting	42	0.646	0.586	0.135
		2 hr Post Lunch	42	0.289	0.854	
	B and C	2 hr Post Breakfast	42	0.662	0.952	0.580
		2 hr Post Lunch	42	0.289	0.854	
25 – 60 years	A and B	Fasting	97	0.010	0.840	0.526
		2 hr Post Breakfast	92	0.001	0.517	
	A and C	Fasting	97	0.010	0.840	0.503
		2 hr Post Lunch	96	0.001	0.533	
	B and C	2 hr Post Breakfast	92	0.001	0.517	0.987
		2 hr Post Lunch	96	0.001	0.533	
>50 years	A and B	Fasting	33	0.841	0.143	0.808
		2 hr Post Breakfast	32	0.605	0.294	
	A and C	Fasting	33	0.841	0.143	0.618
		2 hr Post Lunch	33	0.708	0.593	
	B and C	2 hr Post Breakfast	32	0.605	0.294	0.799
		2 hr Post Lunch	33	0.708	0.593	

Table 16.13. (continuation)

Table 16.14. Comparing means of Fasting, post breakfast and post lunch Serum triglycerides (mg/dL) in age and gender groups. Fasting Serum triglycerides (A); 2 hr Post Breakfast Serum triglycerides (B); 2 hr Post Lunch Serum triglycerides (C)

Age groups	Groups		Sample number (n)	Shapiro-Wilk (Log transformation)		Student t test or Mann Whitney U test (P value)
				Before	After	
Males						
18 – 25 years	A and B	Fasting	20	0.281	0.688	0.038
		2 hr Post Breakfast	20	0.056	0.318	
	A and C	Fasting	20	0.281	0.688	0.022
		2 hr Post Lunch	20	0.002	0.852	
	B and C	2 hr Post Breakfast	20	0.056	0.318	0.656
		2 hr Post Lunch	20	0.002	0.852	
25 – 60 years	A and B	Fasting	48	<0.001	0.742	0.001
		2 hr Post Breakfast	44	0.001	0.150	
	A and C	Fasting	48	<0.001	0.742	<0.001
		2 hr Post Lunch	43	<0.001	0.044	
	B and C	2 hr Post Breakfast	44	0.001	0.150	0.070
		2 hr Post Lunch	43	<0.001	0.044	
>50 years	A and B	Fasting	33	<0.001	0.235	0.001
		2 hr Post Breakfast	27	0.069	0.487	
	A and C	Fasting	33	<0.001	0.235	<0.001
		2 hr Post Lunch	30	0.477	0.186	
	B and C	2 hr Post Breakfast	27	0.069	0.487	0.195
		2 hr Post Lunch	30	0.477	0.186	

Age groups	Groups		Sample number (n)	Shapiro-Wilk (Log transformation)		Student t test or Mann Whitney U test (P value)
				Before	After	
Females						
18 – 25 years	A and B	Fasting	42	<0.001	0.067	0.070
		2 hr Post Breakfast	42	<0.001	0.049	
	A and C	Fasting	42	<0.001	0.067	0.004
		2 hr Post Lunch	42	<0.001	0.013	
	B and C	2 hr Post Breakfast	42	<0.001	0.049	0.308
		2 hr Post Lunch	42	<0.001	0.013	
25 – 60 years	A and B	Fasting	97	<0.001	0.191	<0.001
		2 hr Post Breakfast	92	<0.001	0.536	
	A and C	Fasting	97	<0.001	0.191	<0.001
		2 hr Post Lunch	96	<0.001	0.397	
	B and C	2 hr Post Breakfast	92	<0.001	0.536	0.018
		2 hr Post Lunch	96	<0.001	0.397	
>50 years	A and B	Fasting	33	0.115	0.167	0.006
		2 hr Post Breakfast	32	0.263	0.293	
	A and C	Fasting	33	0.115	0.167	0.001
		2 hr Post Lunch	33	0.153	0.466	
	B and C	2 hr Post Breakfast	32	0.263	0.293	0.268
		2 hr Post Lunch	33	0.153	0.466	

Table 16.14 (Continuation)

Table 16.15. Comparing means of Fasting, post breakfast and post lunch HDL-Cholesterol (mg/dL) in age and gender groups. Fasting HDL-Cholesterol (A); 2 hr Post Breakfast HDL-Cholesterol (B); 2 hr Post Lunch HDL-Cholesterol (C)

Age groups	Groups		Sample number (n)	Shapiro-Wilk (Log transformation)		Student t test or Mann Whitney U test (P value)
				Before	After	
Males						
18 – 25 years	A and B	Fasting	20	0.947	0.627	0.833
		2 hr Post Breakfast	20	0.623	0.225	
	A and C	Fasting	20	0.947	0.627	0.898
		2 hr Post Lunch	20	0.520	0.146	
	B and C	2 hr Post Breakfast	20	0.623	0.225	0.929
		2 hr Post Lunch	20	0.520	0.146	
25 – 60 years	A and B	Fasting	48	0.190	0.829	0.310
		2 hr Post Breakfast	44	0.051	0.416	
	A and C	Fasting	48	0.190	0.829	0.163
		2 hr Post Lunch	43	0.005	0.210	
	B and C	2 hr Post Breakfast	44	0.051	0.416	0.674
		2 hr Post Lunch	43	0.005	0.210	
>50 years	A and B	Fasting	33	0.049	0.740	0.649
		2 hr Post Breakfast	27	0.086	0.567	
	A and C	Fasting	33	0.049	0.740	0.294
		2 hr Post Lunch	30	0.096	0.508	
	B and C	2 hr Post Breakfast	27	0.086	0.567	0.579
		2 hr Post Lunch	30	0.096	0.508	

Age groups	Groups		Sample number (n)	Shapiro-Wilk (Log transformation)		Student t test or Mann Whitney U test (P value)
				Before	After	
Females						
18 – 25 years	A and B	Fasting	42	0.291	0.955	0.622
		2 hr Post Breakfast	42	0.212	0.936	
	A and C	Fasting	42	0.291	0.955	0.449
		2 hr Post Lunch	42	0.606	0.723	
	B and C	2 hr Post Breakfast	42	0.212	0.936	0.806
		2 hr Post Lunch	42	0.606	0.723	
25 – 60 years	A and B	Fasting	97	0.001	0.669	0.306
		2 hr Post Breakfast	92	0.005	0.802	
	A and C	Fasting	97	0.001	0.669	0.101
		2 hr Post Lunch	96	0.005	0.769	
	B and C	2 hr Post Breakfast	92	0.005	0.802	0.529
		2 hr Post Lunch	96	0.005	0.769	
>50 years	A and B	Fasting	33	0.037	0.189	0.492
		2 hr Post Breakfast	32	0.033	0.232	
	A and C	Fasting	33	0.037	0.189	0.217
		2 hr Post Lunch	33	0.097	0.427	
	B and C	2 hr Post Breakfast	32	0.033	0.232	0.577
		2 hr Post Lunch	33	0.097	0.427	

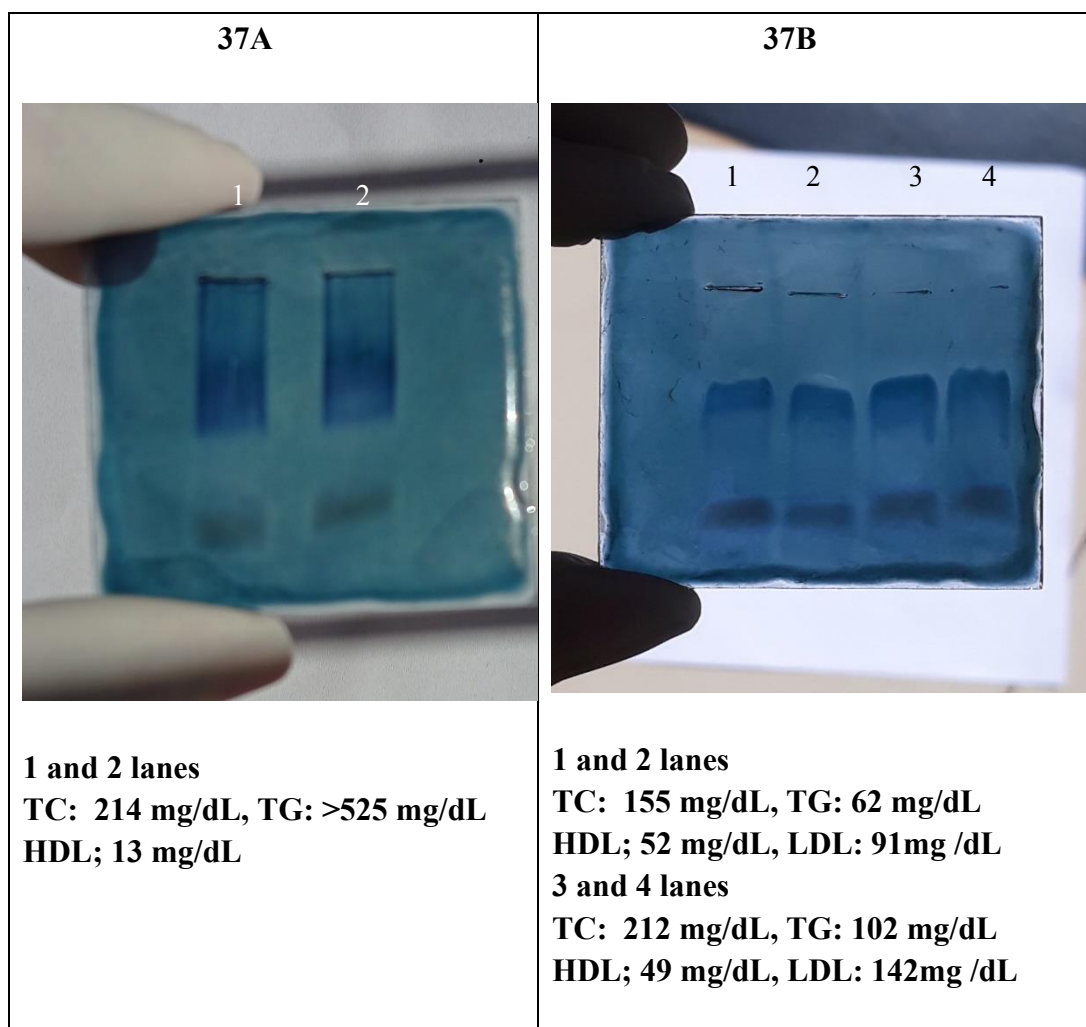
Table 16.15 (Continuation)

Table 16.16. Comparing means of Fasting, post breakfast and post lunch LDL-Cholesterol (mg/dL) in age and gender groups. Fasting LDL-Cholesterol (A); 2 hr Post Breakfast LDL-Cholesterol (B); 2 hr Post Lunch LDL-Cholesterol (C)

Age groups	Groups		Sample number (n)	Shapiro-Wilk (Log transformation)		Student t test or Mann Whitney U test (P value)
				Before	After	
Males						
18 – 25 years	A and B	Fasting	20	0.019	0.422	0.451
		2 hr Post Breakfast	20	0.021	0.394	
	A and C	Fasting	20	0.019	0.422	0.189
		2 hr Post Lunch	20	0.370	0.799	
	B and C	2 hr Post Breakfast	20	0.021	0.394	0.549
		2 hr Post Lunch	20	0.370	0.799	
25 – 60 years	A and B	Fasting	48	0.616	0.021	0.003
		2 hr Post Breakfast	44	0.776	0.519	
	A and C	Fasting	48	0.616	0.021	<0.001
		2 hr Post Lunch	41	0.906	0.001	
	B and C	2 hr Post Breakfast	44	0.776	0.519	0.039
		2 hr Post Lunch	41	0.906	0.001	
>50 years	A and B	Fasting	33	0.137	0.012	0.329
		2 hr Post Breakfast	27	0.408	<0.001	
	A and C	Fasting	33	0.137	0.012	0.123
		2 hr Post Lunch	30	0.570	<0.001	
	B and C	2 hr Post Breakfast	27	0.408	<0.001	0.509
		2 hr Post Lunch	30	0.570	<0.001	

Age groups	Groups		Sample number (n)	Shapiro-Wilk (Log transformation)		Student t test or Mann Whitney U test (P value)
				Before	After	
Females						
18 – 25 years	A and B	Fasting	42	0.049	0.009	0.164
		2 hr Post Breakfast	42	0.079	0.107	
	A and C	Fasting	42	0.049	0.009	0.006
		2 hr Post Lunch	42	0.008	0.009	
	B and C	2 hr Post Breakfast	42	0.079	0.107	0.215
		2 hr Post Lunch	42	0.008	0.009	
25 – 60 years	A and B	Fasting	97	0.001	0.846	0.020
		2 hr Post Breakfast	92	0.002	0.029	
	A and C	Fasting	97	0.001	0.846	0.001
		2 hr Post Lunch	96	<0.001	0.019	
	B and C	2 hr Post Breakfast	92	0.002	0.029	0.268
		2 hr Post Lunch	96	<0.001	0.019	
>50 years	A and B	Fasting	33	0.596	0.022	0.264
		2 hr Post Breakfast	32	0.011	0.484	
	A and C	Fasting	33	0.596	0.022	0.086
		2 hr Post Lunch	33	0.663	0.023	
	B and C	2 hr Post Breakfast	32	0.011	0.484	0.563
		2 hr Post Lunch	33	0.663	0.023	

Table 16.16 (Continuation)

Figure 16.37. Electrophoresis of serum lipoprotein with fasting serum samples.

SUMMARY AND CONCLUSIONS

Chapter-wise summary from the result chapters 5 to 16.

The study employed statistical and graphical methods, including histograms, box-whisker plots, and Q-Q plots, to analyse and classify lipid profile parameters such as Total Cholesterol (TC), Serum Triglycerides (TG), HDL Cholesterol (HDL-C), and LDL Cholesterol (LDL-C). Histograms and Q-Q plots revealed a positive skew in Total Cholesterol and Triglyceride distributions, and box-whisker plots helped identify outliers, which were further categorized into near and far outliers. These graphical tools were used not only for statistical interpretation but also to clinically differentiate common, multifactorial dyslipidemias from rare, severe monogenic forms.

The study found that isolated HDL-C decrease was the most prevalent dyslipidemia across most age and gender groups, except for males aged 26–50 years (who predominantly showed isolated Triglyceride increase) and females of the same age group (who had combined LDL-C and TC increase). The second most prevalent pattern varied by group, with isolated Triglyceride increase common in most male age groups and younger females, while combined TC and LDL-C increase was more common in older females. TG concentrations above 885 mg/dL were identified as indicative of rare dyslipidemia. The highest dyslipidemia prevalence was observed in males aged 26–50 years, whereas the healthiest lipid profiles were found in females under 18 years.

Correlation analysis showed that triglycerides positively correlated with glucose, Total Cholesterol, LDL-C, TSH, and BMI in adult males. While in females, Triglycerides associated with liver enzymes AST and ALP, possibly due to sex-related hormonal differences. Triglyceride was inversely correlated with HDL-C in both sexes. These relationships emphasized the metabolic interconnectedness of lipid profiles with biochemical parameters like TSH, ALT, ALP, and glucose, reinforcing the importance of comprehensive cardiovascular risk assessment.

Parametric methods were used for calculating reference interval for all lipid profile analytes of age group <18 years. For calculating Reference Interval of Total Cholesterol, Serum triglycerides and LDL Cholesterol of age group 18 - 25 years

parametric method was used. Reference interval for Total Cholesterol, Serum triglycerides of age group 26 – 50 and >50 years was calculated using Parametric method. Non-parametric method (Percentile method) was used for calculating reference interval of all lipid profile analytes above 18 yrs. Reference interval for HDL Cholesterol (18 – 25 yrs, 26 – 50 years and, >50 years) and for LDL Cholesterol (26 – 50 yrs and >50 years) was calculated using Non-parametric method (Percentile method). The 90% reference interval calculation is the most acceptable as data is positively skewed.

Regarding fasting versus postprandial measurements, TC showed no significant difference, while TG levels were consistently higher postprandially in all gender age groups. HDL-C showed no significant variation with meal timing, whereas LDL-C demonstrated significant changes in both sexes aged 26–50 years. These findings support the use of both fasting and postprandial measurements in clinical practice and underscore the need for age- and gender-specific diagnostic and therapeutic strategies in lipid management.

The study underlines the importance of **age- and gender-specific reference ranges** and shows how graphical and statistical techniques can effectively classify and analyse lipid disorders. The correlations between lipid parameters and other metabolic markers underscore the need for **holistic cardiovascular risk assessment** and the potential for **personalized treatment strategies**.

CLINICAL APPLICATION AND LIMITATIONS

Clinical applications: Most laboratories in India use reference intervals derived from western populations or kit inserts from the manufactures. This study contributes locally derived reference interval data that may improve interpretation of lipid profile results in the regional population and support future laboratory standardization efforts.

This study provides comprehensive information regarding age- and sex-related changes in lipid profile analytes, contributing to a better understanding of lipid metabolism across different demographic groups. Such information is useful for Clinical Biochemists, General physicians, cardiologists and public health researchers.

The large sample size increases the robustness of the findings and provides valuable real-world evidence regarding lipid profile distribution in individuals undergoing routine laboratory testing.

The correlation analysis contributes to understanding the interrelationships among lipid parameters and may assist in the interpretation of lipid metabolism and cardiovascular risk.

The second set of samples (n = 312) provides evidence regarding the effect of food intake on lipid profile measurements. Postprandial triglyceride analyte showed significant increase over fasting sample compared to other lipid profile analytes

By identifying patterns of lipid abnormality and demographic variations, the study contributes to strategies aimed at early identification of individuals at increased risk.

Increase LDL Cholesterol and Total Triglycerides and decrease in HDL Cholesterol have been shown to increase the risk of atherosclerosis and myocardial infarction (include reference) and also cause skin diseases such as xanthoma and xanthelasma.

Very high increase in triglycerides resulting from type 1 hyperlipoproteinemia has been found to cause acute pancreatitis. During the twenty-four months of sample and test data collection we have obtained twenty-

seven cases of type 1 hyperlipoproteinemia with milky serum samples indicating an incidence of above one case per month.

This study also showed that post lunch samples showed marked increase in triglycerides as compared to fasting sample. This study showed high prevalence of dyslipidemia in the sample population and the relative prevalence increased markedly after 50 years of age in both males and females. The lowest dyslipidemic samples were observed in females aged 18 – 25 years and highest dyslipidemic prevalence was seen in male age group 26 – 50 years.

The identification of isolated increase in any one lipid profile parameter and combined increase of more than one lipid parameter are also unique features of this study. Isolated increase are milder forms and combined increase are more severe forms.

Limitations: The study has not evaluated relative prevalence of atherosclerosis and genetic predisposition related to dyslipidemia.

The lowest sample number was in the <18 years of age group. If the number of samples in the <18 years age group is selectively increased to increase the sample number, it may lead to a biased observation due to preferential accumulation of samples. In this study, we have not evaluated reversal of lipid profile values with diet control, exercise and medical management.

RECOMMENDATIONS

Recommendations

Interpreting cutoff values for moderate and severe dyslipidemia remains clinically challenging across different gender and age groups. Additionally, the initial outlier detection was misleading, as new outliers continued to emerge with each successive removal.

Analysis of genetic factors and DNA sequencing related to both multifactorial and monogenic causes of dyslipidemia has not yet been conducted and should be explored in future studies. Another potential research area is the role of hormonal influences on lipid levels, particularly in individuals aged 18–50 years, which may explain gender-based differences.

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