Surveillance of Iodine in Drinking Water in Relation to Iodine Deficiency Disorders in Northern Kerala

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DOCTOR OF PHILOSOPHY

By

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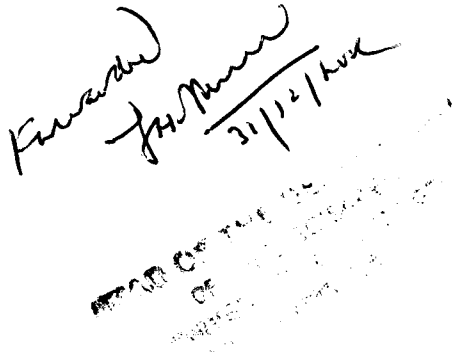

Dedication
to
The Enchanting Guruji

Declaration

I do hereby declare that the thesis entitled ***"Surveillance of Iodine in Drinking water in Relation to Iodine Deficiency Disorders in Northern Kerala"*** is an authentic record of the research work carried out by me under the supervision and guidance of Dr.V.K.Sasidharan, Professor & Head of the Department of Life Sciences, University Of Calicut. No part of this thesis has been previously presented for the award of any degree or diploma.

Minikumary Amma.V

Calicut University Campus
December 2002

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Professor & Head
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Certificate

This is to certify that the thesis entitled ***"Surveillance of Iodine in Drinking water In Relation to Iodine Deficiency Disorders in Northern Kerala"*** is an authentic record of the original research work carried out by Minikumari Amma.V under my supervision and guidance.

Calicut University
December 2002

Dr.V.K.Sasidharan

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INTRODUCTION

Minikumari Amma.V “Surveillance of Iodine in Drinking Water in Relation to Iodine Deficiency Disorders in Northern Kerala ” Thesis. Department of Life Sciences, University of Calicut, 2002

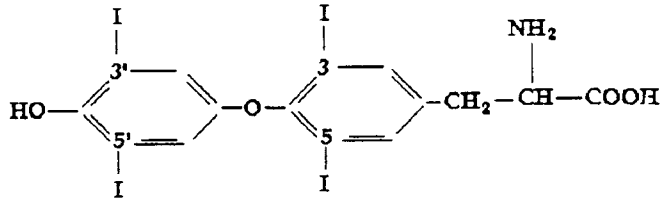
INTRODUCTION

Iodine is one of the most important halogen element, which plays a vital role in the metabolic action of human beings. Iodine is a trace element in both lithosphere and hydrosphere. It is readily liberated from its compounds and it has an appreciable vapour pressure at ordinary temperature. It can easily exist free in the atmosphere. Owing to its high solubility, iodide salts are easily leached from rock matrix and may form a significant hydrochemical constitution in ground water.[1] Thus ground water becomes one of the major iodide source of the majority of rural population in Kerala. The iodine content of the drinking water is a valuable index of the iodine content of the rocks and soils of a region and hence of the locally grown foods, and has been correlated with the incidence of goitre in many areas. The low iodine content in soil and vegetation is caused by severe flooding or intense glaciations. This is followed by washing of iodine through the rivers to the sea which can result in iodine deficiency disorders like severe occurrence of goiter.

Iodine in adequate amount is a pre-requisite for the biosynthesis of thyroid hormones by the thyroid gland. The main hormones secreted by the thyroid gland are triiodothyronine(T_3) and tetraiodothyronine(T_4-Thyroxine). The first step in the biosynthesis is

the uptake and concentration of iodine by the thyroid gland.[2] The thyroid gland can concentrate iodine up to 10,000 times. The uptake is linked to Na^+/K^+ ATPase and so ouabain blocks the ability to concentrate iodine. This step is inhibited by thiocyanate and perchlorate, which compete for the carrier mechanism. TSH stimulates this step. There may be a congenital defect associated with this step. It is called iodine trapping defect and may be treated by large doses of iodine. The next step is the oxidization of iodide to active iodine(I^+) by the enzyme thyroperoxidase. This step is also stimulated by TSH and inhibited by antithyroid drugs such as thiourea, thiouracil and methimazole. The third step is the iodination of thyroglobulin, a tyrosine rich protein synthesized by the thyroid follicular cells. The iodination of tyrosine residues in the thyroglobulin results in the production of mono-iodotyrosine(MIT) and diiodotyrosine(DIT). When two such DIT molecules couple, one molecule of tetraiodothyronine (T_4) is formed. The triiodothyronine may be formed either by coupling of one MIT with one DIT or by de-iodination of T_4. Under normal conditions, 99% of the hormone produced by the thyroid gland is T_4.

Tetraiodothyronine (T_4)



The T_4 residues are now attached to the thyroglobulin molecule, which is stored as colloid in the thyroid acini. When required, the T_4 is liberated by hydrolysis by specific proteases. This hydrolysis is depressed by iodide and therefore potassium iodide is used as an adjuvant in hyperthyroidism.

The MIT and DIT that are not utilized are deiodinised and salvaged for reutilization inside the cell itself. There is an inborn error of metabolism reported affecting at this step. It is called deiodinase defect. In such cases, DIT and MIT are seen in urine. Since iodine is excreted, iodine deficiency is manifested. Treatment is to give iodine.

Almost all the thyroid hormones are transported in plasma by proteins. The bound form is biologically inactive, but they can be rapidly released. The free fraction is only 0.03% of the total plasma iodine, but it is the biologically active portion. Total protein bound iodine (PBI) is about 10 µg/dl. The T_4 constitutes 70 times more than the T_3 concentration. The binding proteins are thyroxine binding globulin (TBG), thyroxine binding pre-albumin and albumin.

Variation in the level of binding protein may produce artefacts while measuring hormones. Since the biologically active free fraction remains normal, there will be no functional abnormality noticed in presence of such artefacts. The protein binding increases the half-life of the hormones, by preventing

degradation and renal excretion. T_4 has a half-life of 4 to 7 days, while T_3 has about 1 day. Thus T_3 is biologically more active. Only the free form can inhibit the secretion of TSH by feedback mechanism.

In the peripheral tissues, deiodination takes place. There is a dehalogenase present in tissues. The iodine goes to the plasma, and may be reutilized by the thyroid. Part of the T_3 and T_4 are conjugated with glucuronic acid and excreted through bile. Deamination of T_4 produces tetraiodothyroacetic acid and T_3 gives rise to triiodothyroacetic acid. These are only one fourth as active as the parent compound.

Calorigenic effect or thermogenesis is the major effect of thyroid hormone. Thyroxine increases cellular metabolism. Protein catabolism results in negative nitrogen balance. Loss of body weight is a prominent feature of hyperthyroidism. Gluconeogenesis and carbohydrate oxidation are increased. Glucose tolerance test shows rapid absorption. Fatty acid metabolism is increased. Cholesterol degradation is increased and hence cholesterol level in blood is decreased which is another hallmark of hyperthyroidism. The requirements for thiamine and vitamin C are increased. Differentiation of fibroblast to fibrocyte as well as osteoblast to osteocyte requires thyroxine. Thyroid hormones are required for the optimal effect of other hormones such as GH, insulin and epinephrine.

Assessment of functional status of the thyroid gland plays an important role not only in diagnosis but also in monitoring the patient under treatment for effectiveness and progress.

1. Measurement of hormones in plasma

Measurement of T_4 and T_3 levels in blood by RIA or ELISA form the basis of laboratory diagnosis of thyroid diseases. In hyperthyroidism, thyroid hormone levels are increased. T_3 and T_4 levels increased and TSH is reduced due to feedback inhibition. In hypothyroidism, T_3 and T_4 are reduced and TSH level is increased. But when hypothyroidism is due to hypothalamic or pituitary defect, then TSH, T_3 and T_4, all are decreased.

2. Measurement of binding proteins

The abnormalities in the level of binding proteins may be reflected as abnormal hormone levels. To avoid the misinterpretation of hormone levels, the binding sites are also measured and the free T_4 (T_3) index is calculated.

T_3 resin uptake measures the free binding sites on TBG. Radioactive iodine labeled T_3 is added to the patient's serum. The labeled T_3 will occupy the free binding sites on TBG. Then a resin is added to the tube, which absorbs the excess of labeled T_3.

The amount of labeled T_3 absorbed by the resin (T_3 resin uptake) is directly proportional to the amount of thyroid hormones (T_3 and T_4) present in serum and is inversely proportional to the free binding sites on the TBG. When the hormone level is increased, the free binding sites on TBG will be low, so more T_3 is taken up by the resin. Similarly when the free binding sites on TBG are high in hypothyroidism, the T_3 resin uptake is also low. Increase in TBG level is noticed in pregnancy and when oral contraceptives are used where estrogen levels are high. Then the free binding sites are high and resin uptake is decreased. The reverse occurs when TBG level is decreased due to malnutrition, nephrotic syndrome (loss of TBG in urine) or inherited deficiency of TBG. In such cases, the free binding sites are low and the resin uptake is increased. Salicylates, phenytoin and such other drugs may also fix on the available sites on TBG, when free sites are reduced and the resin uptake is increased.

3. Measurement of Plasma TSH:

Measurement of plasma TSH is an important test in establishing the cause for hypothyroidism. In primary hypothyrodism, TSH level is elevated due to lack of feedback. But in secondary hypothyroidism, TSH levels as well as T_3 and T_4 levels are low due to a pituitary or hypothalamic cause. Hyperthyroidism due to primary thyroid disease has high T_3 and T_4 levels, but suppressed TSH levels. Hyperthyroidism due to pituitary cause is indicated by high TSH, T_3 and T_4 level. The measurement of TSH is a useful

index for the effectiveness of thyroxine replacement for hypothyrodism.

4. TRH response Test

TRH administration will stimulate the production of TSH, then T_3 and T4 secretion will be increased. An abnormal response is observed in a) Hyperthyroidism, because the negative feedback effect of high T4 overrides the stimulant effect of TRH. Here the thyroid hormone levels are elevated. b) Hypopituitarism, when the pituitary cannot respond to TRH, the plasma thyroid hormone levels are subnormal.

An exaggerated response is observed in primary thyroidism since the negative feedback effect of T4 is reduced.

5. RAIU and thyroid Scanning

RadioActive Iodine Uptake (RAIU) by thyroid gland and thyroid scanning with T_C^{99} are of diagnostic value.

6. Cholesterol level

It is increased in hypothyroidism and decreased in hyperthyroidism. It is not diagnostic, because hypercholesterolemia is seen not only in hypothyroidism, but also in diabetes mellitus, hypertension, obstructive jaundice and nephrotic syndrome. But cholesterol level is a useful index in monitoring the effectiveness of the therapy in thyroid conditions.

7. Detection of thyroid antibodies:

Grave's disease and Hashimoto's thyroiditis are produced by autoimmune mechanism. In these conditions, specific antibodies are detectable in plasma at times even before clinical manifestations occur.

8. Special Test:

Some special tests are used in the diagnosis of different steps involved in hormone synthesis. The perchlorate and thiocyanate discharge tests will assess the iodine uptake and concentration by the gland.

Abnormalities of Thyroid function

Diseases of the thyroid are most common afflictions involving the endocrine systems. The commonest types of thyroid diseases are hyperthyroidism (excess secretion), hypothyroidism (decreased secretion) and goitre (enlargement of thyroid gland) which may or may not be associated with abnormal function (eg: euthyroid goitre (diffuse enlargement), nodular goitre which may lead to hyperfunction, or iodine deficiency goitre which may result in hypothyroidism.

Hyperthyroidism

This is often referred to as thyrotoxicosis which is a syndrome resulting from sustained high levels of thyroid hormones. Hyperthyroidism may be the result of increased synthesis and secretion of thyroid hormone (T_4 and T_3) from the thyroid gland, caused by thyroid gland stimulators in the blood or autonomous thyroid hyperfunction. It can also be caused by excessive release of thyroid hormone from the thyroid gland into the peripheral circulation without increased synthesis of the hormone. This is commonly caused by destructive changes in the thyroid secondary to the various causes of thyroiditis. The last major cause of hyperthyroidism is the conscious or accidental ingestion of excess quantities of thyroid hormone, termed thyrotoxicosis factitia. Patients have an increased rate of metabolism, weight loss, tachycardia, fine tremors, sweating, diarrhea, emotional disturbances, anxiety and sensitivity to heat. Grave's disease is the most common cause of hyperthyroidism. It is an autoimmune disease and has a chronic course with remissions and relapses. The etiology of Grave's disease is an antibody against the thyroid TSH receptor, which results in continuous stimulation of the gland to synthesize and secrete excess quantities of T_4 and T_3. A characteristic symptom observed in Grave's disease is the exophthalmos. All patients with hyperthyroidism have essentially undetectable serum TSH levels except those with a TSH secreting anterior pituitary tumour or those with pituitary resistance to thyroid hormone. Human chorionic

gonadotropin is a weak thyroid stimulator. The level of HCG elevated in conditions like molar pregnancy, choriocarcinoma and hyperemesis gravidarum. Very recently activating point mutations in the TSH receptor, which result in continuous thyroid stimulation, have been described in the solitary nodule. The etiology of familial autosomal dominant syndrome of hyperthyroidism which manifests during infancy is mutations in the gene for the TSH receptor, resulting in constitutive activation of the receptor and continuous stimulation of the thyroid to synthesize and release excess thyroid hormones. Lithium administration can induce goitre with or without hypothyroidism especially in patients with Hashimoto's thyroiditis because of its inhibitory effect on release of iodide from the thyroid. Various clinical types of hyperthyroidism are diffuse toxic goiter, toxic nodular goiter and toxic nodule.[3]

Thyroiditis is the inflammatory disease of the thyroid. Types of thyroiditis include silent lymphocytic thyroiditis, subacute thyroiditis and Hashimoto's thyroiditis. Sporadic lymphocytic thyroiditis is uncommon and earlier reports from the Midwest of its increased frequency may have been confused with ingestion of ground beef contaminated with cattle thyroid. The incidence of hypothyroidism after high dose radiation therapy is high and thyroid function should be evaluated at 6-12 months intervals. Amiodarone and interferon have been reported to induce a wide variety of functional disorders of the thyroid.

Patients with thyrotoxicosis factitia consciously and accidentally ingest excess amount of thyroid hormone, leading to hyperthyroidism in the absence of goitre.

Iodine ingestion is the major cause of hyperthyroidism with a low thyroid radioactive iodine uptake. It is most often seen in patients with underlying nontoxic nodular goitre (especially the elderly) who are given drugs that contain iodine (eg: amiodarone or iodine containing expectorants) or whose radiologic and cardiac studies use iodine rich contrast agents. The hyperthyroidism usually persists as long as the excess iodine remains in the circulation and is more difficult to control than other causes of hyperthyroidism.

There are a number of treatments for hyperthyroidism depending on the etiology.

Iodine in pharmacologic doses inhibits the release of T_3 and T_4 within hours and inhibits the organification of iodine, a transitory effect lasting from a few days to a week. The usual dosage is two to three drops of a saturated potassium iodide solution (300 to 600 mg/day) or 0.5g sodium iodide in 1 litre of 0.9% NaCl solution given IV slowly in 12 hrs. Complications of iodine therapy include inflammation of the salivary glands, conjunctivitis and skin rashes.

Propyl thiouracil and methimazole are antithyroid drugs that decrease the organification of iodide and impair the coupling reaction. Propranolol is also indicated for the prompt management of tachycardia found in other forms of hyperthyroidism.

Radioiodine is recommended by many as the treatment of choice for Grave's disease and toxic nodular goitre in all patients including children. There is no proof that radioiodine increases the incidence of tumors, leukemia, thyroid cancer or birth defects in women who become pregnant later in life.

Surgical treatment is indicated for younger patients with Grave's disease whose disease has recurred after courses of antithyroid drug and who refuse I^{131} therapy, in patients who cannot tolerate other drugs because of hyper sensitivity or other problems, in patients with very large goitres, and in some younger patients with toxic adenoma and multinodular goitre.

Hypothyroidism

This disorder results from low levels of circulating thyroid hormones. Hypothyroidism has been given the following classification:

- Autoimmune thyroiditis: this can be either goitrous (Hashimoto's disease) or non goitrous(primary myxodema).

- Latrogenic: this resulted after thyroidectomy or radio iodine therapy. Drug induced (antithyroid drugs, PAS and iodide in excess) hypothyroidism also comes under this group.
- Dyshormonogenesis
- Due to excess intake of goitrogens
- Secondary to pituitary or hypothalamic disease
- Thyroid agenesis
- Endemic cretinism- often goitrous and due to iodine deficiency.

Most common cause is primary thyroid disease, often autoimmune in nature, leading to myxoedema in adults. Women are more affected than males. Symptoms are lethargy, tolerance to heat, cold intolerance, slow heart rate, weight gain, dry coarse skin, mental dullness and physical slowness.

In children, hypothyroidism produces mental and physical retardation, known as cretinism. The TBG may be elevated due to maternal hyperoestrogenism and therefore total T_4 and T_3 may be normal. The lack of feedback will give elevated TSH level also. Prompt diagnosis and treatment are important in cretinism since any delay in starting replacement may lead to irreversible damage. Maternal hypothyroidism may also cause neonatal cretinism.

Hypothyroidism may result from treatment of hyperthyroidism using antithyroid drugs or radioactive iodine.

Replacement of the hormone will produce immediate effect. Secondary hypothyroidism may result from pituitary or hypothalamic causes. The measurement of TSH test will help to differentiate the different type. Euthyroid goitre can result from iodine deficiency. There is raised TSH level, which would produce continued stimulation of gland, leading to hyperplasia and goitre but hormone levels are often normal. Dishormonogenetic goitres result from congenital deficiency of different steps of hormone synthesis. Diagnostic features are cretinism in early infancy, presence of iodinated tyrosines in plasma with low hormone levels and a positive perchlorate discharge test.

The normal thyroid gland is impalpable. The term goiter is used to describe generalized enlargement of the thyroid gland. A discrete swelling in one lobe with no palpable abnormality elsewhere is termed an isolated (or solitary) swelling. Discrete swellings with evidence of abnormality elsewhere in the gland are termed dominant.[3]

Thyroid enlargement is given the following classification:

1. Simple goiter (euthyroid):
    - Diffuse hyperplastic
        - physiological
        - pubertal
        - pregnancy
    - Multinodular goiter

2. Toxic
    - diffuse- Grave's disease
    - multinodular
    - toxic adenoma
3. Neoplastic
    - Benign
    - Malignant
4. Inflammatory

Dunhill classified thyroid cancer as differentiated and undifferentiated. The differentiated carcinoma is again subdivided into follicular and papillary carcinoma. Papillary carcinoma has frequently followed accidental irradiation of the thyroid in childhood. The incidence of follicular carcinoma is high in endemic goitrous areas, possibly due to TSH stimulation.[3]

Non Thyroidal Illness Syndrome

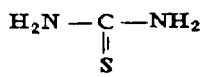
During starvation and in illness, thyroid hormones in blood are reduced to level typical of hypothyroidism. This response has been interpreted over several decades as an adaptive response causing serum total hormone levels to drop while metabolism remains 'normal'. For this reason, it has been described as the "euthyroid sick syndrome". However, in severe illness, the changes in thyroid hormone supply are associated with dramatically increased chances of death. Whether the changes are adaptive or maladaptive remain uncertain.[4,5]

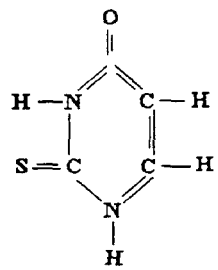
Antithyroid Agents

Antithyroid agents is employed to designate any substance inhibiting thyroid function.[6] Broadly classified into two: those that prevent release of hormone from the thyroid by a feedback mechanism and agents that retard synthesis of hormone. The second group includes thiocyanate and certain anions that inhibit iodide uptake by the thyroid. Sulfonamides interfere with the iodination reaction. The same active pump that pumps iodide ions into the thyroid cells can also pump thiocyanate ions, perchlorate ions and nitrate ions. Therefore the administration of thiocyanate or other ions in high concentration can cause competitive inhibition of the iodide transporting into the cell, that is, inhibition of the iodide-trapping mechanism. Thiocyanate is widely distributed in nature, occurring in blood, saliva, urine and in many plants such as members of the genus Brassica (including cabbage, cauliflower etc). Isothiocyanates, such as mustard oil organic nitriles and the widely distributed cyanogenic glycosides transformed to thiocyanates in mammalian metabolism. Consumption of substantial amounts of foods containing these substances could render iodine-deficient a diet otherwise adequate in iodine and explains the goitre endemic in certain regions of the world.

A number of organic compounds may also be classed as antithyroid substances, these can be divided into two- the most active of which have in common a thiocarbamide grouping, as for example thiourea and thiouracil.

Thiourea





Thiouracil

A second group has an aminobenzene grouping, the best known of which are the sulfonamides; certain chemical groups present in them prevent iodination and subsequent hormone formation by forming molecular compounds with elemental iodine in the gland. Some of the very active aromatic antithyroid substances act by forming stable substitution compounds with the gland iodine.

Iodine deficiency

Iodine, an essential micronutrient, is required for the normal mental and physical well being of mankind. The average iodine requirement for an adult is estimated as 100 µg/day.[7] It is met through sea foods (fish), vegetables, milk, meat and drinking water.

On a worldwide basis, iodine deficiency is the single most important preventable cause of brain damage. Children affected by severe iodine deficiency disorders have an intelligence quotient (IQ) of up to about 13.5 points below that of children from

comparable communities in areas where there is no iodine deficiency.[8] Iodine deficient children suffer from tardy concentration, impaired co-ordination and sluggishness, which result in poor school performance. In addition, their energy and productivity are also be adversely affected. This has an effect on health, the quality of life, and economic productivity of communities. IDD are amongst the easiest and cheapest of all disorders to prevent. Iodine deficiency in humans and animals occurs when iodine intake falls below recommended levels. Iodine deficiency is a naturally occurring ecological phenomenon that is present in many parts of the world. The erosion of soils as a result of glaciation, frequent flooding, rivers changing course over a period of time, heavy rainfall, wide scale deforestation etc, leads to continued and increasing loss of iodine from the soil.[9] Intensive irrigation also results in depletion of soil micronutrients.[10] Thus ground water and locally grown vegetation in these areas lack iodine. The ideal iodine intake as recommended by WHO / UNICEF/ ICCIDD is shown in Table.[11]

Age group	Iodine Requirement (in µg/day)
Infants (0-11 months)	50
Children (12months - 59 months)	90
School Age children (6-12 years)	120
Adults(above 12 years)	150
Pregnant and Lactating women	200

When iodine intake falls below the recommended levels, the thyroid gland is no longer able to synthesize sufficient amount of thyroid hormone. The result is hypothyroidism.

The various reasons for hypothyroidism are:

i. Dietary iodine deficiency
ii. Defect in uptake of iodide by the thyroid gland
iii. Defect of enzymes involved in the synthesis and degradation of thyroid hormones.
iv. Salvage defect (Deiodinase defect)
v. Diseases of thyroid gland
vi. Pituitary diseases
vii. Protein malnutrition
viii. Presence of positive goitrogenic factors in the food.

The resulting low level of thyroid hormones in the blood (hypothyrodism) is the principal factor responsible for the damage done to the developing brain and the other harmful effects known collectively as Iodine Deficiency Disorders.[12] The adoption of this term was significant as it emphasized that the problem extended far beyond goitre and cretinism.

The spectrum of the Iodine Deficiency Disorders(IDD) are:

Adult	Goitre and its complications. Impaired mental function. Iodine induced hyperthyrodism
Child and adolescent	Retarded mental and physical development
Neonate	Neonatal hypothyroidism
Foetus	Increased infant mortality, abortions, still-births, congenital anomalies, Increased perinatal mortality Neurological cretinism (Mental deficiency, deaf mutism, spastic, diplegia squint) myxoedematous cretinism, (mental deficiency, dwarfism, hypothyroidism), psychomotor defects.
All ages	Physical sluggishness. Increased susceptibility to nuclear radiation.

Iodine deficiency can be termed as a problem of human resource development since the loss of energy due to the resultant hypothyroidism and the learning disabilities leading on to poor motivation have serious consequences on child survival and development.

The most critical period of human brain growth and development is from the second trimester of pregnancy to the

third year after birth. It is during this period that 90% of adult human brain development is completed.[13,14] Normal levels of thyroid hormones are required for optimal development of the brain. In areas of iodine deficiency, where thyroid hormone levels are low, brain development is impaired. In its most extreme form, this results in cretinism. However, the greater impact on public health is the more subtle degrees of brain damage and reduced cognitive capacity, which affects the entire population. As a result, the mental ability of apparently normal children and adults living in areas of iodine deficiency is reduced compared to what it would otherwise be. Thus the potential capability of a whole community is reduced by iodine deficiency. Even the domestic animals such as poultry, goats, pigs, cows and buffaloes are affected and as a result, the livestock productivity is dramatically reduced.[15]

Conventional methods of Iodination are:

1. Addition of iodide or iodate to salt

An iodine deficient environment requires the continued addition of iodine. This is most conveniently and cheaply achieved by the addition of iodine to the salt. The entire population groups consume salt in the same quantity everyday, throughout the year. The average intake of salt in India is about 10 grams per person per day. Thus a micronutrient like iodine, introduced through salt will ensure average requirement of 150 µg of iodide everyday,

throughout the year when salt with 15 parts per million of iodine (15 mg of iodine per kg of salt) is available at consumption level. A decrease in salt intake can be readily met by increasing the iodine content. Where a significant amount of processed food is consumed, it is important that the salt used by the food industry is iodised as well. Iodised salt is prepared by adding salt of iodine to common salt. Any of the iodides or iodates of potassium, sodium or calcium can serve the purpose. But in a hot and humid country like India, potassium iodate is preferred because of its stability. Therefore, after purification of common salt, this iodizing agent is added in a proportion of about 30ppm (parts per million). As a result, when the salt reaches the consumer, inspite of all types of losses, it still contains about 15 ppm iodate, which is enough to supply the required amount of 150 µgm of iodine per day. Besides, small quantities of stabilizers and anticaking agents are also added to it to prevent decomposition of potassium iodate and to make the salt free flowing. Usually sodium carbonate and silicon dioxide serve these purposes. However, according to the Food Adulteration Act 1995, these addendants should be limited to an extent of 2% only.

Government has put forward a ban on selling of non-iodized common salt for human consumption. A controversy has generated regarding the justification of use of iodized salt. Universal iodination of salt is recommended because of the increased incidence of iodine deficiency disorders.

On the other side, the universal iodination has other aspects like social, economic and scientific problems. The social and economic problems include unemployment to thousands who are involved in traditional salt manufacturing and economic problems will be mainly because of the uncontrolled price rise on iodised salts by big companies. In addition to these, the scientific problem include i) addition of silicon dioxide as the anticaking agent which has adverse impact on health ii) removal of magnesium and calcium salt essential for the body, during purification of salt and iii) decomposition of iodizing agents during preservation, transportation and cooking thereby loss of iodine.[16]

2. Addition of iodide or iodate to bread.

3. Use of tablets containing potassium or sodium iodide.

4. Administration of iodised oil.

Apart from these conventional methods, in China, iodine repletion of soil through irrigation water is using as an effective and cost efficient way of providing iodine.[17] Southern Xinjiang Province of the People's Republic of China is an area of severe iodine deficiency. Goitre rates ranged from 48-68%, 8-11% of infants had definite neurological deficits, and 25-30% of the infants had microcephaly attributable to iodine deficiency. In these areas, soil iodine concentration ranged from 7-32µg/kg and water iodine

concentrations ranged from 1-1.5 µg/litre. Median urinary iodine excretion in women of child bearing age ranged from <10 µg/litre to 17 µg/litre. Iodination of irrigation water had the potential to reach every household on a sustained basis, given that all farms rely on irrigation. Iodate dripping programme was conducted in groups of villages contained in three separate townships. 5% potassium iodate was dripped at a controlled rate from a 200 litre tank placed on a platform over an irrigation canal. The flow rate in the canal was 1-3m^3/s. Dripping rates adjusted to achieve an iodine concentration of 10-80 µg/litre in the canal water. The concentration of water-soluble iodine in the soil was approximately 3 fold higher than the iodine concentration in the applied water. The high soil concentration of water-soluble iodine declined subsequently with an apparent half-life of 2-4 months. One year after the first dripping, median urinary iodine increased from 14 µg/litre to 49 µg/litre, during the next 2 years, it decreased to 18 µg/litre and 21 µg/litre respectively. Infant mortality fell by 50% in the treated areas of the three townships, and this low level was maintained during the post dripping years. The success of iodine supplementation via irrigation water is the duration of time that added iodine remains in the soil.

Environmental supplementation of iodine at least in the townships studied, was an effective means of achieving the primary goal of overcoming iodine deficiency, thus resulting in improved human health, development and growth. In addition, it can

be regarded appropriately as fertilizer for animals in as much as its immediate economic benefit alone far outweighs its cost.

It is now estimated that nearly 1.6 billion people around the world are at risk of IDD in which approximately 300 million suffer from lower intelligence. The most familiar consequence of iodine deficiency is goitre - an enlargement of the thyroid gland in the neck. Recent studies have proved that there is a wider spectrum of disorders related to growth and development, including brain development. They are now collectively called as IDD. Iodine deficiency has now been recognized as a primary cause of endemic goitre occurring in different parts of the world.[18]

Goiter prevalence and urinary iodine excretion study in school children in an endemic area in Bohemia after twenty years of iodine prophylaxis were carried out.[19] It was revealed that dietary iodine deficiency remains a permissive factor in the development of goiter and is more marked in former endemic foci. Prevention by iodised salt protects newborn infants, but does not prevent the development of the goiter in all subjects in the course of development of the organism. The possibility of reinforced iodisation in treated areas deserves consideration.

In the Randers area of Denmark, urinary iodine excretion was found to be much lower than recommended intake levels, both in women in late pregnancy and in non-pregnant

controls.[20] The increase in serum thryroglobulin level in pregnant women was probably due to the extra iodine requirement of pregnancy, which was not satisfied. The results suggest that pregnant women in this area should receive iodine supplementation and that a general program of iodine supplementation should be considered.

A hospital based study of urinary iodine excretion in residents of Taipei city was carried out in 1991. The study reveals that only 3% of the subjects excreted less than 50μg/g creatinine but 42.7% of subjects excreted more than 300μg iodine/g creatinine. This indicated that iodine deficiency is not a real problem in Taipei city. However about half of the Taipei city residents may take more than the suggested optimum amount of iodine.

Casual urine samples were collected to determine iodine excretion of 1680 Belarus children during 1990-1994.[21] The subjects, 8-16 years old, were from nine different regions of Belarus, 60% were from the Gomel oblast, which has been associated with relatively high levels of radio iodine fallout and increased incidence of thyroid cancer. Most of the median values indicate borderline/ low iodine intake or mild iodine deficiency. Ranges were wide but 163 children excreted <20μg/litre urinary iodide and they should be considered severely deficient in iodine. In a field study in 1997, Liesenkotter et al monitored the iodine supply and its effect on the thyroid gland volume in prepubertal and pubertal children in the eastern and western parts of the city of Berlin.[22] Small thyroid

volumes and normal iodine excretion in Berlin school children indicate full normalization of iodine supply. Iodine status of New Zealand residents as assessed by urinary iodide excretion and thyroid hormones suggest that iodine status of New Zealanders may no longer be considered adequate and may once again be approaching levels of intake associated with clinical iodine deficiency.[23] Urinary iodine excretion studies in school children of rural areas in Cuba reveals that Cuba has a mild iodine deficiency public health problem with respect to WHO criteria, which in the mountainous areas is severe.[24] The Government of Cuba has started a national Plan of Action and funds are being mobilized to introduce nation wide iodized salt.

Korea is a region abundant in foods containing iodine such as seaweed and fish. An adequate amount of iodine consumption is extremely important as both a deficiency and excess of iodine can result in health problems. Study was undertaken to assess the iodine nutritional status of normal Korean adults who consume seaweed and fish, and to determine the relationship between the dietary iodine intake and the urinary excretion of iodine.[25] The study data indicated that the iodine intake and excretion of Koreans depends mostly on the amount of seaweed consumption like sea tangle and sea mustard. As well, the current iodine intake and urinary iodine excretion by Koreans seems to be higher than in other countries.

Iodine intake and Iodine deficiency were investigated in thirty vegans consuming their habitual diet.[26] The findings highlight that vegans are an ' at risk ' group for iodine deficiency.

Goitre prevalence and urinary iodine excretion levels were also assessed in 605 school children living in the Antalya region, a well known endemic goitre area in Turkey.[27] Urinary iodine excretion level revealed mild iodine deficiency in the region. No significant correlation was observed between urinary iodine excretion levels and thyroid volumes.

There is currently no co-ordinated policy on the epidemiology and control of iodine deficiency disorders in many parts of Africa even where these disorders are endemic. One of the studies in this area revealed that there was a gradient in iodine status between the mother and her breast fed infant that is unfavourable to the growing child.[28] The latter may thus require iodine supplementation in spite of the fact that the mother is iodine replete.

Endemic goiter has reemerged in Thailand. This is particularly dangerous for children since iodine deficiency disorders (IDDs) might negatively influence their intellectual and mental development.[29] In order to assess that situation, the iodine content of breast milk was determined. The risk of women with low iodine excretion was 15 fold higher in comparison to women with sufficient

iodine excretion to provide breast milk for their babies with insufficient iodine content. It was concluded that urinary iodine excretion could be used to monitor the IDDs in lactating mothers.

To prevent iodine deficiency disorders in Switzerland, table salt is currently fortified at 15 ppm. However, several recent reports had suggested that urinary iodine excretion is marginal or inadequate among segments of the Swiss population, including school children. Urinary iodine concentration in 243 school children aged 5-13 years from the Zurich area and the Engadine valley were evaluated.[30] The results of the study suggested that the iodine status of some Swiss school children might no longer be adequate. They indicate the importances of intermittent monitoring of iodine status in Switzerland, where dietary habits and food supply patterns are changing. They also supported the recent decision by the Swiss Federal Department of the Interior to increase the concentration of iodide in Swiss table salt, based on the recommendations of the Fluoride- Iodine Commission of the Swiss Academy of Medical Sciences.

An epidemiological study of iodine deficiency disorders covering 4230 pre-adolescent and adolescent school children, together with biochemical analysis of 741 urinary samples, 26 potable drinking water samples and 56 blood samples were conducted in the Plateau state of Nigeria, West Africa.[31] Results indicated that about 2/3rd of the region is goiter endemic due to iodine

deficiency prevailing in the region as reflected by low drinking water iodine concentrations as well as by reduced urinary iodine excretions. Discovery of 9 cretins further signifies the magnitude of the problem in the region. It was concluded that a significant proportion of the children living in these areas might likely to suffer from partial thyroid insufficiency with its attendant consequences.

In order to investigate the prevalence of iodine depletion in chronic inflammatory bowel disease, two separate studies have been performed.[32] 24 hour urinary iodine excretion and radioiodine uptake studies were carried out. The results are compatible with an increased occurrence of iodine deficiency in patients with chronic inflammatory disease.

Iodized salt has a high acceptance in the Potsdam region of Germany. But assessment of iodine intake showed that only 5% of the adult population has an iodine intake of more than 100μgs/day.[33]

Many different factors leading to relative or absolute deficiency of iodine may cause changes in the thyroid gland. May (1935) found that the administration of fluorine to rats produced hypoplasia of the thyroid epithelium with altered staining reaction of the colloid. Having experimented with over 800 patients, he advocated internal fluorine therapy in the treatment of toxic goitre. The evidence of alteration in thyroid structure in the experimental

production of chronic fluorosis in animals is conflicting (Roholm 1937). In North India, a focus of endemic goitre has long been known in the Punjab plains (McCarrison 1913). The report of the Punjab Government hospitals and dispensaries showed how numerous were the cases of goitre seeking treatment from this area. Macnamara (1880) studied the distribution of goitre in the Punjab plains before the introduction of the present extensive system of canal irrigation, at a time when the majority of the town people were dependant on local wells. In certain towns he found nearly all the inhabitants with goitre and saw numerous cretins and dwarfs. Dogs and goats also had goitre. He observed that poverty and bad food led to increase in the size of the goitre, but noted that a colony of river fisherman did not show any thyroid enlargement. In this goitrous area of the Punjab (Wilson, 1939) observed a high degree of human dental fluorosis, as evidenced by mottled enamel among villagers still using well water in the neighborhood of Hundewali, where Heron(1913) found that the older Aravalli rocks emerge through the alluvium. Samples of these rocks were found to have a wide range of values for fluorine content, varying from 30 to 3200 parts per million. Geodetic investigations suggest that these older rocks extend between the Chenab and Ravi rivers, in an area coinciding with the downward extension of goitre in the Punjab plains.

In England, the decrease of endemic goitre in recent times has been attributed to the improvement of village water supplies and the closing of Wells(Ash,1926). Cretinism was formerly

associated with areas where the incidence of goitre was high (Norris, 1848) but Joll (1932), found no focus of endemic cretinism remaining. Stocks (1928), reviewing the results of the Board of Education survey of goitre amongst English school children, concluded that there was a belt where goitre was comparatively prevalent, extending, chiefly in rural areas, from Cornwall north-eastwards through Somerset, into Oxfordshire between the Cotswold and Chiltern hills, on into Northamptonshire, thence northwards to Derbyshire and up the Pennine chain.

The distribution of endemic goitre in the Punjab and in England is related to the geological distribution of fluorine and to the distribution of human dental fluorosis.[34] Enquiry showed the presence of dental fluorosis among school children in two areas of Somerset where two previous observers had recorded a high incidence of goitre, and the absence of dental fluorosis in an adjoining area selected as control where endemic goitre was absent. Goitre prevalence in a number of endemic areas has been shown to depend on factors other than iodine deficiency.[35-37] The role of water hardness in endemic goitre in man and experimental goitre in animals is well established. Goitre has been produced in experimental animals by feeding fluoride salts [38-40] and fluoride exerts an antithyroid effect in man when used in the treatment of thyrotoxicosis.[41-42] While areas in which the water contains an unusually high level of fluoride or in which dental fluorosis is pronounced have been shown to coincide with the regional

distribution of endemic goiter [43-46] and the absence of endemic goitre in other areas where the fluoride levels are equally high has been noted.[47-49] The prevalence of goitre in 17 Himalayan villages has been studied in 1972.[50] Water samples from each village were taken and levels of iodine, fluoride and hardness determined. In 13 villages, wide variations in goitre prevalence were not attributable to differences in iodine intake, which remained constant within a narrow range.

Instead, variations in goitre prevalence were found to correlate closely with the fluoride content and with the hardness of the water in each village. The highest prevalence of goitre is in villages with both hard water and high fluoride levels. The effects of fluoride and water hardness seem to be independent. Water hardness was derived from the calcium and magnesium levels.

In most endemic areas, soil and rock contain both calcium and magnesium. The effect of magnesium has not been investigated to any great extent. Hence the influence of these divalent ions in producing goitre has been studied and after inducing thyroidal deficiency states with the antithyroid drugs, propyl thiouracil and neomercazole in experimental rats.[51] When combined with the antithyroid drugs, calcium appears to increase the goitrogenic effect while magnesium reduces the goiter.

The widespread use of fluoride to reduce dental caries has aroused considerable controversy because of the toxicity of this halogen. Under the conditions of relative iodine deficiency of Central Europe, a possible thyroid inhibiting action of fluorine has elicited the interest of physicians and public health officers. The administration of larger doses of fluoride over a longer period of time caused a noticeable reduction of several parameter of thyroidal biological activity. Prolonged administration of a daily dose of 5-10 mg of fluoride to patients with hyperthyroidism may cause clinical improvement together with a significant fall in the level of plasma protein bound iodine and a reduction in the basal metabolic rate.[52] Studies with radioactive fluorine failed to demonstrate any important accumulation of fluorine within the thyroid in vivo. Thyroidal, blood and urinary radioiodine studies suggest that fluorine inhibits the thyroid iodide concentrating mechanism. Fluorine does not impair the capacity of the gland to synthesize thyroid hormone when there is an abundance of iodide in the blood. However inhibition of the total iodide pool will impose a critical limitation of hormonal synthesis, and may explain the therapeutic effect.

Observational studies primarily based on diet questionnaires or food records have reported that vegetarians can have a very low iodine intake. Increased risk of iodine deficiency with vegetarian nutrition was analytically ascertained by Neubert and colleagues.[53] Six healthy adult volunteers participated in the experimental diet study carried out in four separate 5 day diet

periods. Iodide analysis were performed in the urine samples and in representative samples taken from all ingested diets. Urinary iodide excretion was significantly lower with lactovegetarian diet and extremely low intake was determined for strict vegetarians.

The presence of large goitre belts in the United States, led to an experiment in the 1920s in which schoolgirls were given iodine supplements. As a result, there was a marked decrease in the incidence of goitre. The follow-up showed that these girls suffered from a lower incidence of aggressive thyroid cancers, compared to the general population. Further in those who developed cancer, it was curable. Moreover, there was a decrease in the evidence of follicular cancer.[54] Many who migrated from iodine-poor regions to iodine-rich regions showed decreased trends of development of follicular cancer. In South India, excess iodine in diet is related to the higher incidence of papillary cancer compared to other more malignant subtypes of thyroid cancer. Thus, iodine has a protective effect on the development of a more curable form of thyroid cancer. Shellfish and other seafood, which form the staple diet in countries like Iceland and Norway, are associated with the highest incidence of thyroid cancer in the world. In women, certain factors such as the size of the family, the use of oral contraception, late age of first childbirth (above 30 years) are said to constitute an increased risk.

A pooled analysis of case control studies of thyroid cancer showed that relatively elevated fish consumption does not appreciably increase thyroid cancer risk and may have a favourable influence in areas where iodine deficiency was common.[55]

Although thyroid cancer is not preventable, avoiding exposure to radiation during childhood and screening for cancer in susceptible families go a long way in early diagnosis and cure. In families affected by the medullary subtype, bio-chemical and genetic screening has resulted in early detection. Calcitonin, a hormone produced by 'C' cells in the thyroid gland can be considered a marker. Genetic screening for the RET proto-oncogene further helps to confirm the risk of developing this cancer.

Ultrasound of the thyroid can pick up millimeter-sized nodules in the thyroid and ultrasound guided fine needle aspiration cytology is a useful screening procedure. In familiar predisposal to thyroid cancer, regular check up and a genetic study are necessary. FNAC is a reliable, effective and relatively painless outpatient procedure done without anesthesia. For thyroid cancers, the patient needs surgery at the earliest. Early surgery is safe and the results are good.

Evidence of an association between sub-clinical hypothyroidism and cardiovascular disease in mounting. The impact of thyroid hormone in lipid levels is primarily mediated through

triiodothyronine-bound protein and activation of the promoter regions of the low density lipoprotein receptor and 3 hydroxy – 3-methyl glutaryl COA- reductase genes, leading to a reduction in serum cholesterol levels. Thus the decreased T_3 seen in hypothyroidism may result in increased serum cholesterol. Although a clear correlation exists between overt hypothyroidism and clinically significant hypercholesterolemia, there is a logarithmic relationship between thyroid-stimulating hormone and cholesterol. Current data suggested that normalizing even modest thyroid stimulating hormone elevations might result in improvement in the lipid profile.[56]

The relationship between serum cholesterol, thyrotropin, thyroxine and triiodothyronine was investigated in 456 male patients with suspected hypothyroidism. The correlation between serum cholesterol and serum thyroxine and between serum cholesterol and serum triiodothyronine were not significant, but the correlation between serum cholesterol and TSH was significant.[57] A study carried out in Netherlands showed that long term amiodarone treatment is associated with a dose dependent increase in plasma cholesterol that is independent of thyroid function.[58]

In order to investigate the variation in iodine content in drinking water in Denmark, a study was carried out. Iodine content in tap water was varied from 2.1-30.2 µg/l. The iodine content was highest in the eastern part of Denmark and lowest in the western part of Denmark.[59]

In order to verify a relationship between congenital hypothyroidism (CH) and iodine deficiency, Parito. G. et al analyzed the iodine concentration in drinkable water from 148 localities in Calabria. The high incidence of congenital hypothyroidism in rural and mountainous areas were corresponded with a high percentage of water sample with iodine concentration less than 2 μg/l.[60]

High incidence of goitre in human together with low level of iodine in water and cow milk had been observed in southern Poland.[61] A negative correlation had been obtained between goitre incidence in human and iodine concentration in water (r=0.43). A low correlation coefficient suggested that iodine is not a solely factor responsible for goitre development. Studies on cows had indicated that thiocyanate may have effect on goitre development as well. It had been observed that enhanced level of plasma thiocyanate following feeding with brassica plants increased proportionally the goitrogenic action as well as the accumulation of I^{131} by the thyroid and its conversion into organic form.

Thiocyanate analysis in serum and urine from a human population sample in Port Harcourt, Nigeria was done.[62] Thiocyanate levels were determined in serum and urine samples obtained from a human population sample of healthy non-smoking volunteers of both sexes known to eat gari based meals at least once a day. Average increases of 18 – 20 % were observed, for serum

and urine thiocyanate followed by a gari based meal. No significant effect of sex or age on the thiocyanate levels was observed. The gari samples used in the study , as well as random samples from the locality of study, had no detectable thiocyanate but contained between 0.013 to 0.015 mg cyanide per kg of gari. These findings indicated that conversion of cyanide to thiocyanate is a significant pathway in the metabolism of HCN and contributes significantly to thiocyanate found in body fluids and tissues of man . In addition , support was provided for the possible involvement of the sulphur - transferases in the process of cyanide detoxication.

Sera from pregnant and non pregnant women were analyzed for thiocyanate.[63] Serum thiocyanate concentration was higher in Nigerians compared with Caucasians and was significantly higher also in pregnancy. Hypoalbuminemia and dietary consumption of cyanogenic substances in pregnancy probably contributed to the higher thiocyanate levels. This might have a possible significant effect on the fetal well-being, particularly fetal birth weight.

Urinary thiocyanate concentration is an indicator of cyanide exposure. A large epidemic of spastic paraparesis in Mozambique during a drought was attributed to cyanide exposure from cassava.[64] A marked seasonal variation in thiocyanate concentration was present, with the highest value coinciding with the dry season, the period of the epidemic and the cassava harvest.

Lower values were found in the neighboring unaffected semi-urban center. As cassava cultivation increases in many drought- affected countries, monitoring of urinary thiocyanate concentration to estimate cyanide exposure and identifying population at risk for spastic paraparesis should be done.

Interaction between thiocyanate and nitrosamine precursors ingested in food may enhance nitrosamine carcinogenesis.[65] An experimental study in growing pigs showed that 112.2 – 117.3 mg/kg hydrocyanic acid affected serum but not organ and muscle thiocyanate in protein sufficient diets.

Meal prepared from unheated rape seed (Brassica napus) showed the presence of thiocyanate ion, while meal from heated seed of the same cultivar did not show detectable amounts.[66]

In India, the widespread incidence of endemic goitre and cretinism have been first observed in the turn of the century by Mc Carrison[67] and confirmed by other workers.[68-69] A broad belt of high incidence of goitre with cretinism has been described extending from the mountain ranges of Afganisthan to the sub-Himalayan region of both India and Pakistan and including Nepal, Assam, Bangladesh, Burma etc and ending with Malaysian archiopelago and the Indonesian islands. In Ceylon, endemic goitre is prevalent in the South Western provinces of the island. Apart from

this, no large-scale reports of goitre in other parts of India are available.

Epidemiological surveys have been conducted to study the prevalence of hypothyroidism in the population inhabiting the foothills of the Himalayas (Terai region) and Assam only, where the prevalence of hypothyroidism has been reported to be very high. There is practically no information on prevalence of hypothyroidism (either clinical or sub clinical) in the state of Meghalaya. In order to study the prevalence of hypothyroidism in the women of reproductive age group (15-45 years) of Meghalaya, serum samples collected from non-pregnant and pregnant women were screened for total T_4(Thyroxine) and TSH (Thyrotropin) with the help of radioimmunoassay and immunoradiometric assay.[70] A prevalence of 1.11% and 1.43% was noted in the non-pregnant and the pregnant women respectively. An average prevalence of 1.25% was recorded among the women of the reproductive age irrespective of status. Thus the prevalence of hypothyroidism in the women of Meghalaya seems to be very high as compared to that of the non-endemic goitre belt of India (0.071%).

Geographically, Kerala lies in the Peninsular India. A significant proportion of the population in the state lives in the coastal areas. The state of Kerala has achieved near 100 percent literacy and is known to be a better performing state with respect to health indicators. The population of Kerala has traditionally consumed sea fish and tapioca on a regular basis. Sea fish is rich in

iodine. While the thiocyanate present in tapioca interferes with iodine utilization by the thyroid gland. Thus, sea fish and tapioca consumption have had a significant effect on the iodine nutrition of Kerala over the years. Over a period of time, the tapioca cultivation and consumption has declined. Fish, however, remains an important food item consumed by the population.

In Kerala, the occurrence of thyroid adenoma in increased frequency and acting as a precancerous lesion has been observed. The increased frequency of nodular lesions among hospital admissions in Kerala has been known for sometime past and the ICMR established a Research cell for a study of this problem in 1965 and the increased frequency was confirmed. Being a coastal state, it was then presumed that iodine deficiency may not be a possible factor and the attention was drawn to the high background radiation from the monozite sand. Tulpule while analyzing water samples from different parts of India for fluoride and iodine content surprisingly noted that the 17 water samples received from Kerala had low iodine content comparable to those from Bihar and Uttar Pradesh. But no attempt was made to correlate this with the increased frequency of nodular goitre in the state.

Very low levels of iodine in subsoil water was observed in the samples derived from the hilly regions of this state from where large number of multinodular goitre were reporting to the thyroid clinic of the Medical College Hospital, Thiruvananthapuram.

An attempt was then made to outline the various lesions and correlate this with the low iodine content in drinking water. Based on a report to the Indian Council of Medical Research (ICMR) in 1967, Kochupillai et al in 1976 conducted the initial study. They studied the overall prevalence of thyroid nodules in the coastal areas of Kerala. Subsequent studies have been restricted to districts or hospital based studies. Since 1989, the Goitre Control Cell of the Directorate of Health Services conducted district wise surveys all over the state and reported the prevalence of goitre. It ranged from 4.7% to 27.3%. These studies have helped researches and public health officials to assess the status of iodine nutrition in Kerala. The studies have been listed in the table in chronological order.

Sl No	Author & Study Questions	District	Period Of survey	Goitre Prevelance Rate(%)	Other Parameters	Remarks
1	Kochu pillai et al[71] Prevalence of thyroid nodules	Limited to coastal areas	1979	Overall prevalence not done	Prevalence of thyroide nodules-13% Solitary-68% MNG-20%	Only looked at thyroid noldules
2	Ramachandran[72] Prevalence of thyroid swelling in hospital setting	Thyroid Clinic in Medical College Hospital	1990	Adenoma-16% Nultinodualr Goitre-61% Carcinoma-6.5%	Low levels of iodine in the subsoil water of midland and highland areas.	Hospital based study
3	Paulose[73] Goitre prevalence in villages of Kottayam, Medical College Kottayam Inpatient and Outpatients Clinics and school Camps	Kottayam	1992	Kottayam District 6-28% MCH Outpatent-30.0% MCH Inpatient -3.7% Peripheral - 9 to 17% School campus-16 to 39%	Low levels of iodine in water of highland areas - $\frac{1}{3}^{rd}$ of that of lowland areas	Regionalized Need further analysis
4	Goitre Control Cell, Directorate of Health Services(DHS)[74] Goitre Prevalence	Trivandrum	1989-1992	17.3 - 27.3	Not done	Only goitre prevalence assessed

Sl No	Author & Study Questions	District	Period Of survey	Goitre Prevelance Rate(%)	Other Parameters	Remarks
5	Goitre Control Cell, (DHS) [74] Goitre Prevelance	Kollam	1992	5.8 – 12.9	Not done	Only goitre prevalence assessed
6	Goitre Control Cell, (DHS) [74] Goitre Prevelance	Ernakulam	1992	9.1	Not done	Only goitre prevalence assessed
7	Goitre Control Cell, (DHS) [74] Goitre Prevelance	Alappuzha	1992	4.7	Not done	Only goitre prevalence assessed
8	Goitre Control Cell, (DHS) [74] Goitre Prevelance	Pathanamthitta	1992	12.6	Not done	Only goitre prevalence assessed
9	Goitre Control Cell, (DHS) [74] Goitre Prevelance	Idukki	1992	17.8	Not done	Only goitre prevalence assessed
10.	Goitre Control Cell, (DHS) [74] Goitre Prevelance	Kottayam	1994	21.0	Not done	Only goitre prevalence assessed
11	Goitre Control Cell, (DHS) [74] Goitre Prevelance	Thrissur	1994	14.0	Not done	Only goitre prevalence assessed

Sl No	Author & Study Questions	District	Period Of	Goitre Prevelance Rate(%)	Other Parameter	Remarks
12	Goitre Control Cell, (DHS) [74] Goitre Prevalence	Palakkad	1994	6.0	Not done	Only goitre prevalence assessed
13	Goitre Control Cell, (DHS) [74]Goitre Prevalence	Manjeri	1994	11.0	Not done	Only goitre prevalence assessed
14	Goitre Control Cell, (DHS) [74]Goitre Prevalence	Calicut	1994	13.0	Not done	Only goitre prevalence assessed
15	Goitre Control Cell, (DHS) [74] Goitre Prevalence	Wayanad	1994	21.0	Not done	Only one indicator assessed
16	Goitre Control Cell, (DHS) [74] Goitre Prevalence	Kannur	1994	11.0	Not done	Only one indicator assessed
17.	Goitre Control Cell, (DHS) [74] Goitre Prevalence	Kasargode	1994	10.0	Not done	Only one indicator assessed
18	National Nutrition Monitoring Bureau(NNMB)	Rural Survey	1996	Boys<18years-6.8% Girls<18years- 8.8%	Not done	Physiological effect
19	Kapil [75] Goitre Prevalence Urinary Iodine	Ernakulam School survey	1998	Goitre – 1% Median UIE- 200µg/L	Not done	District study

OBJECTIVES OF THE PRESENT INVESTIGATIONS

The prevalence of thyroid diseases is very high in Kerala. Calicut Medical College is the major referral hospital catering 6 districts of North Kerala. A large number of thyroid patients are coming in the medicine and surgery OP of Calicut Medical College and the numbers of patients are increasing every year. Assessment of the urinary iodine excretion is believed to give the best index of the prevalence of IDD in the community.[76]

From the above review on Iodine Deficiency Disorders (IDD) in Kerala, it was decided to evaluate the status of IDD quantitatively, using clinical and biochemical indicators through case study of Calicut Medical College. The specific study includes: -

1. To evaluate the current status of Iodine deficiency disorders among the people who attended the medicine and surgery clinic of Calicut Medical College during the year 1999 – 2000.
2. To evaluate the strict implementation of iodination of salt
3. To determine the iodide content of common food items and thiocyanate content in tapioca and cabbage.
4. To establish the relation between water iodide content to various thyroid abnormalities.
5. To establish the relation between serum cholesterol to various thyroid abnormalities.

METHODOLOGY

Minikumari Amma.V “Surveillance of Iodine in Drinking Water in Relation to Iodine Deficiency Disorders in Northern Kerala ” Thesis. Department of Life Sciences, University of Calicut, 2002

METHODOLOGY

The methodology followed for the study was the one recommended by WHO/ UNICEF and accepted internationally.

Study Design and Study Population

During the two years of the study period, a total number of 2950 subjects attended the surgery and medicine OP of Calicut Medical College with thyroid complaints. They were examined by the experts in the field and subjected to thyroid hormone analysis. Based on the hormone results, 250 patients with hypothyroidism and 250 patients with hyperthyroidism were included in the study. For each test group, 250 normal subjects from the same household eating same food items and using same drinking water source were included as control.

Instructed the persons under study to use only common salt for one month, not eating items containing antithyroid agents (cabbage, tapioca etc) and not taking any medicines. Then collected 24-hour urine sample, and blood sample. Drinking water used by the persons was also collected into a 50ml high-density polyethene bottle, which had been washed in distilled water. The bottles did not contaminate or absorb ions from test solutions.

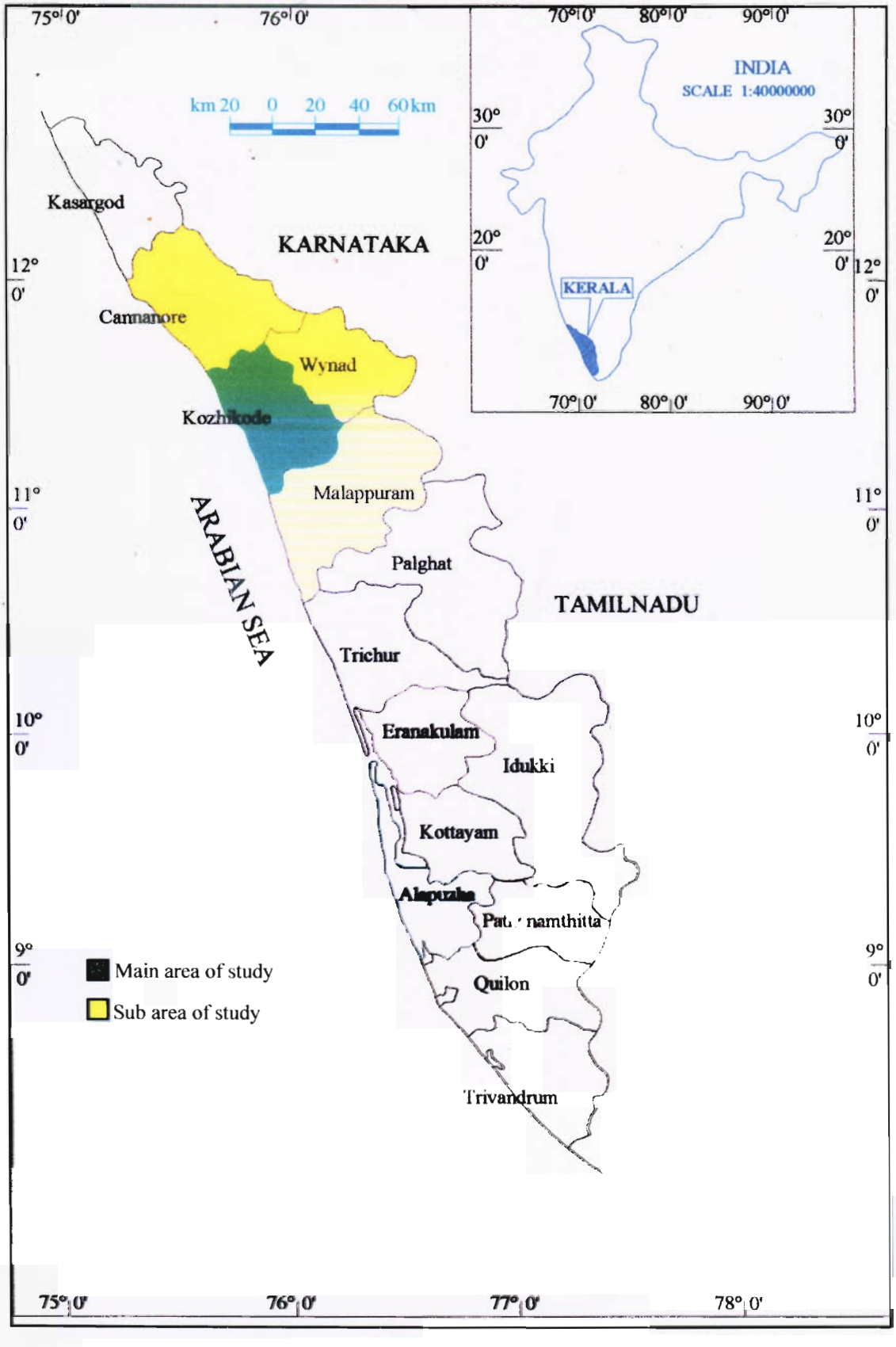
24-hour urine sample and water samples were analyzed for iodide content. The analysis was done in the Central Water Analysis Laboratory of Centre for Water Resources Development and Management, Kunnamangalam, Calicut. Quality assurance (colorimetric confirmation) was done in the Biochemistry laboratory of Calicut Medical College. Each sample was analyzed for 5 times to establish the reliability and validity of the process in the laboratory. Normal controls using iodised salt (no diet restriction) are also subjected to urinary iodide analysis.

Hormonal assay (T_3, T_4, TSH) was done in the Nuclear Medicine Department of Calicut Medical College. Cholesterol estimation was also done in the blood sample. Vegetables and fish samples from different parts of the study area were also analyzed for their iodide content. Thiocyanate estimation in tapioca and cabbage was also done.

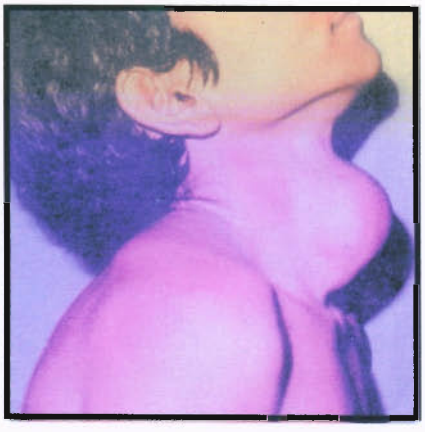
Qualitative Components

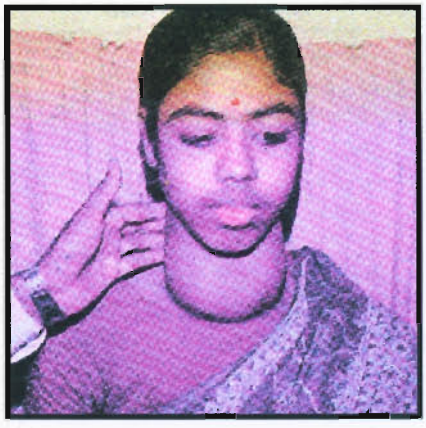
Details about the patients were collected from the OP after direct questionnaire with the patients. The details of the questionnaire included place of residence, age, family history, type of thyroid abnormality, symptoms, food habits, Cytology reports and Histopathology reports, which are available from the patients during the follow up.

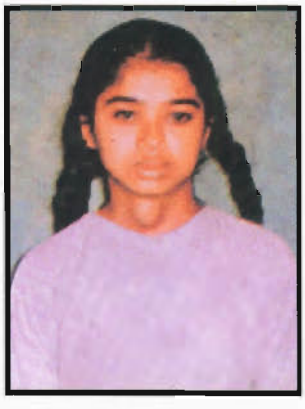
Map of Kerala showing study area

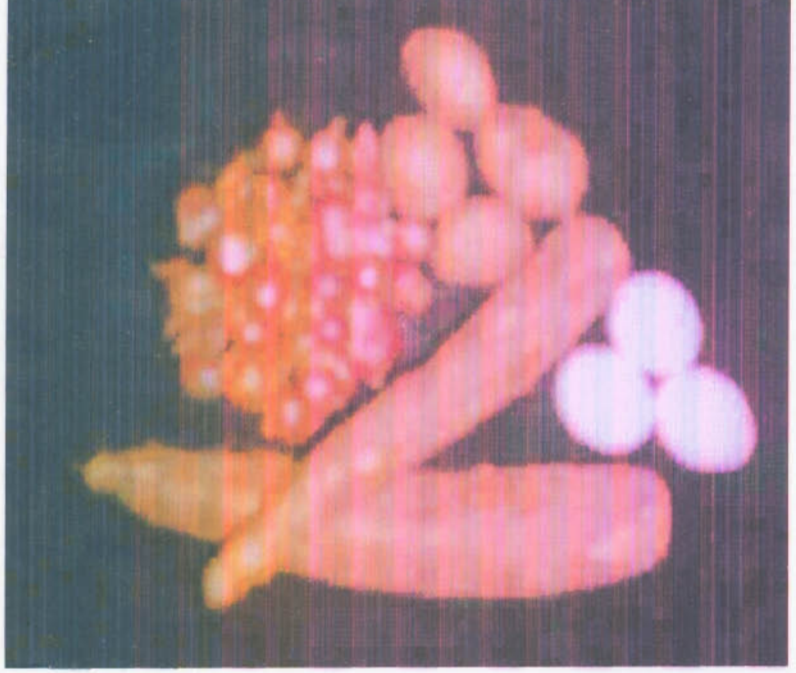


THYROID PATIENTS WITH ENLARGED GLAND









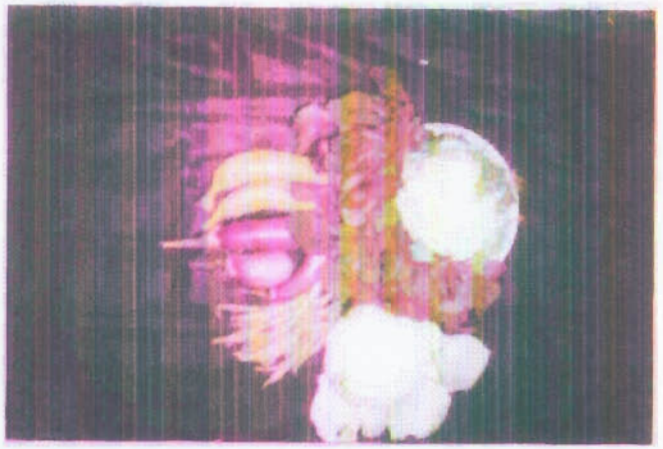


Fig. Different food items analysed for iodide

Materials and Methods

I Determination of iodide content in water

Two different methods were used for the determination of iodide concentration in water.

a) Colorimetric method using UV visible spectrophotometer

The water iodide content is determined by photometric method using ceric ammonium sulphate and arsenious acid[77]. Iodide can be determined in water using its ability to catalyze the reduction of ceric ions by arsenious acid in sulphuric acid solution. The effect is proportional nonlinearly to the amount of iodide present. Photometric determination of the loss of ceric ion colour directly is difficult without a recording device. The colour fades rapidly while it is being read in the photometer. The reaction is stopped after a specific time interval by the addition of ferrous ammonium sulfate. The resulting ferric ions, which are directly proportional to the remaining ceric ions. The ferric ions develop a colour complex with potassium thiocyanate that is relatively stable. This method has the advantage of requiring only small water samples, eliminating distillation procedures, minimizing certain interferences and giving stable colours for spectrophotometric determination. Digestion with chromic acid and distillation must be undertaken where an estimate is desired of the

organically bound and other nonsusceptible forms of iodine in addition to the usual iodide ion.

An excess of NaCl is added to the sample to eliminate the interference of chloride already present in the water by attaining a stable maximum chloride concentration that sensitizes the reaction. The formation of non-catalytic forms of iodine and the inhibitory effects of silver and mercury are reduced by this addition.

Requirements

1. Water bath (30 ± 0.5°C).

2. Colourimeter or Spectrophotometer.

3. Reagants.

Store all the following stock solutions in tightly stoppered containers in a dark place.

a) 20% NaCl solution

Dissolve 200gm of NaCl in distilled water and dilute to 1litre. Recrystallize the NaCl if an interfering amount of iodine is present using a water ethanol mixture.

b) Arsenious Acid-0.1N

Dissolve 4.946g AS_2O_3 in dis.water, add 0.2ml con. H_2SO_4 and dilute to 1000ml.

c) con. H_2SO_4

d) Ceric ammonium sulphate-0.02N

Dissolve 13.38g $Ce(NH_4)_4(SO_4)_4.2H_2O$ in distilled water, add 44ml con. H_2SO_4 and make upto 1 litre.

e)Ferrous ammonium sulfate reagant-

Dissolve 1.5g Fe $(NH_4)_2(SO_4)_2$. $6H_2O$ in 100ml dis. water containing 0.6ml con. H_2SO_4.

Prepare daily

f) Potassium thiocyanate solution-

Dissolve 4.00g KSCN in 100ml dis water.

g) Stock iodide solution-

Dissolve 201.6mg anhydrous KI in dis. water and dilute to 1000ml [200 ppm].

h) Intermediate iodide solution [4 ppm]

Dilute 20 ml stock iodide solution to 1000ml with dis.water.

i) Working iodide solution(0.1ppm)

Dilute 25ml intermediate iodide solution to 1000 ml with dis. water.

Procedure

Take 10 ml of water sample in a dry test tube and add the following reagents:- I ml NaCl solution, o.5ml arsenious acid and 0.5ml con. H_2SO_4

Place the reaction mixture and the ceric ammonium sulfate solution in the 30°C water bath and allow coming to temperature equilibrium. Add 1.0ml of ceric ammonium sulphate

solution, mix the contents of the test tube by inversion, and start the stopwatch to time the reaction. Use an inert clean test tube stopper when mixing. After 15 ± 0.1 min, remove the sample from the water bath and add immediately, 1.00 ml ferrous ammonium sulphate reagent with mixing whereupon the yellow ceric ion colour should disappear. Then add, with mixing 1ml potassium thiocyanate solution. Replace the sample in the water bath. Within 1 hour after the thiocyanate addition, read the red colour as percentage transmittance or absorbance (optical density) in a photometric instrument at 510nm

Maintain the temperature of the solution and the cell compartment at 30 ± 0.5^{0}C, until the transmittance or absorbance is determined. If several samples are run, start the reaction at one minute intervals to allow time for addition of ferrous ammonium sulphate and thiocyanate.

Calibration standards

Treat high concentration standards containing 20,40,60,80...µg/litre and low concentration standards containing 2,4,6,8...µg/litre of iodide in the same manner with each set of samples to establish calibration curves and are shown in figures I and II .

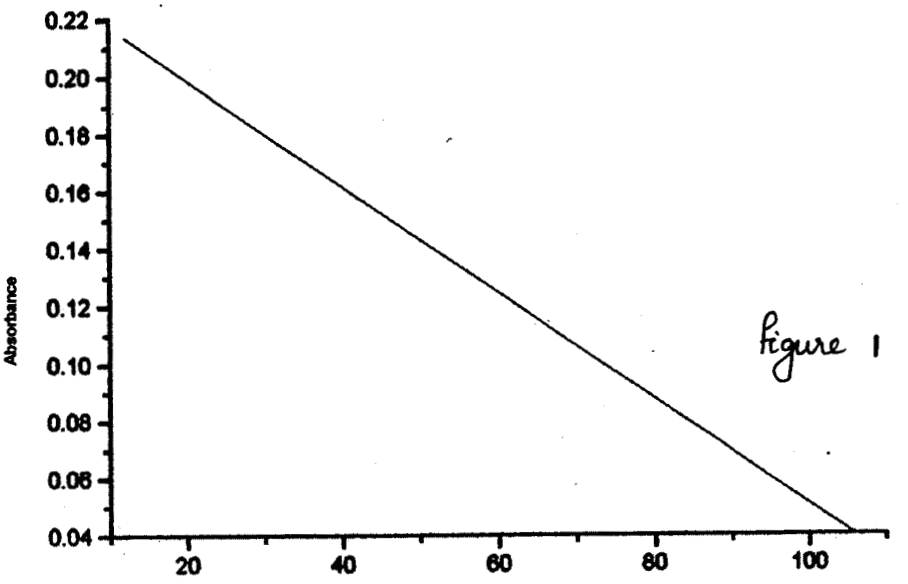


Figure I

Callibration curve for Iodide estimation at high concentration

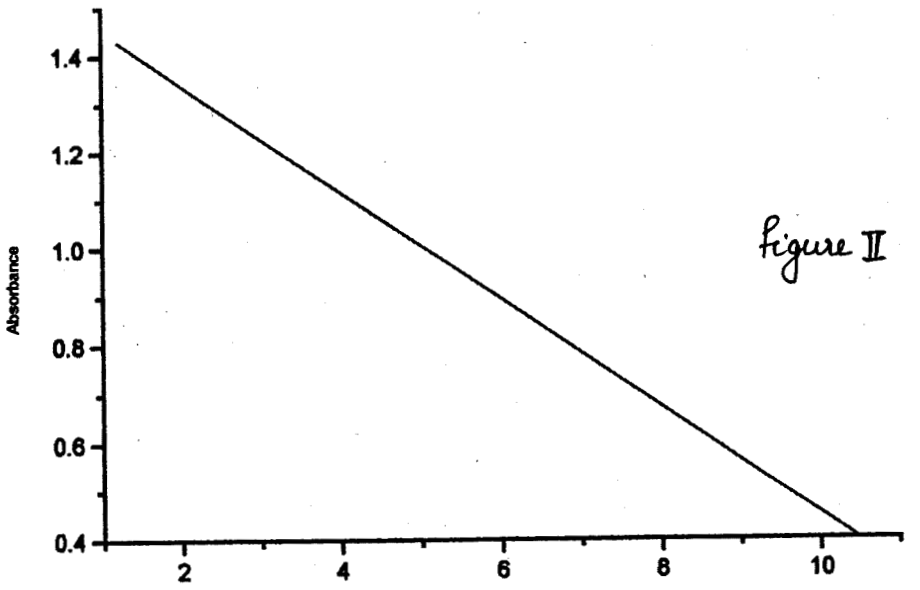


Figure II

Callibration curve for Iodide estimation at low concentration

Test concentrations can be directly obtained from the graph. Results obtained by this method are reproducible on samples of Los Angels source of water and have been reported to be accurate to ± 0.3µg iodide.

b) Electrometric method by Ion meter using Iodide selective electrode

Ion selective electrodes measure ion activities, the thermodynamically effective free ion concentration, not concentrations. Activity measurements are valuable because the activities of ions determine rate of reaction and chemical equilibria. For example, ion activities are important parameters in predicating corrosion rates, extent of precipitation, formation of complexes, degree of acidity, solution conductivity, effectiveness of metal pickling baths and electroplating bath solutions and physiological effects of ions in biological fluids. In dilute solutions, ion activity usually approaches the concentration. Ion selective electrodes have several advantages over conventional methods of determining ionic concentration. They do not affect the solution being studied, they are portable, not too expensive and they are suitable both for direct determination of ion activities and as sensors in titration. Ion selective electrodes are classified into:

1. Glass electrodes.
2. Solid state and precipitate electrodes.
3. Liquid- liquid membrane electrodes.
4. Enzyme and gas sensing electrodes.

Preparation of Stock solution (1000ppm)

Weighed out exactly, 1.308 g of KI in a one litre standard flask and made up to the mark with dis. Water. From the (1000 ppm) stock solution, working standards (0.02, 0.04, 0.06ppm etc) was prepared. The instrument was calibrated using standard solutions. The sample solution was taken in a 100ml beaker. Shaken well and reading was noted.

II Determination of iodide in 24hr urine

The above two methods were used for the urinary iodide estimation also.

Urine contains a number of substances that interfere with the iodide determination by photometric method and so prevent iodide from being measured directly. Dilute urine 1ml with 4ml dis. water or buffer. The interfering substances are removed by extraction in a column containing activated charcoal prior to assessment of iodide

III Iodide analysis in food samples

The sample preparation for the determination of iodide was done as per standard procedure.[78]

Cabbage: 100g of pickled cabbage was mixed with 200ml of deionized water at 60°C and crushed with a home mixer. The crushed picked cabbage was transferred into a big beaker (about 1litre)

quantitatively with deionized water and heat on the steam bath to 70-80°C for 1hr. After cooking transferred the mixture into a 500ml volumetric flask and made upto 500ml with deiconised water. Filtered off the undissolved parts of the pickled cabbage with a fluted filter. .

Cauliflower: 50gm floral part of cauliflower accurately weighed and mixed with 100ml dis. water, crushed with a home mixer. The crushed cauliflower was transferred into a beaker quantitatively with deionized water and heated on steam bath to 75-80°C for 1 hr. After cooking, the mixture was transferred into 500ml volumetric flask and filled upto the volume with deionized water. The undissolved part of the cauliflower was filtered with a fluted filter paper.

Potato: 10g of potato was accurately weighed and mixed with 100ml deionized water, which was heated at 60°C and Crush with a home mixer. Transfer the crushed potato into a beaker quantitatively with deionized water and heat on a steam bath to 75-80°C for 1hr. After cooking, transfer the mixture into 50ml volumetric flask and fill up to the volume with deionized water. Filter of the undissolved parts of the potato with a fluted filter paper.

Iodine content of cabbage, cauliflower, potato, tapioca, beans, amaranthus, brinjal and raw banana in 10% concentrations were also analysed for iodide content using Ion meter.

Onion : 5gm of onion was accurately weighed and mixed with 100ml of deionised water. The remaining steps are same as that of above.

Milk : Two types of sample were collected. One was pasteurized and the other was not pasteurized. Before estimation of iodide, 1ml of milk is diluted to 10ml with distilled water.

Egg : From well-mixed egg, 20-g of sample was accurately weighed into a beaker. Added 20ml 10% Na_2CO_3 solution, mix and evaporated to dryness in an oven (over night) at 100°C. The beaker was transferred while hot to furnace at 500°C (faint red) and kept for 1 hour. Cooled, added few drops of water and the residue was broken up with glass rod. Added 50ml water, covered the beaker with watch glass. Added 20 ml HNO_3 (1+3) slowly and washed watch glass, mixed, filtered the content and washed the charred material and filter paper thoroughly with water.

coconut : Weighted out accurately 20 g of coconut flesh in a beaker. It was then mixed with 50 ml distilled water and crushed in a home mixer. The filtrate was collected in a 100ml standard flask and made up to the mark. Iodine content of onion, milk, egg and coconut were also analysed as per above.

Fish: 10g of fish meat was mixed in a blender with distilled water. Filtered off the undissolved part through a fluted filter. Collected the filtrate into 250ml standard flask. Prepared 0.1 to 0.3 ppm standard iodide solution. Then calibrated ion meter with these standards. Sample is introduced and the reading was noted.

Details of the food items used for the determination of iodide content are shown below.

Sl. No	Common name	Botanical name	Part used for determination
1	Amaranthus		
	Green	Amaranthus viridis	Leafy part
	Red	Amaranthus gangeticus	
2	Beans	Phaseolus vulgaris	Whole part
3	Banana	Musa paradiciaca	Peeled banana
4	Cabbage	Brassica oleracea	
		Species – capitata	Outer leafy part
5	Brinjal	Solanum melongena	Whole part
6	Cauliflower	Brassica oleracea	
		Variety – bortrytis	Florets
7	Coconut flesh	Cocos nucifera	Fleshy part
8	Onion	Allium cepa	Whole part
9	Tapioca	Manihot esculenta	Root tuber
10	Potato	Solanum tuberosum	Stem tuber
11	Milk		
	Pasteurized		
	Non pasteurized		
12	Egg		

Details about fishes

Sl No	Common Name	Scientific Name	Parts Used
1	Sardine	Sardinella fimbriata.	Fleshy part
2	Mackeral	Rastrelliger kanagurta	Fleshy part
3	Nemepterus	Synagris japonicus	Fleshy part
4	Oil sardine	Sardinella longiceps	Fleshy part
5	Anchovy	Engraulis inaicus	Fleshy part
6	Caraputty	Equuta ruconius	Fleshy part

IV. Determination of Thiocyanate in Cabbage and Tapioca

Thiocyanate in tapioca and cabbage were determined by photometric method using ferric nitrate solution[79].

Determination of Thiocyanate in Tapioca

a) Preparation of stock solution:

Dissolved 1.673g KSCN in a litre standard flask using distilled water. This corresponds to 1000 ppm KSCN stock solution.

b) Preparation of ferric nitrate solution:

Dissolved 10g $Fe(NO_3)_3$ in 50 ml distilled water. Add 2.5ml conc. HNO_3 and dilute to 100ml.

c) Nitric acid solution-0.1N

Mix 6.4 ml con. HNO_3 in about 800ml distilled water and diluted to 1L.

Apparatus:

UV Visible Spectrophotometer at λ_{max} of 460 nm was used.

Procedure

a) Preparation of standards:

Prepared a series of standards containing 0.01 to 0.04-ppm thiocyanate by pipetting measured volumes of standard KSCN solution and diluting with distilled water.

b) Colour development:

A filtered sample was used that contains the concentration of SCN and the pH was adjusted to 2 by adding HNO_3 drop wise. Pipetted 50ml portion of sample to a beaker, added 2.5 ml ferric nitrate and mixed. Filled the absorption cell and measured the absorbance against a reagent blank at 460nm.

Method

To the standard solutions of SCN, added concentrated HNO_3 drop wise and adjusted the pH. Then taken 50ml of standard solution into the beaker and added 2.5ml $Fe(NO_3)_3$ and mixed. Calibrated the instrument with these standards and a calibration curve was plotted. A linear plot was obtained. Then sample was introduced. Absorbance

VS concentration curve was plotted. From the curve, concentration of thiocyanate in the sample was obtained.

Determination of thiocayante in Cabbage

Thiocyanate in cabbage was also determined by the same procedure.

Preparation of stock solution

1000ppm KSCN stock solution was prepared by dissolving 1.673g of KSCN in 1litre-distilled water.

Preparation of ferric nitrate solution

10g of $Fe(No_3)_3$ was dissolved in 50ml distilled water. Added 2.5ml concentration HNO_3 and diluted to 100ml.

Method

The 1000ppm KSCN stock solution was diluted quantitatively with distilled water and prepared 10,20,22,24-ppm KSCN solution.

Apparatus:

UV-Visible Spectrophotometer at 460nm is used.

Procedure

a)Calibration curve was plotted using prepared standards.

b)Colour development.

A filtered sample was used that containing the concentration of SCN^- and the pH is adjusted to 2 by adding concentrated HNO_3 drop wise. Pipette 50ml proportion of sample to a beaker, add 2.5ml ferric nitrate and mix. Filled the absorption cell and measured the absorbance against a reagent blank at 460 nm.

Method

To the standard solutions of SCN^- added Concentrated HNO_3 drop wise and adjusted the pH. To 50ml of standard solution in the beaker, added 2.5ml $Fe(No_3)_3$ and mixed very well. A calibration curve was plotted. A linear plot should be obtained. The sample was introduced and noted absorbance. From the curve, the concentration of SCN^- in the sample can be obtained.

V Estimation of Triiodothyronine (T_3) - Radioimmunoassay Method (RIA Kit method)

Principle:- Unlabelled endogenous T_3 competes with radiolabelled T_3 for the limited binding sites on the antibody made specifically for T_3. The antibody is in the form of complex with second antibody. At the end of incubation, the T_3 bound to antibody-second antibody complex and free T_3 are separated by the addition of polyethylene glycol. The amount bound to the antibody complex in the assay tube is compared with values of known T_3 standards and the T_3 concentration in the

patient sample can be calculated. 8- anilino 1- naphthalene sulphonic acid (ANS) is used in this kit for displacing T_3 bound to BG. [80-85]

Reagents

1. T_3 standard (lyophilized)
2. T_3 antisera complex (lyophilized)
3. I^{125} labelled T_3 (lyophilized)
4. T_3 free human serum (lyophilized)
5. Control serum A and B (lyophilized)
6. Concentrated assay buffer solution
7. Polyethylene glycol (dry powder)

Kit is stored at 2-8°C. Assay buffer vial can be at room temperature.

Specimen collection and preparation

Collected 5ml of blood without anticoagulant in a glass vial or in a test tube. Allowed the blood to clot at room temperature. After rimming the clot, centrifuged and collected the serum. Store at 2-8°C for assay on the same day or -20°C if the storage is expected to be more than a day. Allowed the sample to thaw prior to assay. Mixed thoroughly.

Reconstitution of reagents

1. Assay buffer : Add 100ml double distilled water to the contents of the concentrated buffer vial. This reconstituted assay buffer contains 0.1% gelatin in 0.14 M Tris-hydroxymethyl Amino Methane buffer, pH adjusted to 8.6.

1. T_3 standard : Add 5ml of assay buffer to the standard. Mix gently. The reconstituted standard is 2.4 ng/ml. A series of standards are prepared from it (1.2, 0.6, 0.3, 0.15 ng/ml).
2. T_3 antisera complex : Add 10 ml of buffer and mix gently.
3. I^{125}- T_3 : Add 10ml of buffer and mix gently.
4. T_3 free serum : Add 2ml of ouble distilled water and mix gently.
5. Control serum A and B : Add 0.5 ml of double dis. water and mix gently.
6. Polyethylene glycol(PEG)
   Transfer PEG powder to a 125ml reagent bottle containing 100ml of 1% (W/V) NaCl. Mix well.

T_3 ASSAY FLOW CHART

Volumes in micro litres (µl)

Arrange 20 tubes and label as 1-18, T_1 and T_2 and take the reagents as follows:

Tube No	Buffer	Free human serum	Standard or sample	Antiserum complex	I^{125}-T_3
1,2(Blank)	400	50	-		100
3,4(Zero standard)	300	50	-	100	100
5,6(0.15ng/ml)	200	50	100	100	100
7,8(0.30 ng/ml)	200	50	100	100	100
9,10(0.6 ng/ml)	200	50	100	100	100
11,12(1.2 ng/ml)	200	50	100	100	100
13,14(2.4 ng/ml)	200	50	100	100	100
15,16(test1)	300	-	50	100	100
17,18(test2)	300	-	50	100	100
T_1,T_2(Total)	-	-	-	-	100

Mixed the tubes gently and incubated either at 37°C for 45 mins or at room temperature for 3 hours. Added 1.0 ml of PEG solution to all tubes except T_1 & T_2. Mixed and centrifuged at 2000 x g for 20 minutes. G= $(RPM)^2$ x r x 1.18 x 10^{-5} where r = radius of the rotor in cms. Discarded the supernatent, carefully removed the last traces of the supernatent without touching or disturbing the precipitate.

Calculation

Content	Tube No.	CPM	Corrected average cts	B/Bo
Total	T_1, T_2	37790		
		37932	37511	
Blank	1,2	1612		
		1697	1405	
Zero std	3,4	17248		
		17199	15569(B_0)	100.0
Std E (0.15)	5,6	15354		
		15162	13604	87.4
Std D (0.3)	7,8	12978		
		13167	11418	73.3
Std C (0.6)	9,10	9953		
		9892	8268	53.1
Std B (1.2)	11,12	7107		
		6873	5595	35.9
Std A (2.4)	13,14	4986		
		5047	3362	21.3
Sample 1	15,16	11367		
		11616	9837	63.2
Sample 2	17,18	7114		
		7110	5507	35.4

Calculation of Results

1. Calculated the average counts per minute (CPM) for all duplicate tubes. Correct for background. Average counts of tubes 1& 2 is called blank counts.

$$\% \text{ Blank} = \frac{\text{Blank Counts}}{\text{Total counts}} \times 100$$

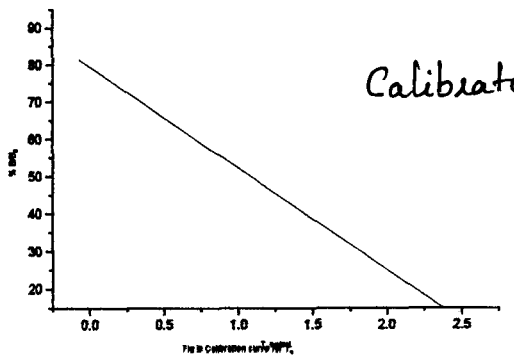
2. Subtracted blank counts per minute from the average of all the remaining duplicates. This is called average counts. Calculate Zero binding

$$\%B / B_0 = \frac{\text{Corrected average counts of 3 \& 4}}{\text{Corrected total counts}} \times 100$$

3. Calculated

$$\%B/ B_0 = \frac{\text{Corrected average counts of std./ sample}}{\text{Corrected average counts of zero std}} \times 100$$

4. Ploted the standard curve
   a) Corrected average counts against concentration of T_3 (ng/ml) on a linear graph
   b) %B/ B_0 on a logit scale against concentration of T_3 ng/ml on a log sale of a logit- log graph



Calibration curve for T_3

5. Read the sample value from the standard curve. T_3 concentration of the sample can be expressed as ng/ml by multiplying the value by 2.

Normal Values

The normal values were 0.7 – 2.0 ng/ml.

VI *Radio immuno assay Procedure for T_4 (Kit Method)*

Principle :- Unlabelled endogenous T_4 competes with radiolabelled T_4 for the limited binding sites on the antibody made specifically for T_4. At the end of incubation, the T_4 bound to antibody and free T_4 are separated by the addition of polyethylene glycol. The amount bound to the antibody complex in the assay tube is compared with values of known T_4 standards and the T_4 concentration in the patient sample can be calculated. 8- anilino 1- naphthalene sulphonic acid (ANS) is used in this kit for displacing T_4 bound to TBG.[86-90]

Reagants

1. T_4 standard (lyophilized)
2. T_4 antisera (lyophilized)
3. I^{125} labelled T_4(lyophilized)
4. T_4 free human serum (lyophilized)
5. Control serum A and B (lyophilized)
6. Concentrated assay buffer solution
7. Polyethylene glycol (dry powder)

Kit is stored at 2-8°C. Assay buffer vial can be stored at room temperature.

Specimen collection and preparation

Collected 5ml of blood without anticoagulant in a glass vial or in a test tube. Allowed the blood to clot at room

temperature. Rim the clot, centrifuge and collect the serum. Store at 2-8°C for assay on the same day or -20°C if the storage to than prior to assay. Mix thoroughly. Haemolysed and lipemic samples should be avoided.

Reconstitution of reagents

1. Assay buffer:- Add 100ml double distilled water to the contents of the concentrated buffer vial. This reconstituted assay buffer contains 0.1% gelatin in 0.14 M Trishydroxymethyl Amino Methane buffer, pH adjusted to 8.6.
2. T_4 standard:- Add 4ml of assay buffer to the standard. Mix gently. The reconstituted standard is 2.4 ng/ml. A series of standards are prepared from it (20, 10, 5, 2.5 ng/ml).
3. T_4 antisera :- Add 10 ml of buffer and mix gently.
4. I^{125}- T_4 :- Add 10ml of buffer and mix gently.
5. T_4 free serum:- Add 5ml of double distilled water and mix gently.

6. Control serum A and B

   Added 5 ml of assay buffer and mixed gently.

7. Polyethylene glycol (PEG):

Transfer PEG powder to a 125ml reagent bottle containing 90ml. of 1% (w/v) NaCl. Mix it well till complete dissolution is obtained.

T_4 ASSAY FLOW CHART

Volumes in micro litres (µl)

Tube No.	Buffer	Free serum	Std/dil sample (1:10)	^{125}I- T_4	Anti serum
T_1,T_2(Total)	-	-	-	100	-
1,2 (NSB)	200	100	-	100	-
3,4 (Zero)	100	100	-	100	100
5,6	-	100	100(D)	100	100
7,8	-	100	100(C)	100	100
9,10	-	100	100(B)	100	100
11,12	-	100	100(A)	100	100
13,14	100	-	100(S1)	100	100
15,16	100	-	100(S2)	100	100

Mixed the tubes gently. Incubated either at 37°C for 30 mins or at room temperature for 75 mins. Added 1.0 ml of PEG solution to all tube except T_1 & T_2. Mixed and centrifuged at 2000xg for 20 minutes $g=(RPM)^2 \times r \times 1.18 \times 10^{-5}$ where r=radius of the rotor

in cms. Discarded the supernatent, carefully removed the last traces of the supernatent without touching or disturbing the precipitate, with a wick cut out from absorbent sheet.

Calculation

Content	Tube No.	CPM	Corrected average cts	%B/B_0
Total	T_1, T_2	63541		
		64382	63962 (T)	
Blank	1,2	1444		
		1347	1396 (BL)	
Zero std	3,4	33974		
		34484	32835 (Bo)	100.0
Std D	5,6	23301		
(2.5ng/ml)		23912	22211	67.7
Std C	7,8	17108		
(5 ng/ml)		17155	15736	47.9
Std B	9,10	12066		
(10 ng/ml)		11880	10578	32.2
Std A	11,12	7491		
(20 ng/ml)		7304	6002	18.3
Sample 1	13,14	16475		
		15507	14596	44
Sample 2	15,16	10520		
		10635	9182	28

Calculation of Results

1. Calculated the average counts per minute (CPM) for all duplicate tubes. Corrected for background. This is called corrected average counts.

2. Calculated % Blank

$$\% \text{ Blank} = \frac{\text{Corrected average counts of 1 \& 2}}{\text{Corrected total counts}} \times 100$$

3. Calculated Zero Binding

$$\% B_0 = \frac{\text{Corrected average counts of 3 \& 4}}{\text{Corrected total counts}} \times 100$$

4. Calculated % Blank

$$\%B / B_0 = \frac{\text{Corrected average counts of std/ sample}}{\text{Corrected average counts of zero std.}} \times 100$$

5. Plot the standard curve
   a. Corrected average counts against concentration of T_4 (ng/ml) on a linear graph.
   b. $\%B/B_0$ on a logit scale against concentration of T_4 ng/ml on a log sale of a logit- log graph (standard curve in the Figure).

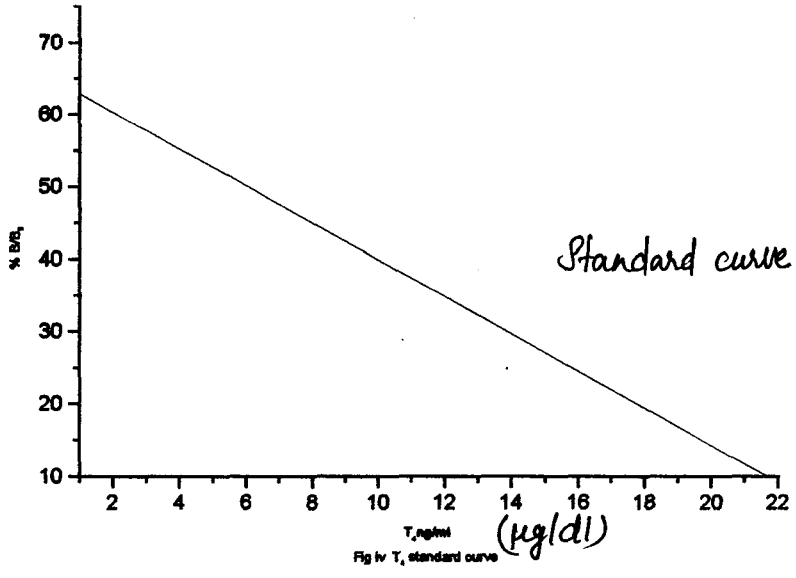


Fig iv T_4 standard curve

6. Read the sample value from the standard curve.

T_4 concentration of the sample can be expressed as ng/ml by multiplying the value by 10.

Normal Values

The normal value were 5.5 – 13.5 µg/dl (55 - 135 ng/ml)

VII *Estimation of TSH Radioimmunometric Assay- Kit Method*

Principle

In immunoradiometric assay (IRMA), two antibodies generated against different portions [epitopes] of the same antigen are used. One antibody is bound to a solid phase, usually a tube, while the other antibody is labeled with I^{125}. Thus when an antigen is present, it simultaneously binds both antibodies in a

"bridge" or "sandwich" fashion. This entire complex remains bounded to the tube. The radioactivity in the bound fraction may then be quantitated using a Gamma Counter.[91-95]

In this procedure, the sample is pipetted into the antibody coated tube along with the complementary antibody labeled with 1- 125. After incubation for the stipulated period, the tubes are emptied and washed to remove unbound I^{125} labelled antibody. The emptied tubes are counted in a Gamma counter, a standard curve is constructed, and from which unknown concentrations of human Thyroid Stimulating Hormone can be readout.

Specimen Collection And Handling

Serum: Collected blood into a clean tube. Allowed the blood to clot for at least thirty minutes at room temperature. Separated the serum by centrifugation and store in a refrigerator (up to one week) or store frozen for extended use.

Plasma: Collected blood into a clean tube containing heparin. Separated the plasma by centrifugation and stored the plasma in a refrigerator (up to one week or store frozen).

Equipment and Reagents Required

1. Pipette that can accurately and precisely deliver the required volumes (100 and 200 microlitres)
2. Test tube rack.

3. Laboratory vortex mixer.
4. Repipetter calibrated to deliver 1ml for tube washing.
5. Absorbent paper for blotting test tubes.
6. Gamma counter calibrated for 1- 125.
7. Horizontal test tube rack circular shaker.

Kit Contents and Their Reconstitution

REAGENT	RECONSTITUTION	STORAGE
hTSH monoclonal antibody coated tubes	Ready to use	2 - 8°C with desiccant
Anti hTSH-1-125	Ready to use	2 - 8°C
hTSH standards in animal serum 0-100µIU/ml (2^{nd} IRP 80/558)	Ready to use	3 weeks at 2-8°C For extended storage preserve frozen
Wash diluent	Dilute with 950ml double distilled water.	2 - 8°C
Controls	Dilute with 1ml doubledistilled water stand for 30minutes. Gently vortex.	-15°C, aliquoted suitably.

Assay Procedure

a. Setup the assay in duplicates. Add all solutions in the quantities specified directly from the reagent vials.

a. Assay conditions

1. Bring all standards samples, controls, coated tubes and anti- hTSH 1-125 to room temperature prior to use.
2. Place the required number of anti- hTSH coated tubes, serially numbered, in a test tube rack. Reseal the unused tubes in the plastic bag along with the desiccant and refrigerate.
3. Pipette 200µ l of each standard, control or samples into its respective tubes.
4. Pipette 100µ l of anti hTSH 1-125 into each tubes and vortex all tubes. Incubate the tubes for 2 hours with gentle shaking (350rpm) at room temperature (18-25°C) on a shaker.

Quality Control:

Commercially available controls or serum pools containing low, normal and elevated levels of hTSH should routinely be assayed as unknowns. Their results should be then plotted and monitored to determine the performance and reliability of the assay.

Normal values

The normal values were 0.5-6.0µ IU/ml

Assay Protocol

Tube No.	Std/Sample (µl)	Anti-hTSH I^{125} (µl)		Washing	Description (µIU/ml) (µIU/ml)
1,2	200 (µl)	100 (µl)	Incubated for	Added 2ml diluted	0
3,4	200	100	2 hours	wash solution	0.15
5,6	200	100	at RT	to each tube.	0.5
7,8	200	100	with gentle	Mixed and decanted the	1.5
9,10	200	100	shaking	Solution.	5.0
11,12	200	100	(350 rpm)		15.0
13,14	200	100		Repeat the wash	50.0
15,16	200	100		Step once more.	100
17,18	200	100			Control-A
19,20	200	100			Control-B
21,22	200	100		Count tubes in	Sample-1
23,24	200	100		Gamma counter	Sample-2
				set for 1^{125} for one minute	

Calculations

Take the average of the background corrected counts of all duplicates. Plotted the averaged counts(Y-axis) versus the standard concentration(X-axis) on 3 cycle log-log paper. For reading samples with very low TSH, plot the first five standards including 0 µIU/ml on a linear –linear graph paper. Using the standard curve constructed above, determined the hTSH concentrations of each sample.

SAMPLE (µIU/ml)	CORRECTED CPM	AVERAGE CPM	RESULTS
0µIU/ml	62 85	73	
0.15µIU/ml	270 234	252	
0.5µIU/ml	619 706	663	
1.5µIU/ml	1637 1625	1631	
5.0µIU/ml	4879 5136	5008	
15µIU/ml	13874 14017	13946	
50.0µIU/ml	33207 33423	33315	
100.0µIU/ml	43979 44370	44174	
CONTROL A	1492 1373	1433	1.30µIU/ml
CONTROL B	14841 15288	15064	16.7µIU/ml

A Typical Standard Curve (Log-Log Graph)

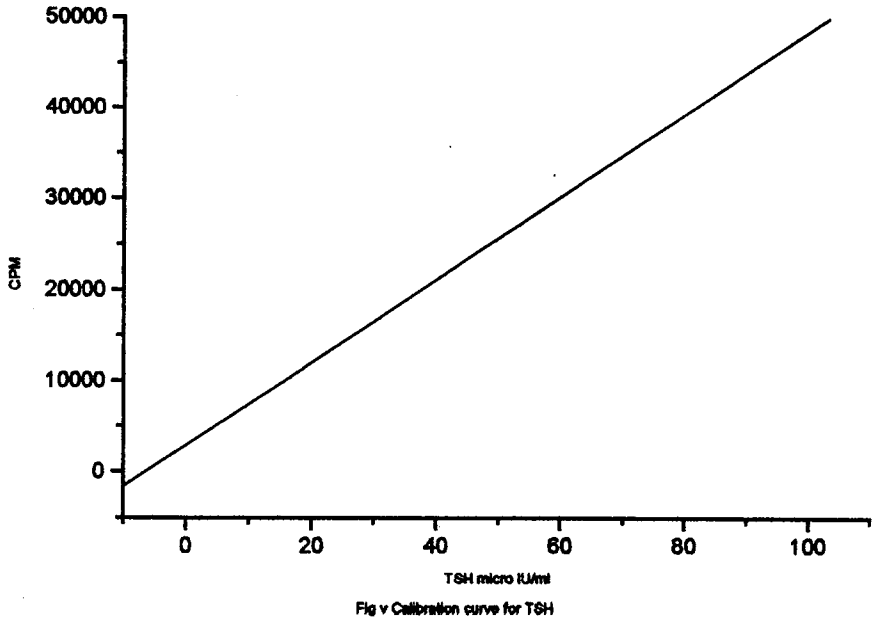


Fig v Calibration curve for TSH

VIII **Cholesterol Estimation**

Methodology

Serum cholesterol was estimated by modified Roeschlau's method.[96-97]

Principle

The estimation of cholesterol involves the following enzyme catalyzed reactions.

1. Cholesterol ester $\xrightarrow{CE}$ Cholesterol + Fatty acid

2. Cholesterol + O_2 $\xrightarrow{CHOD}$ Cholesterol-4-en-3-one+H_2O_2

3. $2H_2O_2$ + 4AAP + Phenol $\xrightarrow{POD}$ $4H_2O$+ Quinoneimine

CE : Cholesterol esterase

CHOD : Cholesterol Oxidase

4AAP : 4-Aminoantipyrine

Absorbance of Quinoneimine so formed is directly proportional to Cholesterol concentration.

Reagent composition

1. Cholesterol Reagent

Cholesterol esterase (Pancreatic)	>200IU/L
Cholesterol oxidase (microbial)	>150 IU/L
Peroxidase (horseradish)	>2000 IU/L
Sodium Phenolate	20 mmol/L
4-Aminoantipyrine	0.5 mmol/L
Phosphate buffer (pH 6.5 ± 0.1)	68 mmol/L
Lipid clearing agent	-

2. Cholesterol standard

Cholesterol	200 mg/dl (5.2 mmol/L)

3. Aqua-4

Double deionized, 0.2 micron, membrane filtered, particle- free water for reconstitution of Reagent 1

Reagent Reconstitution

Allow Reagent 1 and Reagent 3 to attain room temperature. Add 100ml of Aqua4 to contents of each vial of Reagent 1, swirl to dissolve, do not shake vigorously.

Storage and Stability

a) Prior to reconstitution

Unopened Reagents are stable till the expiration date stated on the label when stored at 2-8°C.

b) After reconstitution

The working Cholesterol reagents is stable for 365 days at 2-8°C. Discard reagent if it turns turbid or of its absorbance is greater than 0.4 at 510 nm against distilled water.

Sample

Unhemolyzed serum separated from the cell as soon as possible. Plasma may be used with heparin or EDTA as the anticoagulant. Fluoride or oxalate will interfere with the assay. Samples are stable for 7 days at 2-8°C.

General Assay Parameters

Mode	End Point
Wavelength 1(nm)	510
Wavelength 2(nm)	630
Sample Volume (µl)	20
Reagent Volume (µl)	1000
Incubation Time (Mins)	10
Incubation Temp (°C)	37
Normal Low (mg/dl)	140
Normal High (mg/dl)	250
Linearity Low (mg/dl)	0
Linearity High (mg/dl)	600
Concentration of Standard (mg/dl)	200
Blank with	Reagent
Absorbance Limit Max.	0.400
Units	(mg/dl)

Programme parameters for specific analyzers are available on request.

Assay Procedure

Pipette into tubes marked	Blank	Standard	Sample
Working Reagent	1000 µl	1000 µl	1000 µl
Distilled Water	20 µl	-	-
Standard	-	20 µl	-
Sample	-	-	20 µl

Mix well incubate at 37°C for 10 minutes. Aspirate Blank followed by standard and Tests.

Calculation

$$\text{Cholesterol} = \frac{\text{Absorbance of Sample x Concentration of Standard (mg/dl)}}{\text{Absorbance of Standard (mg/dl)}}$$

Linearity

The assay is linear up to 600 mg/dl. For higher values dilute the samples with normal saline and repeat the assay. Multiply the results with the dilution factor.

Notes

The colour developed at the end of the test is Stable for 30 minutes.

Normal values

140-250 mg/dl.

RESULTS AND DISCUSSION

Minikumari Amma.V “Surveillance of Iodine in Drinking Water in Relation to Iodine Deficiency Disorders in Northern Kerala ” Thesis. Department of Life Sciences, University of Calicut, 2002

RESULTS AND DISCUSSION

I Details of the study people

Qualitative Components

Among the total thyroid complaint population, 17% of them are typical hypo and hyperthyroid patients and is shown in figure 1.

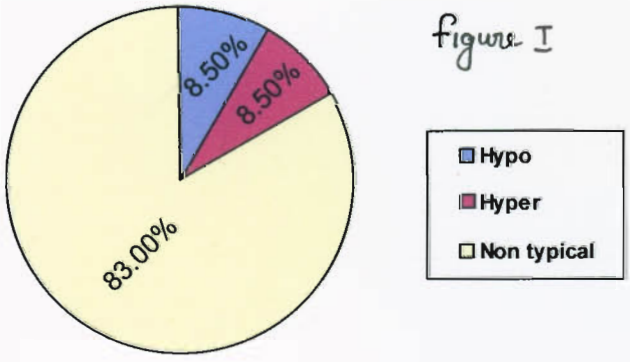


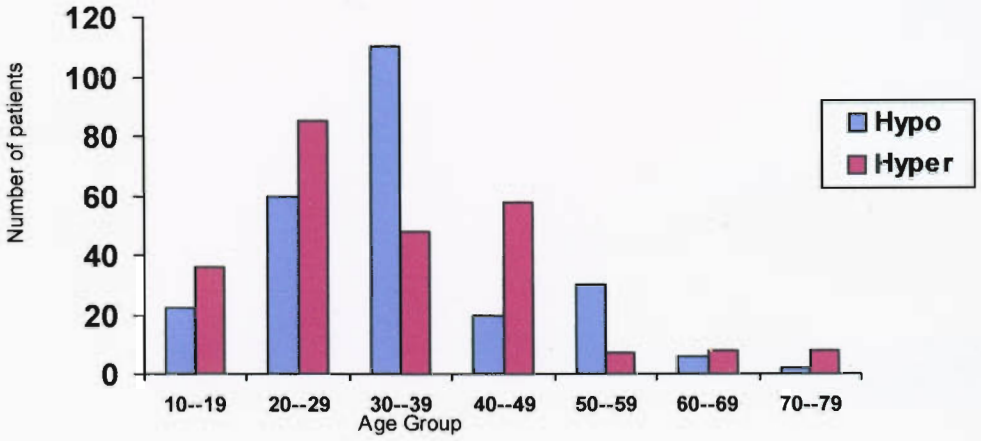
figure I

The no. of hypothyroid patients and hyperthyroid patients in each age group is shown in table 1 and figure 2.

Table 1

No.	Age group	No. of total thyroid patients	No. of hypothyroid patents	No. of hyperthyroid patients
1	10-19	58	22	36
2	20-29	145	60	85
3	30-39	158	110	48
4	40-49	78	20	58
5	50-59	37	30	7
6	60-69	14	6	8
7	70-79	10	2	8

Figure 2



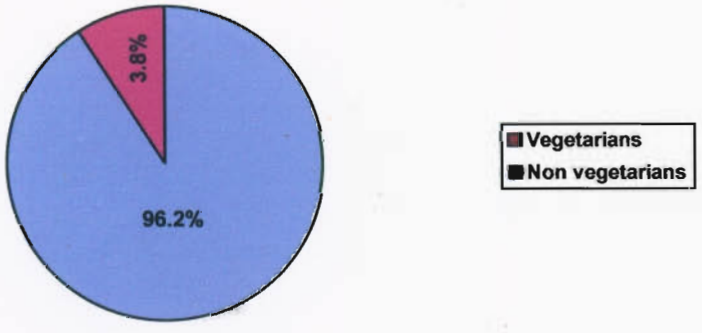
The no. of hypothyroid patients are more in 30-39 age group and the no. of hyperthyroid patients are more in 20-29 age group.

Total no. of vegetarians and Non-vegetarians is shown in table 2a and figure 3.

Table 2a

Food Habit	Total Number	Percentage of total
Vegetarians	38	3.8%
Non- vegetarians	962	96.2%

Figure 3



No. of vegetarians and non-vegetarians in each age group of hypothyroid patients are shown in table 2b.

Table 2 b

Age group	Total of hypothyroid	Non-vegetarians	Vegetarians	Lacto vegetarians
10-19	22	20	2	
20-29	60	56	3	1
30-39	110	108	2	
40-49	20	20	-	
50-59	30	30	-	
60-69	6	6	-	
70-79	2	2	-	
	250	242(97%)	7 (3%)	1 %

Only 3 % of hypo thyroid patients are vegetarians.

No. of vegetarians and non-vegetarians in each age group of hyperthyroid patients are shown in table 2 c.

Table 2 c

Age group	Total no. of hyperthyroid	Non- vegetarians	Vegetarians
10-19	36	31	5
20-29	85	81	4
30-39	48	47	1
40-49	58	58	-
50-59	7	7	-
60-69	8	8	-
70-79	8	8	-
Total	250	240(96%)	10(4%)

Only 4% of hyperthyroid patients are vegetarians.

No. of vegetarians and non-vegetarians in each age group of hypothyroid patients are shown in table 2b.

Table 2 b

Age group	Total of hypothyroid	Non-vegetarians	Vegetarians	Lacto vegetarians
10-19	22	20	2	
20-29	60	56	3	1
30-39	110	108	2	
40-49	20	20	-	
50-59	30	30	-	
60-69	6	6	-	
70-79	2	2	-	
	250	242(97%)	7 (3%)	1 %

Only 3 % of hypo thyroid patients are vegetarians.

No. of vegetarians and non-vegetarians in each age group of hyperthyroid patients are shown in table 2 c.

Table 2 c

Age group	Total no. of hyperthyroid	Non- vegetarians	Vegetarians
10-19	36	31	5
20-29	85	81	4
30-39	48	47	1
40-49	58	58	-
50-59	7	7	-
60-69	8	8	-
70-79	8	8	-
Total	250	240(96%)	10(4%)

Only 4% of hyperthyroid patients are vegetarians.

No. of hypothyroid patients having family history according to age group are shown in table 3a

Table 3a

Age group	Total no. of hypothyroid	No. of patients having no family history	No. of patients having family history
10-19	22	17	5
20-29	60	52	8
30-39	110	88	22
40-49	20	15	5
50-59	30	27	3
60-69	6	5	1
70-79	2	2	-
Total	250	206 (82%)	44 (18%)

No. of hyperthyroid patients having family history is shown in table 3b

Table 3b

Age group	Total No. of hyperthyroid	Having no family history	Having family history
10-19	36	25	11
20-29	85	70	15
30-39	48	40	8
40-49	58	40	18
50-59	7	5	2
60-69	8	7	1
70-79	8	8	-
Total	250	195 (78%)	55 (22%)

Percentage of total hypo and hyperthyroid patients having family history and not having family history are shown in figure 4a and 4b respectively.

Figure 4a

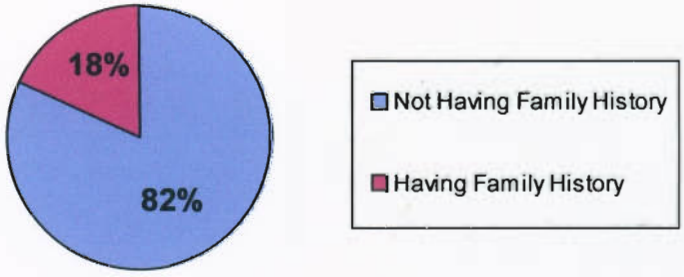


Figure 4b

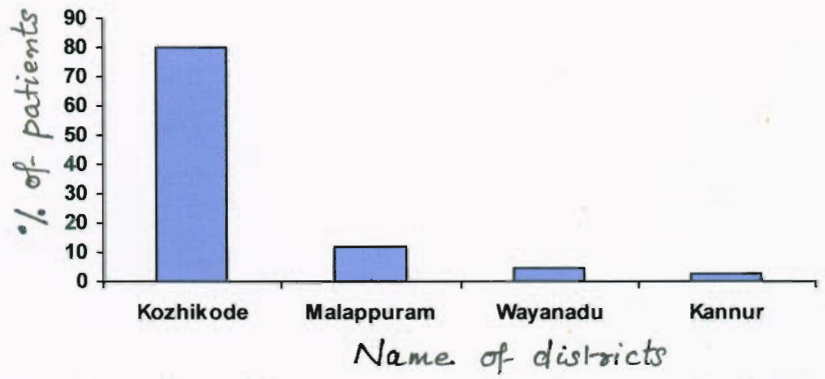
18 % of hypothyroid patients and 22 % of hyperthyroid patients had family history of goiter.

Percentage of hypothyroid patients coming from each district is shown in table 4a and figure 5a.

Table 4a

Age group	No. of hypothyroid patients	No. of patients from each district			
		Kozhikode	Malappuram	Wayanadu	Kannur
10-19	22	16	6	-	-
20-29	60	38	14	4	4
30-39	110	99	4	5	2
40-49	20	10	6	4	-
50-59	30	30	-	-	-
60-69	6	6	-	-	-
70-79	2	2	-	-	-
Total(%)		80 %	12 %	5 %	3 %

Figure 5a

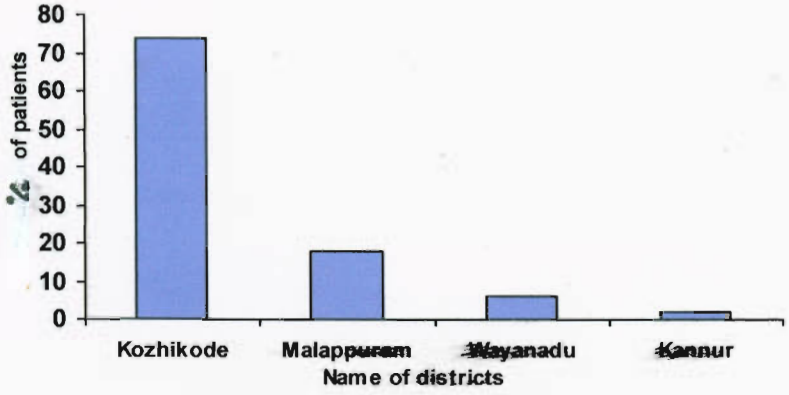


Percentage of hyperthyroid patients coming from each district is shown in table 4b and figure 5b.

Table 4b

Age group	No. of hyperthyroid	No. of patients coming from each district			
		Kozhikode	Malappuram	Wayanadu	Kannur
10-19	36	30	6	-	-
20-29	85	50	29	4	2
30-39	48	35	4	7	2
40-49	58	48	6	4	-
50-59	7	7	-	-	-
60-69	8	8	-	-	-
70-79	8	8	-	-	-
Total (%)		74	18	6	2

Figure 5b



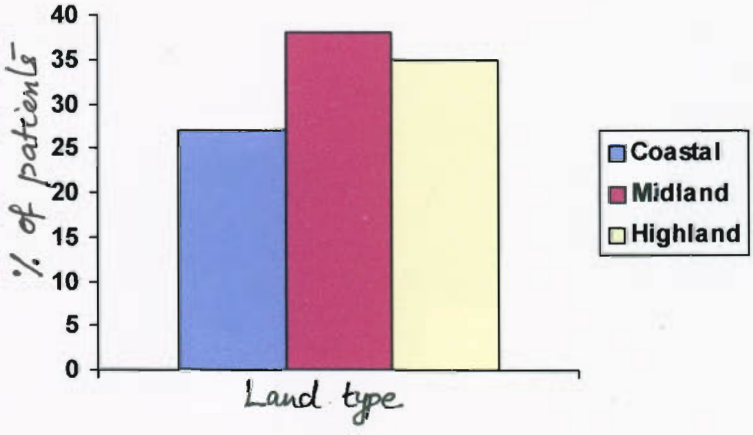
More than 70% of the study patients are coming from Kozhikode district.

No of hypothyroid patients coming from different land types in each age group is shown in table 5a and figure 6a.

Table 5a

Age group	No. of hypothyroid patients	Type of land area		
		Coastal	Midland	Highland
10-19	22	3	7	12
20-29	60	12	20	28
30-39	110	25	40	35
40-49	20	5	10	5
50-59	30	10	15	5
60-69	6	2	3	1
70-79	2	1	-	1
Total (%)		27%	38%	35%

Figure 6a

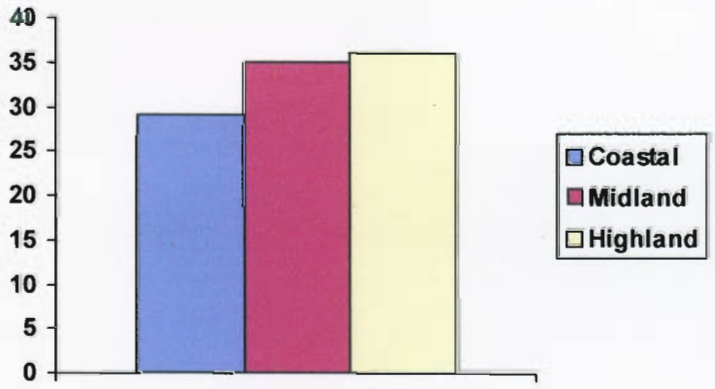


No. of hyperthyroid patients coming from different land types in each age group is shown in table 5b and figure 6b.

Table 5b

Age group	Hyperthyroid patients	Type of land area		
		Coastal	Midland	Highland
10-19	36	15	8	13
20-29	85	30	30	25
30-39	48	12	12	24
40-49	58	10	25	23
50-59	7	5	-	2
60-69	8	1	5	2
70-79	8	-	7	1
Total(%)		29%	35%	36%

Figure 6b



Total % of thyroid patients coming from different land areas is shown in table 6.

Table 6

Type of thyroid abnormality	Total no. in each group	Land area of coming		
		Coastal	Midland	Highland
Hypo thyroid	250	27 %	38 %	35 %
Hyper thyroid	250	29 %	35 %	36 %

There is no significant difference in percentage of hypo and hyperthyroid patients coming from different land areas(coastal, midland and highland).

Comparison of baseline characteristics

The baseline characteristics namely age, sex, family history, food habit, drinking water source, family background, place of coming, use of Tapioca and cabbage of the thyroid patient group is compared with their corresponding normals using chisquare test. The value of chisquare and corresponding 'p' values are shown below in tables 7a-15c.

Sex

Table 7a

Sex	No of hypothyroid patients	No of Normal persons	Total	Chisquare value	'p' value
Male	32	32	64	0.00	1.00
Female	218	218	436		

Table 7b

Sex	No of hyperthyroid patients	No of Normal persons	Total	Chisquare value	'p' value
Male	31	31	62	0.00	1.00
Female	219	219	438		

Table 7c

Sex	No of hypothyroid patients	No of hyperthyroid patients	Total	Chisquare value	'p' value
Male	32	31	63	0.02	0.89
Female	218	219	437		

Family History

Table 8a

Family History	No.of hypothyroid patients	No.of Normal patients	Total	Chisquare value	'p' value
Not having family history of thyroid abnormality	206	206	412	0.00	1.00
Family history of one member	218	218	436		
Family history of 2 members	10	10	20		
Family History of 3 members	1	1	2		

Table 8b

Family History	No.of hyperthyroid patients	No.of Normal patients	Total	Chisquare value	'p' value
Not having family history of thyroid abnormality	195	195	390	0.00	1.00
Family history of one member	43	43	86		
Family history of 2 members	11	11	22		
Family History of 3 members	1	1	2		

Food Habit

Table 9a

Food Habit	Hypo	Normal	Total	Chisquare value	'p' value
Vegetarian	9	9	18		
Non – Vegetarian	241	241	482	0.00	1.00

Table 9b

Food Habit	Hyper	Normal	Total	Chisquare value	'p' value
Vegetarian	10	10	20		
Non Vegetarian	240	240	480	0.00	1.00

Drinking water source

Table 10a

Drinking Water Source	Hypo	Normal	Total	Chisquare value	'p' value
Well water	214	214	428	0.00	1.00
Pipe water	36	36	72		

Table 10b

Drinking Water Source	Hyper	Normal	Total	Chisquare value	'p' value
Well water	212	212	424	0.00	1.00
Pipe water	38	38	76		

Family background

Table 11a

Family Background	Hypo	Normal	Total	Chisquare value	'p' value
Poor	194	194	388	0.00	1.00
Moderate	53	53	106		
Rich	3	3	6		

Table 11b

Family Background	Hyper	Normal	Total	Chisquare value	'p' value
Poor	195	195	390	0.00	1.00
Moderate	41	41	82		
Rich	14	14	28		

Tapioca consumption

Table 12a

Tapioca consumption	Hypo	Normal	Total	Chisquare value	'p' value
Not eating	2	2	4	0.00	1.00
Occassional consumption	225	225	450		
Routine consumption	23	23	46		

Table 12b

Tapioca consumption	Hyper	Normal	Total	Chisquare value	'p' value
Not eating	7	7	14	0.00	1.00
Occassional consumption	220	220	440		
Routine consumption	23	23	46		

Cabbage consumption

Table 13a

Cabbage consumption	Hypo	Normal	Total	Chisquare value	'p' value
Not eating	9	9	18	0.00	1.00
Occassional consumption	240	240	480		
Routine consumption	1	1	2		

Table 13b

Cabbage consumption	Hyper	Normal	Total	Chisquare value	'p' value
Not eating	22	22	44	0.00	1.00
Occassional consumption	228	228	456		
Routine consumption	-	-	-		

Land Area of coming

Table 14a

Land area of coming	Hypo	Normal	Total	Chisquare value	'p' value
Coastal	68	68	136	0.00	1.00
Midland	95	95	190		
Highland	87	87	174		

Table 14b

Land area of coming	Hyper	Normal	Total	Chisquare value	'p' value
Coastal	73	73	146	0.00	1.00
Midland	87	87	174		
Highland	90	90	180		

Actual age of the study group

Table 15a

Group	Age Mean±SD	Median	't' value	'p' value
Hypo	34±12	34	0.15	0.88
Normal	34±12	34		

Table 15b

Group	Age Mean±SD	Median	't' value	'p' value
Hyper	33±14	30	0.08	0.948
Normal	33±14	30		

Table 15c

Group	Age mean±SD	Median	't' value	'p' value
Hypo	34±12	34.00	2.92	0.087
Hyper	33±14	30.00		

The baseline characteristics of hypothyroid patients are compared with their normals and there is no significant difference in number between the two. The baseline characteristics of hyperthyroid patients are compared with their normals and there is no significant difference in number between the two. So the groups are comparable. There is no significant difference in age between the study groups.

II Comparison of Thyroid hormone levels

i) Mean value of thyroid hormone levels in hypothyroid, its normal, hyper thyroid and its normal in each age group.

The results are shown in tables 16a to 16d.

Normal T_3 = 0.7 – 2.0 ng/ml

T_4 = 5.5 – 13.5 µg/dl (55-135 ng/ml)

TSH = 0.5 – 6.0 µIU/ml

a. Hypothyroid patients

Table 16a

Age group	T_3 (ng/ml)	T_4 (ng/ml)	TSH (µIu/ml)
10-19	1.17 ± 0.74	82.5 ± 48.9	12.1 ± 19.4
20-29	0.99 ± 0.43	85.0 ± 39.5	12.9 ± 15.8
30-39	0.99 ± 0.43	85.9 ± 33	12.6 ± 9.5
40-49	1.17 ± 0.45	83.3 ± 45.2	12.6 ± 10.9
50-59	1.20 ± 0.50	87.8 ± 32.7	14.7 ± 12.0
60-69	1.04 ± 0.21	108.2 ± 25.8	8.6 ± 1.7
70-79	1.47 ± 0.75	78.5 ± 41.7	6.95 ± 0.49

b. Normal control of hypothyroid patients

Table 16b

Age group	T_3 (ng/ml)	T_4 (ng/ml)	TSH (µIu/ml)
10-19	1.3 ± 0.42	106 ± 23.4	1.67 ± 1.1
20-29	1.24 ± 0.34	89.5 ± 22.3	3.27 ± 1.4
30-39	1.2 ± 0.33	87.4 ± 20.8	5.47 ± 1.94
40-49	1.4 ± 0.56	70.75 ± 8.27	2.85 ± 2.42
50-59	1.27 ± 0.30	92.5 ± 28.61	5.78 ± 1.82
60-69	1.09 ± 0.13	105.83 ± 6.37	3.75 ± 0.97
70-79	1.32 ± 0.12	104.5 ± 23.33	3.75 ± 2.33

c. Hyperthyroid patients

Table 16 c

Age group	T_3 (ng/ml)	T_4 (ng/ml)	TSH (μIu/ml)
10-19	2.48 ± 1.67	135.63 ± 31.54	0.22 ± 0.21
20-29	2.13 ± 1.27	166.82 ± 52.10	0.25 ± 0.82
30-39	2.03 ± 1.67	160.52 ± 44.30	0.71 ± 0.10
40-49	2.06 ± 0.93	140.52 ± 32.52	0.36 ± 1.04
50-59	2.37 ± 0.63	140.14 ± 46.35	0.03 ± 0.02
60-69	2.23 ± 0.34	131.5 ± 30.87	0.30 ± 0.17
70-79	2.90 ± 1.36	124.88 ± 20.82	0.14 ± 0.19

d. Normal control of hyperthyroid patients

Table 16d

Age group	T_3 (ng/ml)	T_4 (ng/ml)	TSH (μIu/ml)
10-19	1.41 ± 0.36	99.17 ± 26.66	2.46 ± 1.49
20-29	1.37 ± 0.41	106.92 ± 27.68	3.13 ± 1.60
30-39	1.30 ± 0.37	113.90 ± 20.70	2.18 ± 1.10
40-49	1.41 ± 0.33	100.26 ± 25.86	2.53 ± 1.33
50-59	1.27 ± 0.32	101.29 ± 26.22	3.66 ± 1.59
60-69	1.2 ± 0.4	98.5 ± 27.3	2.13 ± 1.30
70-79	1.53 ± 0.34	111.5 ± 16.88	2.24 ± 1.24

ii) Mean value of thyroid hormone levels in total hypothyroid, normal, hyper thyroid and its normal (values are mean ± S.D from 250 values)

The result is shown in table 17.

Table 17

Group	T_3 (ng/ml)	T_4 (ug/ml)	TSH (µIu/ml)
Hypothyroid Patients	1.04 ± 0.48	85.90 ± 36.94	13.65± 2.84
Normal control of hypothyroid	1.24 ± 0.36	89.40 ± 23.27	4.38 ± 1.43
Hyperthyroid patients	2.18 ± 1.32	152.38 ± 44.02	0.44 ± 0.16
Normal Control of hyperthyroid patient	1.38 ± 0.37	105.32 ± 25.84	2.67 ± 1.46

iii) Comparison of thyroid hormone values of hypothyroid, normal, hyperthyroid and its normal.

The results are shown in tables 18a to 20c.

1) Total hypothyroid patients and its normal control

a) T_3

Table 18a

Group	T_3 (ng/ml)		Kruskal-Wallis H test	'p' value
	Mean±SD	Median		
Hypothyroid patients	1.06±0.5	2.0	25.138	0.000001
Normal control	1.24±0.36	1.2		

There is highly significant difference between the T_3 values of hypothyroid patients and its normal controls.

b) T_4

Table 18b

Group	T_4 (ηg/ml)		Kruskal-Wallis H test	'p' value
	Mean±SD	Median		
Hypothyroid patients	85±36	90	0.046	0.83
Normal control	89±23	89		

There is no significant difference in T_4.

c) TSH

Table 18c

Group	TSH (μIU/ml)		t value	p value	Kruskal-Wallis H test	'p' value
	Mean±SD	Median				
Hypothyroid patients	13.6± 2.8	9.04	7.62	0.00	302.46	0.00
Normal control	4.4±1.43	3.10				

The analysis is done both by t test and K.H test. There is highly significant difference between the TSH values in both tests.

2) Total hyperthyroid patients and its normal control.

a) T_3

Table 19a

Group	T_3 (ng/ml)		Kruskal-Wallis H test	'p' value
	Mean±SD	Median		
Hyperthyroid patients	2.18±1.32	1.85	80.54	0.00
Normal control	1.34±0.37	1.40		

There is highly significant difference in T_3 between hyperthyroid patients and its normal control.

b) T_4

Table 19b

Group	T_4 (ng/ml)		Kruskal-Wallis H test	'p' value
	Mean±SD	Median		
Hyperthyroi patients	152.4±44.0	148	168.38	0.00
Normal control	105.32±25.84	109		

c) TSH

Table 19c

Group	TSH (µIU/ml)		Kruskal-Wallis H test	'p' value
	Mean±SD	Median		
Hyperthyroid patients	0.44±0.16	0.10	329.77	0.00
Normal control	2.67±1.46	2.3		

3) Total hypothyroid and hyperthyroid patients

a) T_3

Table 20a

Group	T_3 (ng/ml)	Kruskal-Wallis H test	'p' value
	Mean±SD		
Hypothyroid patients	1.06±0.5	172.5	0.00
Hyperthyroid control	2.18±1.32		

T_3 value of hypothyroid patients is compared with that of hyperthyroid patients. There is highly significant difference between the two.(H=172.5, P=0.00).

b) T_4

Table 20b

Group	T_4 (ηg/dl) Mean±SD	Kruskal-Wallis H test	'p' value
Hypothyroid patients	85.9±36.0	226.84	0.00
Hyperthyroid control	152.4±44.0		

c) TSH

Table 20c

Group	TSH (μIU/ml) Mean±SD	Kruskal-Wallis H test	'p' value
Hypothyroid patients	13.6± 2.8	363.9	0.00
Hyperthyroid control	0.44±0.16		

There is highly significant difference between T_4 and TSH values of hypothyroid patients and hyperthyroid patients.

III Comparison of urinary Iodide Excretion in various thyroid abnormalities

The details regarding experimental procedures and analytical methods are given in chapter 2. The studies were carried out for two years, 1999 and 2000.

i) Comparison of urinary iodide excretion in total study population.

The results along with 't' values and 'p' values are given in tables 21a to 21c and the mean values are shown in figure 7.

a) Hypothyroid patients and normal control (Values are mean ± SD of 250 numbers and expressed as µg/24hr)

Table 21a

Group	Urinary iodide (Mean ±SD)	't' value	p value	H value	p value
Hypothyroid patients	190±16.6	1.2	0.23	2.19	0.14
Normal control	188.2±17.5				

b) Hyperthyroid patients and its normal control

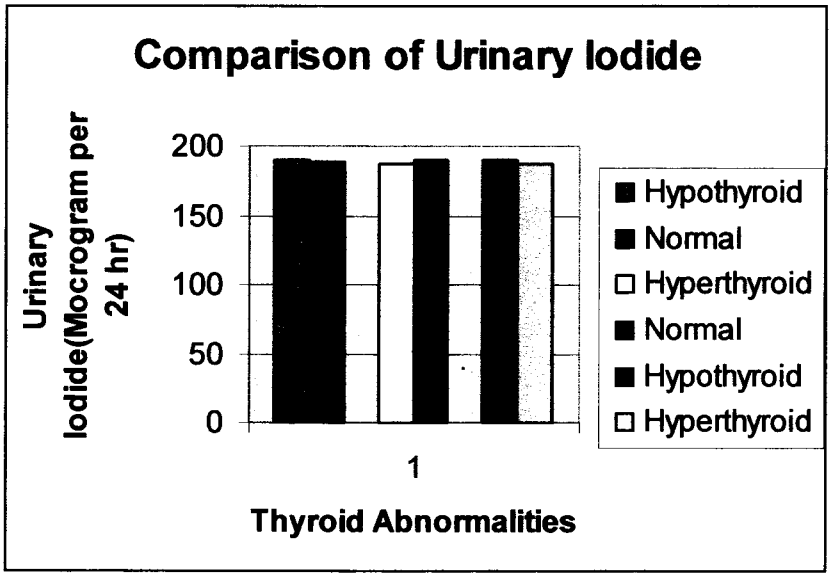
Table 21b

Group	Urinary iodide (Mean ±SD)	't' value	p value	H value	p value
Hyperthyroid patients	187.9±17.4	1.16	0.25	1.60	0.21
Normal control	190±21.6				

c) Hypothyroid and hyperthyroid patients

Table 21c

Group	Urinary iodide (Mean ±SD)	't' value	p value	H value	p value
Hypothyroid patients	190±16.6	1.33	1.78	2.62	0.11
Normal control	187.9±17.4				



The average urinary iodide excretory value of hypothyroid patients are compared with their normals and there is no significant difference(t=1.2, p=0.23). The analysis is confirmed by Kruskal-Wallis H test(H value=2.19, p=0.14).

The average urinary iodide excretory value of hyperthyroid patients are compared with their normals and there is no significant difference when tested by 't' test (t=1.16, p=0.25) and also by 'H' test (H value=1.6, p=0.21).

The average urinary iodide excretory value of hypothyroid patients are compared with hypothyroid patients and there is no significant difference in both tests (t=1.33, p=1.78 and H value=2.62, p=0.11).

ii) Comparison of urinary iodide variation within group and between groups

An Anova was done to compare the urinary iodide excretory level within and between the study groups and is shown in table 22.

Table 22

	Age groups	F	Significant level
Hypothyroid	Between groups Within groups	0.995	0.429
Normal Control	Between groups Within groups	0.546	0.773
Hyperthyroid	Between groups Within groups	0.103	0.996
Normal Control	Between groups Within groups	0.933	0.472

There is no significant variation within and between the groups.

iii) Comparison in urinary iodide excretion in each age group

The results along with 't' values are given in table in each age group and is shown in tables 23a to 23g.

a) Age Group(10-19)

Table 23a

No.	Age group (10-19)	Urinary iodide (µg/24hr)	't' between groups	't' value	Significant level
1.	Hypothyroid patients	190.5±18.5	1 and 2	0.048	>0.05
2.	Normal control	190.8±18.0	1 and 3	0.604	>0.05
3.	Hyperthyroid	187.5±18.2	3 and 4	0.66	>0.05
4.	Normal control	184.3±22.2			

b) Age Group(20-29)

Table 23b

No.	Age group (20-29)	Urinary iodide (µg/24hr)	't' between groups	't' value	Significant level
1.	Hypothyroid	191.7±17.9	1 and 2	0.673	>0.05
2.	Normal control	189.5±17.9	1 and 3	1.29	>0.24
3.	Hyperthyroid	187.8±17.5	3 and 4	1.67	>0.095
4.	Normal control	192.8± 21.2			

c) Age Group(30-39)

Table 23c

No.	Age group (30-39)	Urinary iodide (µg/24hr)	't' between groups	't' value	Significant level
1.	Hypothyroid	189.9±15.3	1 and 2	1.022	0.36
2.	Normal control	187.5±17.7	1 and 3	0.41	>0.05
3.	Hyperthyroid	188.8±17.3	3 and 4	0.22	>0.05
4.	Normal control	189.6± 21.2			

d) Age Group (40-49)

Table 23d

No.	Age group (40-49)	Urinary iodide (µg/24hr)	't' between groups	't' value	Significant level
1.	Hypothyroid	191.7±18.3	1 and 2	1.035	0.36
2.	Normal control	186.2 ± 14.5	1 and 3	078	0.46
3.	Hyperthyroid	188.1±17.7	3 and 4	0.555	>0.05
4.	Normal control	190.1 ± 21.6			

e) Age Group(50-59)

Table 23e

.	Age group (50-59)	Urinary iodide (µg/24hr)	't' between groups	't' value	Significant level
1.	Hypothyroid	187.5±16.3	1 and 2	0.54	>0.05
2.	Normal control	185.2±17.0	1 and 3	0.48	>0.05
3.	Hyperthyroid	191.1±19.6	3 and 4	1.00	>0.05
4.	Normal control	180.7 ±16.1			

f) Age Group(60-69)

Table 23f

No.	Age group (60-69)	Urinary iodide (µg/24hr)	't' between groups	't' value	Significant level
1.	Hypothyroid	187.5±16.7	1 and 2	0.69	>0.05
2.	Normal control	195.8±21.3	1 and 3	0.22	>0.05
3.	Hyperthyroid	185.5±14.5	3 and 4	0.80	>0.459
4.	Normal control	194.1± 24.5			

g) Age Group (70-79)

Table 23g

No.	Age group (70-79)	Urinary iodide (µg/24hr)	't' between groups	't' value	Significant level
1.	Hypothyroid	165.5±3.5	1 and 2	1.08	0.45
2.	Normal control	192.5±24.7	1 and 3	1.38	0.27
3.	Hyperthyroid	185.0±18.7	3 and 4	0.46	>0.05
4.	Normal control	191.5± 25.5			

In each age group there is no significant variation in urinary iodide excretion between hypothyroid patients, and its normal control as well as hyperthyroid patients and its corresponding control.

The comparison is same in all the age group. The average urinary excretion in hypothyroid patients in the age groups (20 –29),(30-39),40-49),(50-59) is more than that of normal control. In 10-19 age group, there is no difference between hypo thyroid patients and its normal control. In higher age groups (60-69) and (70-79), the urinary iodide excretion in hypothyroid patients in lower than that of normal control.

The average urinary excretion in hyperthyroid patients in the age groups (20-29) ,(30-39),(40-49), ,(60-69) and (70-79) is lower than that of normal control. In age groups (10-19) and (50-59) there is slight increase in the urinary iodide excretion in hyperthyroid patients than the normal control. In almost all the age groups except (50-59) and (70-79), the average urinary iodide excretion in hypothyroid patients are higher than that of hyperthyroid patients.

iv. Comparison of urinary iodide with age groups

The results are shown in tables $24a_1$-$24b_2$

a) Hypothyroid Patients

Table $24a_1$

Age group	Urinary iodide (μg/24hr)	ANOVA	
		F statistic	'p' value
10-19	190.5 ±18.5		
20-29	191.7 ±17.9		
30-39	189.9 ±15.3		
40-49	191.8 ±18.3	0.995	0.43
50-59	187.6 ±16.3		
60-69	187.5 ±16.7		
70-79	165.5 ± 3.5		

An ANOVA was done (table-24a_1) for hypothyroid patients and hyperthyroid patients (table-24b_1) for the comparison of average 24hr urinary iodide excretory value with different age groups and there is no significant difference.

A regression analysis was also done (table-24a_2 and b_2) taking urinary iodide excretory value as the dependant variable and age as the independent variable. The regression coefficient is (-0.119) for hypothyroid patients which was not significant and its 95% confidence interval was (-0.29) to (0.049), which again indicates that there is no significant linear relation between age and urinary iodide excretion. Similarly there is no significant linear relation between age and urinary iodide excretion in the case of hyperthyroid patients also.

Table 24a_2

Independent variable	Dependant variable	β	95% confidence limit	
			Lower	Upper
Age group	Urinary iodide	-0.119	-0.29	0.049

b) Hyperthyroid patients

Table 24b_1

Age group	Urinary iodide (µg/24hr)	ANOVA	
		F statistic	'p' value
10-19	187.5 ±18.2	0.103	0.99
20-29	187.8 ±17.5		
30-39	188.8 ±17.3		
40-49	188.1 ±17.7		
50-59	191.1 ±19.6		
60-69	185.5 ±14.5		
70-79	186 ±18.7		

Table 24b_2

Independent variable	Dependant variable	β	95% confidence limit	
			Lower	Upper

Age groups	Urinary iodide	-0.037	-0.189	0.116

V. Comparison of urinary iodide with family history

The results are shown in table25a to 25b.

a) Hypothyroid

Table 25a

	History	Urinary Iodide Mean ±SD (µg/24hr)	H value	p value
1	Having no family history	190.0±17.0	0.773	0.86
2	Having family history of one member	190.4±15.0		
3	Having family history of two members	189.6±14.4		
4	Having family history of three members	177±0.0		

Urinary iodide of hypothyroid patients having family history is compared with that of not having family history. There is no significant difference among the groups (p=0.86).

b) Hyperthyroid

Table 25b

	Family History	Urinary Iodide (µg/24hr)	H	p
1	Having no family history	186.8 ±17.1	8.3	0.04
2	Having family history of one member	193.8 ±17.7		
3	Having family history of two members	183.5 ± 18.2		
4	Having family history of three members	210 ± 0.0		

There is significant difference between the groups.

vi. Comparison of urinary iodide with sex

The results are shown in table 26a, 26b

a) Hypothyroid patients

Table 26a

Sex	Urinary Iodide	't'	p	H	p
Male Female	191.8±13.6 189.7±17.0	0.67	0.50	0.66	0.42

There is no significant difference between the urinary iodide excreted by male and female patients.

b) Hyperthyroid patients

Table 26b

Sex	Iodide (µg/24hr)	'F'	p	H value	p
Male Female	187.6 ± 18.9 188.0 ± 17.3	0.11	0.91	0.14	0.71

There is no difference with sex.

vii. Comparison of urinary iodide with drinking water source.

The results are shown in table 27a to 27c

a) Hypothyroid

Table 27a

Source of drinking water	Iodide (µg/24hr)	't'	p	H	P
Well water Pipe water	190.8± 16.3 185.5± 17.6	1.76	0.08	3.01	0.08

There is no significant difference between the two.

b) Hyperthyroid

Table 27b

Drinking water source	U-Iodide (µg/24hr)	t	p	Kruskal Wallis H	'p' value
Well water Pipe water	187.9 ±17.4 188.1 ±17.9	0.04	0.97	0.00	0.99

There is no significant difference between the two.

c) Total study group

Table 27c

Drinking water source	Urinary Iodide (µg/24hr)	't' value	'p' value
Well water Pipe water	189.5 ± 18.3 185.9 ± 18.4	2.29	0.023

There is significant difference between the two.

viii. Comparison of urinary iodide with Food habit

Results are shown in table 28a to 28c.

a) Hypothyroid

Table 28a

Family History	Urinary Iodide (µg/24hr)	t	p	Kruskal Wallis H	'p' value
Vegetarian Non Vegetarian	184.4 ± 18.7 190.2 ± 16.5	1.02	0.31	0.81	0.37

b) Hyperthyroid

Table 28b

Family History	U-Iodide (µg/24hr)	t	p	Kruskal Wallis H	'p' value
Vegetarian Non Vegetarian	186.3 ± 14.4 188.1 ± 17.6	0.31	0.76	0.004	0.95

There is no difference with food habit.

c) Total Study Group

Table 28c

Family History	Urinary iodide	't' value	Significant level
Vegetarian Non Vegetarian	184.1±17.9 189.2±18.4	1.69	0.093

24 hour urinary excretion has no significant variation between vegetarians and non-vegetarians.

ix. Comparison of urinary iodide with thyroid hormone levels

The relation between 24 hr urinary iodide excretory level and the hormone levels had been looked into by regression analysis and there is no significant relationship. The values of β and confidence limits are as shown below in tables 29a and 29b.

a) Hypothyroid patients

Table 29a

Independent variable	Dependant variable	β	95% confidence limits	
			Lower	Upper
T_3	Urinary iodide	3.39	-0.94	7.71
T_4		-0.031	-0.025	0.087
TSH		0.052	-0.109	0.214

b) Hyperthyroid patients

Table 29b

Independent variable	Dependant variable	β	95% confidence limits	
			Lower	upper
T_3	Urinary iodide	0.405	-1.243	2.054
T_4		-0.005	-0.054	0.044
TSH		-0.023	-0.350	0.304

x. Comparison of 24 hr urinary iodide excretion with variation in land area.

The results along with 't' values are given in table 30. There is no significant variation (at 5% level) of urinary iodide excretion with variation in land area.

Table 30

No.	Group	Urinary iodide	't' between groups	't' value	Significant level
1.	Coastal	187.5 ±18.3	1 and 2	1.19	0.29
2.	Midland	189.2 ±18.5	1 and 3	1.48	0.16
3.	Highland	189.8 ±18.3	2 and 3	0.32	>0.05

xi. Comparison of urinary iodide excretion in hypothyroid patients, normal control, hyperthyroid patients and corresponding normal coming from different land areas.

The results are shown in tables 31a to 31c.

(1) Coastal

Table 31a

No	Group	Urinary iodide	't' between groups	't' value	Significant level
1.	Hypothyroid	189.3 ± 15.7	1 and 2	1.30	0.24
2.	Normal control	185.6 ± 16.7	1 and 3	0.096	>0.05
3.	Hyperthyroid	189.2 ± 17.5	2 and 3	1.027	0.35
4.	Normal control	186.1 ± 22.4			

There is no significant variation between different groups

(2) Midland

Table 31b

No	Group	Urinary iodide	't' between groups	't' value	Significant level
1.	Hypothyroid	189.1 ± 16.1	1 and 2	0.24	>0.05
2.	Normal control	188.6 ± 16.5	1 and 3	0.66	>0.05
3.	Hyperthyroid	187.5 ± 17.9	2 and 3	1.54	0.14
4.	Normal control	192.3 ± 22.8			

(2)Highland

Table 31c

No.	Group	Urinary iodide	't' between groups	't' value	Significant level
1.	Hypothyroid	191.5 ± 17.9	1 and 2	0.63	>0.05
2.	Normal control	189.2 ± 19.0	1 and 3	1.64	0.10
3.	Hyperthyroid	187.2 ± 17.0	2 and 3	1.39	0.20
4.	Normal control	190.9 ± 19.3			

There is no significance at 5% level

Comparison of Urinary Iodide excretion in Iodised salt taking controls.

The results are shown in Table 31 d.

Table 31 d

Group	Urinary Iodide (Mean ± SD) (µg/24hr)
Hypothyroid patients	**275 ± 16.5**
Normal Control (Hypo)	**260 ± 18.5**
Hyperthyroid patients	**250 ± 20**
Normal Control (Hyper)	**280 ± 10**

The values are well above the recommended levels. (100 µg / 24 hr) and there is no significant variation between the study groups.

IV. Comparison of water iodide

A. Comparison of water iodide with baseline characteristics

Drinking water source

Comparison of water iodide content in well water and pipe water (shown in tables 32a-c).

a) Total study group (values are mean ±SD from 1000 numbers and expressed as µg/l).

Table 32a.

No.	Group	Water iodide	't' between groups	't' value	Significant level
1. 2.	Well water Pipe water	10.2± 13.3 8.8 ± 10.7	1 and 2	1.25	0.26

There is no significant variation in iodide content in pipe water and well water.

b) Hypothyroid patients (total number= 250)

Table 32b

No.	Group	Water iodide (µg/litre)	't'	P	H value	p
1. 2.	Well water Pipe water	9.9±14.4 9.9± 11.2	0.001	0.99	1.06	0.30

There is no significant difference in water iodide used by hypothyroid patients with source of water.

c) Hyperthyroid patients (total number=250)

Table 32c

No.	Group	Water iodide (µg/litre)	't'	P	H value	P
1. 2.	Well water Pipe water	10.5±12 7.6± 10	1.37	0.17	1.35	0.25

There is no significant difference in water iodide used by hyperthyroid patients with source of water.

B) Comparison of water iodide with land types (coastal, midland & highland)

The results are shown in tables 33a-c.

a) Total study group(values are mean ±SD from 1000 numbers and expressed as µ g/l).

Table 33a

No.	Group	Water iodide (µg/litre)	't' between groups	't' value	Significant level
1.	Coastal	27.3 ± 3.9	1 and 2	33.04	0.00
2.	Midland	5.2 ± 10.7	2 and 3	7.3	0.00
3.	Highland	1.09 ± 0.5	1 and 3	123.9	0.00

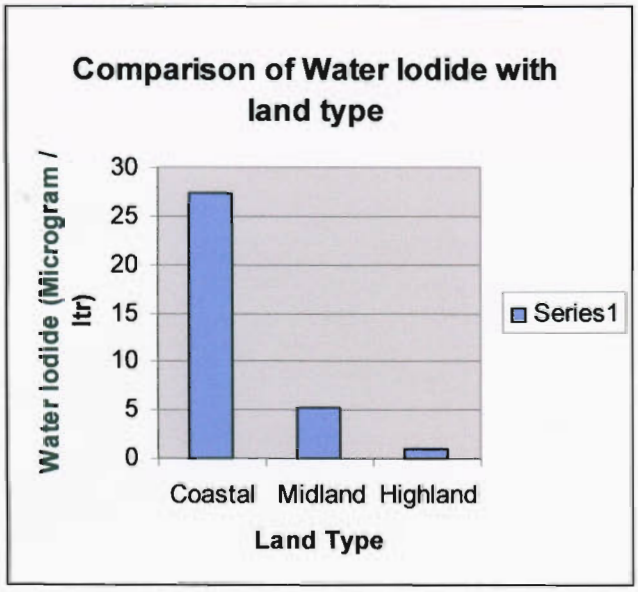


Figure - 8

b) Hypothyroid patients(total number= 250)

Table 33b

Land type	Water iodide (μg/litre)	Kruskal Wallis H value	p value
Coastal Midland highland	27.0 ± 4.1 5.8 ± 14.4 1.1 ± 0.8	205.58	0.00

c) Hyperthyroid patients (total number=250)

Table 33c

Land type	Water iodide (μg/litre)	Kruskal Wallis H value	p value
Coastal Midland Highland	27.6 ± 3.8 4.6 ± 3.8 1.1 ± 0.41	215.3	0.00

There is significant difference in iodide content of water collected from coastal, midland and highland areas irrespective of nature of thyroid patients.

C Comparison of iodide content in water used by hypothyroid patients and hyperthyroid patients

The reults are shown in tables 34a-d.

Table 34a. Total study group

No.	Group	Water iodide (µg/litre)	't'	P	H value	p
1.	Hypothyroid patients	9.93 ± 13.99	0.097	>0.05	0.014	0.91
2.	Hyperthyroid patients	10.05 ± 11.79				

Group 1 is compared with group2. There is no significant variation of iodide content in water brought by total hypothyroid and hyperthyroid patients.

Comparison of water iodide content according to land area

a) Coastal

Table 34b

No.	Group	Water iodide (µg/litre)	't'value	P
1.	Hypothyroid patients	27.0±4.1	0.869	0.42
2.	Hyperthyroid patients	27.6±3.7		

Midland

Table 34 c

No.	Group	Water iodide (µg/litre)	't'value	P
1.	Hypothyroid patients	5.78±14.4	0.737	0.474
2.	Hyperthyroid patients	4.6±3.8		

c) Highland

Table 34 d

No.	Group	Water iodide (µg/l)	't'value	P
1.	Hypothyroid patients	1.13±0.75	0.496	0.62
2.	Hyperthyroid patients	1.08±0.41		

There is no significant variation in iodide content of water used by hypothyroid and hyperthyroid patients from coastal, midland and highland areas.

V. The comparison of Serum Cholesterol

A. Comparison of Serum Cholesterol in various thyroid abnormalities are shown in table 35 a – c.

Table 35 a (results are expressed as mg %)

Group	S. Cholestrol Mean ± SD	H. value	P value
Hypothyroid patients	270 ± 55		
Normal control	192 ± 35	217. 87	0.00

The average serum cholesterol of hypothyroid patients are compared with their normals and there is highly significant difference between the two. (H value = 217.87, p = 0.00)

Table 35 b

Group	S. Cholestrol (mg%) Mean ± SD	H. value	P value
Hyperthyroid patients	133 ± 29		
Normal control	205 ± 48	288. 37	0.00

The average serum cholesterol of hyperthyroid patients are compared with their normals and there is highly significant difference between the two. (H value = 288.37, p = 0.00)

Table 35 c

Group	S. Cholestrol (mg%) Mean ± SD	H. value	P value
Hypothyroid patients	270 ± 55		
Hyperthyroid patients	133 ± 29	354. 7	0.00

The average serum cholesterol of hypothyroid patients are compared with their hyper thyroid patients and there is highly significant difference between the two. (H value = 354. 7, p = 0.00)

B) Comparison of cholesterol level with sex.

The results are shown in table 36 a & b.

Table 36 a (Hypothyroid)

Sex	S. Cholestrol (mg%) Mean ± SD	H. value	P value
Male	265.8 ± 41.3		
Female	271.5 ± 57.8	0.162	0.69

Table 36 b (Hyperthyroid)

Sex	S. Cholestrol (mg%) Mean ± SD	H. value	P value
Male	131.4 ± 28.4		
Female	133.8 ± 29.4	0.12	0.73

There was no significant difference with sex

C) Land wise comparison of serum cholesterol in hypo, normal, hyper and its normal

a) Coastal

Group	Serum cholesterol (mg/l.)	t between groups	T value	P value
Hypothyroid patients	273 ± 52	1 and 2	9.27	0.00
Normal control	199 ± 39	1 and 3	19.96	0.00
Hyperthyroid patients	132 ± 28	2 and 3	13.15	0.00
Normal control	204 ± 37			

b) midland

Group	Serum cholesterol (mg/l.)	t between groups	t value	P value
Hypothyroid patients	270±56	1 and 2	11.17	0.00
Normal control	193±37	1 and 3	19.26	0.00
Hyperthyroid patients	139±30	2 and 3	10.96	0.00
Normal control	213±54			

c) Highland

Group	Serum cholesterl (mg/l.)	t between groups	t value	P value
Hypothyroid patients	268±58	1 and 2	11.31	0.00
Normal control	187±31	1 and 3	20.13	0.00
Hyperthyroid patients	128±28	2 and 3	11.77	0.00
Normal control	199±49			

There is significant increase in serum cholesterol in hypothyroid patients and significant decrease in hyperthyroid patients than their corresponding control in coastal, midland and highland areas.

VI. Iodide Content Food Items

The results are shown in table 37 & figures 9 and 10.

Table 37

Food Items	Iodide content (mean± SD) (µg/kg)
Amaranthus	
(green)	272 ± 38
(red)	372 ± 38
Beans	385 ± 29
Banana	372 ± 33
Cabbage	238 ± 23
Brinjal	371 ± 25
Cauliflower	438 ± 25
Coconut Flesh	398 ± 39
Onion	625 ± 41
Tapioca	150 ± 29
Potato	110 ± 12
Milk	
(pasteurized)	81 ± 9
(non–pasteurized)	112 ± 9
Egg	79 ± 23
Sardine	530 ± 233
Mackeral	670 ± 272
Nemepterus	830 ± 210
Oil Sardine	905 ± 177
Anchovy	930 ± 486
Caraputhy	1026 ± 79

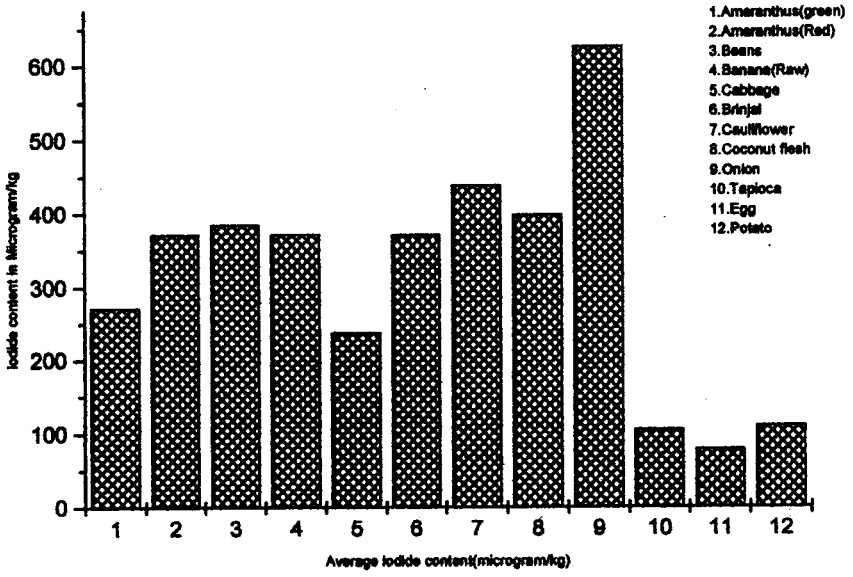


Figure 9

Fig 9 Conc.of iodide in food items

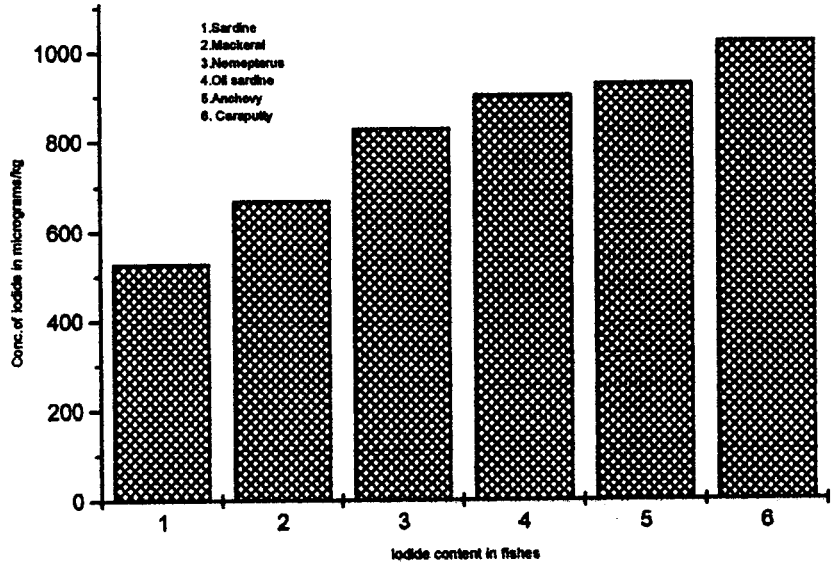


Figure 10

Fig 10 Conc.of iodide in fish samples

For a non-vegitarien who daily uses sea fish, the iodine intake is sufficient to fulfill the requirement.

VII. Thiocyanate content in Cabbage and tapioca

Table 38

Food items	Thiocynate content (mg/kg)
Cabbage	110 - 150
Tapioca	45-100

Discussion

Iodine deficiency in humans and animals occurs when iodine intake falls below recommended levels. If excess of iodine is consumed, it is eliminated by passing out through urine. The two traditional methods for the assessment of iodine deficiency in a given area are the estimation of urinary iodide excretion and the prevalence of goiter. Urinary iodine excretion is a good marker of very recent dietary iodine intake. An equilibrium is established between iodine intake from food and urinary iodine exertion. In individuals, urinary iodine exertion can vary somewhat from day to day and even within a given day, but this variation tends to dampen out in populations. In field studies, the estimation of urinary iodine excretion in random urine specimens provides an adequate assessment of a population's iodine nutrition. Since there is significant variation in the volume of daily urine excretion, it is better to estimate the total iodide in 24 hr urine.

Annette Neubert reported that vegetarian diet can increase the risk of iodine deficiency[53]. The study was based on analysis of dietary iodine of the various food items and urinary iodine excretion. They observed that among volunteers, consuming

lactovegetarian diet had the lowest urinary iodide excretion. However iodine was restricted in all other forms such as sea food, iodized salt etc and only the source of iodine was dietary iodine. They concluded that due to low iodine content of vegetable diets, the consumption of these for prolonged periods could increase the prevalence of hypothyroidism.

In the present study, no significant difference could be observed between vegetarian and non vegetarian subjects as indicated in table28c. However it should be added that the vegetarian group was far lesser in no compared to non- vegetarian group and a comparison with larger and equal no in both the groups can alone make a conclusive decision.

A number of studies had been carried out to establish the existence of iodine deficiency using urinary iodide as the indicator. An epidemiological study using 4230 pre-adolescent and adolescent school children in the Plateau state of Nigeria had shown that in goitrogenic endemic area, there is iodine deficiency due to low drinking water iodine content which correlated well with low urinary iodine.[31] The study also showed no significant gender differences in urinary iodine content. The urinary iodide studies in 243 school children aged 5–13 years from the Zurich area revealed that the mean urinary iodide excretion was 11.3 ± 8.7µg/dl , below the threshold suggested by W.H.O.[31]

The breast milk analysis showed that milk of 46.7 % of mothers from 12 villages of Thailand contained iodine below recommended standards.[29] Iodine intake and iodine deficiency were investigated in thirty vegans consuming their habitual diet.[26] 36% males and 63 % females had iodine intake below the lower reference nutrient intake.

Urine samples of primary school students in a Southeastern urban area, Brazil were collected and analysed for iodide content[98]. Urine iodine biochemical determinations were normal in 92.2 % of private school children and 42.6 % of public school children. In Germany, iodine deficiency is common .In a representative group of 2500 Germans, Study were carried out and iodine concentration in spot urine samples were determined. The median urinary iodine concentration was 9.4µg/dl in 566 adults aged 50–70 yrs, 8.3 µg/dl in 772 conscripts and 5.6µg/dl in 739 breast fed newborns[99]. Changes in urinary iodine excretion and in serum thyroid function parameters during the course of pregnancy, at term and post partum in Soudi women have been demonstrated[100] . Sub clinical iodine deficiency was evident in 28.8% of pregnant women at term and 11.5 % of women at 6-10 weeks postpartum.

Iodine deficiency diseases remain a health problem even in some developed countries. Examinations of iodine intake in 4649 randomly selected participants from two cities in Denmark showed that iodine intake was lower than recommended level in both cities if iodine intake from supplements was not included[101].

Tyrotropin and thyroglobulin can be used as surveillance indicators for assessing iodine deficiency disorders. A study was carried out in Austria to compare the relation between TSH and Tg , free triiodothyronene and thyroxine serum levels with urinary iodine excertion in 2311 untreated euthyroid patients[102]. Iodine intake of approximately 250 µg/day is associated with the lowest TSH stimulation to thyrocytes . Iodine status was improved in Austria since 1984, however , in 1996, iodine excretion in one – third of the investigated patients was under 100 µg/g of creatinine and more than 80 % had less than 200 µg/g of creatinine. In order to establish whether the breast – fed child of an iodine replete mother was

protected from iodine deficiency disorders in Nigeria, urinary iodide excretion was estimated in 68 healthy mother-child pairs. In all the breast fed children , the mean value of urinary iodide excretion was 9.9 µg/dl (fell in the iodine deficiency range <10µg/dl) although all the mothers were iodine replete (mean urinary iodine excretion 14.5µg/dl) despite the fact that all resided in a non-iodine deficient area[28]

The iodine intake level in a population in Denmark determined in cross-sectional studies[103]. Average urinary iodine excretion was 57µg/l and varied from 29-81 µg/l. 7% of individual urine samples indicated severe iodine deficiency. Participants with moderate iodine deficiency showed clear signs of substrate deficiency for thyroid hormone synthesis while participants with mild iodine deficiency did not.

Semiz S etal assessed goitre prevalence and urinary iodine exertion in 605 school children living in the Antalya region. Median iodine/creatnine ratios of all subjects, and goitrous and non goitrous subjects were 64.1 ± 20.1, 62.8 ± 21.8 and 64.9 ± 19.1 µg/g respectively[27] .

In a study carried out in korea, the average iodine intake of Korean adults was 479 µg /day[104]. There was no significant difference in age or sex. The dietary iodine intake was positively correlated with the urinary excretion of iodine .

Iodine excretion studies in rural school children in Cuba revealed that the median urinary iodine excretion was 95µg/l for the whole sample[24]. But in the mountainous stratum, the median urinary iodine excretion values was less than 20 µg/ltr. Before the iodization of salt, goiter was endemic in New Zealand due to low soil iodide.[23] In a study carried out in 1993, median urinary iodide

excretions for 2 areas in New Zealand were considerably lower (60 and 76 µg/day) than those reported previously

Leisen kotter et al monitored the iodine supply and its effect on the thyroid gland volume in prepubertal and pubertal children in the eastern and western parts of the city of Berlin .[22] The mean iodine excretion was 115.8 µg/g creatinine, with no significant differences between eastern parts with 114.5 µg iodine/g creatinine vs 116.7 µgs iodine per gm creatinine in the western parts of the city

Urine sample study in Belarus showed that there is severe iodine deficiency in this region.[23] 10% of the study children excreted <20 µg/litre of iodide.

In Taipei city, iodine deficiency is not a real problem.[105] About half of the Taipei city residents may take more than the suggested optimum amount of iodine. An iodine excretion study was carried out by the Institute of Endocrinology, Bucharest, Romania. The average 24 hr urine excretion was 91± 5 µg .

The above studies showed that in almost all the countries out side India, there is mild iodine deficiency (Urinary iodide excretion was below 100µ g/ltr) . An assessment of iodine deficiency in Ernakulam district was done in 1998 and the median urinary iodide excretion was found to be 200µg/litre. A study carried out by community medicine department of Trivandrum Medical College revealed that the mean urinary excretion was 123µg/24hr which is higher than the recommended level.

Analysis of drinking water iodine and urinary iodine in various areas included in the study did not show any direct correlation. Even in subjects consuming drinking water with low iodine, there was no significant decrease in urinary iodine as

compared to controls. On the other hand, the urinary iodine was found to be well above the recommended normal level. (table21a-c). This could be probably due to the fact that there may be adequate iodine intake due to the intake of iodine rich foodstuffs namely sea fish, diary products, onion, egg which are commonly taken by even low income group. Hence inspite of the low water iodine intake, the total iodine from different food items may be sufficient to meet iodine demand and hence urinary iodine excretion remaining normal.

Since there is no correlation between urinary iodide and iodine disorders, a study was conducted with controls consuming iodized salt along with iodine rich food. The urinary iodide of iodized salt consuming controls was much higher than the group of control not consuming iodized salt (table 31d).

The analysis of the results obtained in the present work also indicate that there is no correlation between baseline factors with the percentage of hyper and hypo thyroidism. The consumption of iodine through drinking water did not correlate with the incidence of thyroid disorders and urinary iodine. The subjects included in the present study as hyper and hypothyroid are genuine since the protein (TSH), hormone and cholesterol levels correlated well with the literature reports. Yet, there was no statistical significance with the conditions and urinary iodine. This probably could mean that in majority of the cases of the study, iodine intake is not the cause for the condition. The causes may be other than iodine intake. Further, the incidence of hypo and hyper thyroidism was found to be the same extend in regions with drinking water having varying concentration of iodine. (Table 34 b – d).The urinary exertion of iodine in restricted controls (avoiding iodized salt and antithyroid

agents containing food item) was not significantly different from urinary iodine of hypo and hyperthyroid patients on a similar diet.

In hypothyroid patients, the cholesterol values are significantly elevated than their corresponding control and in hyperthyroid patients the cholesterol levels are lowered. (Table 35 a-b) The impact of thyroid hormone on lipid levels is primarily mediated through triiodothyronine – bound thyroid protein binding and activation of the promoter regions of the low-density lipoprotein receptor and 3 – hydroxyl – 3 methylglutaryl coenzyme A – reductase genes, leading to a reduction in serum cholesterol levels in hyperthyroid patients. [56]

Thiocyanate content of tapioca and cabbage are higher (table 38) which can compete with iodine uptake and hence consumption of these items especially by poor socioeconomic group could increase the risk of Iodine deficiency disorders. However in the present study, the hyper and hypo thyroid patients are advised not to consume such items during the study period. Hence there was no fall in urinary iodine from normal levels. In the present study the thiocynate content in tapioca is lower than that of other areas. The cyanide content in casava varieties used by Kani tribals in Nedumangadu taluk there found out by Leena Mohanan and K.T. Shenoy. [106] The results showed that there is greater variation in cyanide content. (142 – 761 mg/kg)

Probably a study including hyper and hypothyroid patients, and their controls consuming thiocyanate rich foods during the experimental period could elucidate whether thiocyanate has any role in inducing hypothyroidism.

CONCLUSION

Minikumari Amma.V “Surveillance of Iodine in Drinking Water in Relation to Iodine Deficiency Disorders in Northern Kerala ” Thesis. Department of Life Sciences, University of Calicut, 2002

IV. Conclusion

The number of thyroid patients are increasing day to day. One of the major reasons for this is the deficiency of iodine since iodine is a substrate for the synthesis of thyroid hormones.

The overall results point out that inspite of the prevalence of 17% established iodine disorders among subjects with thyroid complaints there is no deficiency of iodine intake as judged by urinary iodine. On the other hand, on comparison of the above group with that of iodized salt consuming group, it was noticed that the intake and excretion of iodine is much higher than any of the group included in the study. The consumption of iodized salt along with iodine rich food like sea fish, egg, milk etc., could, enhance the iodine intake to well above the optimum level and even to toxic level. Prolonged intake of iodine at or near toxic level could induce hyperthyroidism and autoimmune thyroid diseases, thyrotoxicosis and even thyroid carcinoma. So periodical and epidemiological studies should be conducted on the levels of the iodine intake and disorders due to high iodine in take.

A final word on the compelled intake of iodised salt can be said only after several of such studies.

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