PREVALENCE OF TORCH INFECTIONS IN PREGNANCY RELATED COMPLICATIONS IN THE MALABAR REGION OF KERALA

Thesis submitted to the
University of Calicut
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY
IN
MICROBIOLOGY

UNDER THE FACULTY OF SCIENCE

By

DENOJ SEBASTIAN

Department of Life Sciences
University of Calicut
Kerala -673 635

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CERTIFICATE

This is to certify that the thesis entitled ***"Prevalence of TORCH infections in Pregnancy related complications in the Malabar region of Kerala"*** is an authentic record of research work carried out by Sri. Denoj Sebastian under my supervision and guidance in the Department of Life Sciences, University of Calicut in partial fulfillment of the requirements for the degree of Doctor of Philosophy and that no part of this work has been presented before for the award of any degree, diploma, associateship in any University or institution.

Thenjipalam
June 2006

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DECLARATION

I hereby declare that the thesis entitled ***"Prevalence of TORCH infections in Pregnancy related complications in the Malabar region of Kerala"*** is a genuine record of the research work done by me under the supervision of Dr. Fathimathu Zuhara, K, Reader in Microbiology, Department of Life Sciences, University of Calicut and that no part of this work has previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title of any University or institution.

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Calicut University
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Introduction

Every minute, one woman somewhere in the world dies from a complication related to pregnancy or childbirth with a total death toll of almost 6 lakh women a year.[1] Ninety-nine percent of these deaths occur in developing countries[2,3]. Of these, India accounts for 1.36 lakh maternal deaths.[1] That is, in India, one woman dies every five minutes from a pregnancy-related cause. For every three deaths of women in their reproductive years in some developing countries, one is the result of complications from pregnancy and childbirth. About 15% of deaths of women in the reproductive age in India are maternal deaths. Complications related to pregnancy, childbirth and complications arising out of unsafe abortion are leading causes of death in adolescent girls. In India, 50 percent of maternal deaths of girls in the 15-19 years age group are due to complications arising out of unsafe abortion [4].

A successful pregnancy outcome, in any given community, is a powerful indicator of the health status of its women and of the quality of health care available to them during pregnancy and birth. Poor maternal health resulting in repeated miscarriages, stillbirths and early infant death is a well known fact. Fetal death in uterus, if not detected in time and followed up with prompt hospitalisation, can endanger the mother's life. [5]

Negative pregnancy outcome means all pregnancy outcomes other than a live birth and a voluntarily terminated pregnancy. It includes spontaneous abortions (miscarriages), stillbirths, intrauterine growth retardation (IUGR),

intrauterine fetal death (IUD), early neonatal mortality and congenital disorders/malformations. **Miscarriage** is defined as the premature expulsion from the uterus of the product of conception before 28 weeks of pregnancy. The term **Intrauterine Growth Retardation (IUGR)** is the most common generic term that is used to describe the fetus with a birth weight at or below the 10th percentile for gestational age and sex.[6] while **Intrauterine Fetal Death (IUD)** includes all fetal deaths occurring during pregnancy, after 28th week of gestation and during labour. Early neonatal mortality refers to death of the infant within the first seven days after birth.[5] Stillbirths or IUD and early neonatal deaths are often associated with premature births and low birth weights. A **Congenital disorder/malformation** is a medical condition that is present at birth. Congenital disorder can be recognized before birth (prenatally), at birth, or many years later.[7]

Pregnancy wastage rates in India are high when compared to a number of developing countries. Miscarriage usually happens in the first three months. Early miscarriage is mainly due to the fetus failing to develop normally. Later miscarriage is more likely to be the result of the placenta not functioning properly or a weak cervix. Symptoms include bleeding; but this is not always the case and about half of all women who bleed in the early stages of pregnancy do not go on to miscarry. Bleeding may also not be related to the fetus at all but could be caused by lesions in the vagina or cervix.

Almost eight million low birth weight infants were born in India every year, accounting for around 40 per cent of low birth weight infants in the world.[1] It is also said that 75 per cent of neonatal deaths occurred in infants with low birth weight[1,8]. A study in the Tamilnadu district showed a rate of 1.35% stillbirth, 3.53% neonatal mortality and 4.2% perinatal mortality.[9] The

stillbirth rate in India was 30 to 35 per 1,000 births and the perinatal mortality rate was around 60 to 70 per 1,000 live births.[1]

In the rural part of Tamil Nadu, women had a controlled reproductive pattern. The excess neonatal mortality among girls constitutes about one third of the perinatal mortality rate as a result of their preference to sons.[9] According to a joint study [1] conducted by the World Health Organization (WHO), the National Neonatology Forum and the United Nations Children's Fund (UNICEF) on the "State of newborns in India," out of the 26 million newborns every year, 1.2 million die within the first four weeks. This constitutes 30 per cent of the 3.9 million neonatal deaths worldwide. Neonatal Mortality Rate of 44 deaths per 1,000 live births as reported by *The Hindu* in 2004 accounts nearly two-thirds of the global infant mortality and half of the global child mortality. The undivided States of Uttar Pradesh, Madhya Pradesh and Bihar together accounted for over 50 percent of neonatal deaths in India in the year 2000. This was roughly 15 per cent of the global neonatal deaths. The number of deaths was as low as 10 per 1,000 live births in Kerala, whereas, it was around 60 in Orissa and Madhya Pradesh. Infections, birth asphyxia, and premature birth were identified as the leading causes of neonatal deaths.

Congenital anomalies were another significant negative outcome of pregnancy. The ratio of major congenital anomalies to the total number of deliveries reported by Henry and Varma in 1996 was 1:400.[10]

High-Risk Pregnancy and Risk Factors

Pregnancy in which the mother, fetus, or newborn is or will be at increased risk for morbidity or mortality before or after delivery is termed high risk pregnancy.

Most pregnancies proceed normally and result in a healthy baby. The most important step in ensuring a safe and healthy pregnancy is identifying women at risk of complications and the best time to identify them is before they get pregnant. Factors such as lifestyle, family health history and the mother's overall health offer important information about potential risks. For instance, women over age of 35 are considered at higher risk than younger women for pregnancy-related complications. Chronic health problems such as asthma, diabetes, heart problems, lupus and Rh disease also require particular care during pregnancy. Some factors, such as the mother's advanced age, anemia and bleeding in pregnancy are considered as low-risk.[11]

Maternal Infections

Intrauterine Infections

Anne, T. and Elizabeth, R [12] opined that serious infectious illness in the mother can have non-specific fetal or obstetric effects and lead to miscarriage, premature labor or fetal death. These infections must be treated as any other serious illness. Infections acquired in utero or during the birth process are a significant cause of fetal and neonatal mortality and an important contributor to early and later childhood morbidity.

Usually the fetus is infected by transplacental spread after maternal infection, in which the organism circulates in the mother's blood. These

infections, acquired in utero, can be severe enough to cause fetal loss or can result in intrauterine growth restriction, prematurity, or chronic postnatal infection. In most cases the maternal illness is mild but the impact on the developing fetus is more severe. The degree of severity is dependent on the gestational age of the fetus when infected, the virulence of the organism, the damage to the placenta, and the severity of maternal disease. For example, a primary maternal infection such as *Herpes simplex* is more likely to be vertically transmitted and cause a more severe disease than recurrence of same infection in the mother.[13] Wilcox, A.J. et al., [14] observed the difficulty to determine the percentage of fetal loss due to infection during early pregnancy.

Different workers[13,15] listed the unique pathogenic mechanisms of these infections. Because of their relatively low virulence, the organisms involved seldom lead to fetal death beyond the earliest stages of embryogenesis. Since the fetus is essentially a graft of foreign tissue in the uterus, the placenta constitutes a protective immunologic barrier that shields the fetus from the mother's humoral and cell-mediated immune responses. This makes the fetus especially susceptible to infection during the first trimester and the perinatal period. Early in pregnancy, the most complex events in embryogenesis take place, making sensory organs such as the eyes and ears vulnerable. The immature fetus lacks the immunologic mechanisms necessary to completely eliminate an infecting organism. Therefore, a state of immunologic tolerance is often established, which results in persistence of organisms that ordinarily would be eliminated by a normal child or adult.

Clinical evidence of infection may be seen at birth, soon afterward, or not until years later. The infected newborn infant may display growth retardation, developmental anomalies, or multiple clinical and laboratory abnormalities. Progressive tissue destruction is seen in Rubella, HSV, CMV,

Toxoplasma and *Treponema pallidum* as the infective agents continue to survive and replicate in the tissues for months or years after initial infection. This is particularly unfortunate when treatment is possible. The sequelae of these diseases can also progress over time, *e.g.,* the hearing loss that is secondary to rubella infection can progress or develop even after years of normal hearing.[13]

According to Anne, T. and Elizabeth,[12] routine screening of pre-pregnancy or antenatal cases for the presence of, or susceptibility to, these infections and appropriate management can prevent adverse fetal or perinatal outcomes. This screening should include infection with TORCH agents, Hepatitis B virus, *Treponema pallidum*, and HIV.[16] When a TORCH test or screening is ordered on a newborn, it is suspected that the child has been exposed in utero to one of several organisms that can cause mild or subclinical disease in the mother but devastating damage to the infant. Routine screening of pregnant women for TORCH titers at the first prenatal visit is commonplace in many parts of the world. However, the value of this testing has been questioned by workers like Garland, S.M *et al.,*[17,18] Khan, N.A *et al.,*[17,18] and many others, while screening of both maternal IgG and IgM antibodies are performed routinely.

The acronym TORCH has become one of the most recognized in the field of neonatal/perinatal medicine. The original concept of the TORCH perinatal infections was to group five infections with similar presentations, and etiology. These are the infections with;

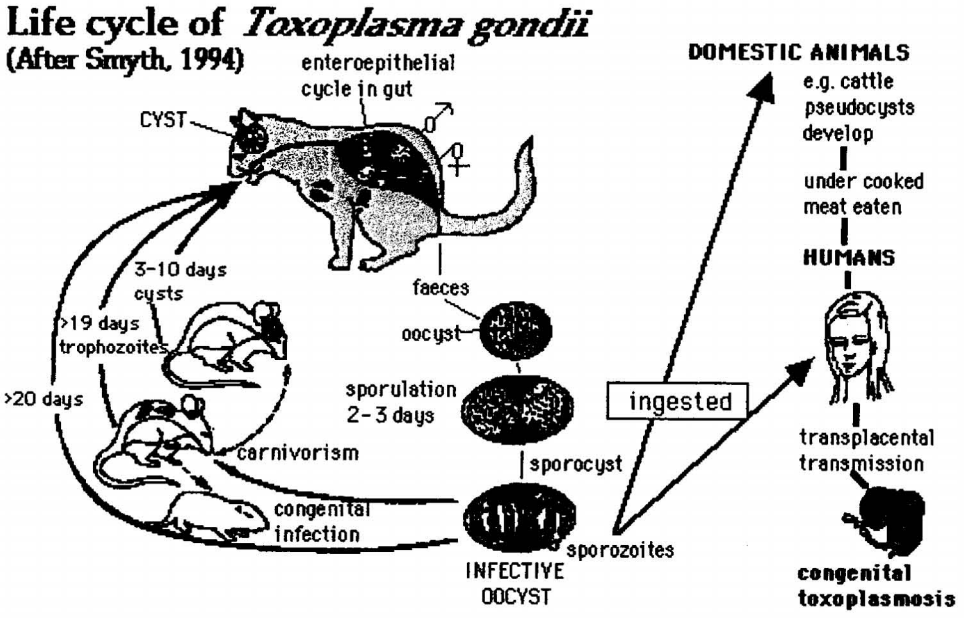
- *Toxoplasma gondii*
- Other diseases (Paravovirus B19, *T. pallidum, Varizella zoster*)
- Rubella Virus

- Cytomegalovirus (CMV)
- *Herpes simplex* Virus (HSV)

Toxoplasma gondii

Toxoplasma, an apicomplexan parasite, is and obligate intracellular parasite that has evolved a very different survival strategy from the extracellular trypanosomes. The life cycle of *Toxoplasma* is very complex but breaks down into two parts, one sexual and the other asexual.

The sexual cycle occurs exclusively in cats and is initiated when a cat eats an infected prey or accidentally ingests feces contaminated with oocysts. Following a typical process of gamete formation and fusion in the intestinal epithelium, the zygote is formed. This ultimately develops into an immature oocyst which, after being shed in the feces will mature into an extraordinarily resistant entity containing 8 sporozoites. The oocysts are highly infectious not only to other cats but to virtually any warm-blooded animal. Herbivorous grazing animals, of course, will be particularly susceptible but it's also found in strict carnivorous animals, as well. Once in the herbivore, the sporozoites are released from the oocyst in the intestine and invade the intestinal epithelium. There they differentiate into the rapidly dividing tachyzoite form which is capable of indefinite replication *in vivo* and *in vitro.* These disseminate through the host, infecting virtually any cell in any tissue. As the host's immune response rises to the challenge, the parasites encyst and differentiate to the very slowly dividing form, the bradyzoite. These are very stable and infectious if tissue from the animal is eaten.



The most serious problems occur in either of two situations. First, if a woman becomes infected for the first time during pregnancy, her fetus is at risk of severe neurological problems, even death. Second, if a chronically infected person develops AIDS or is immuno-suppressed for any other reason, the disease can reactivate with the quasi-latent tissue cysts releasing the bradyzoites which rapidly differentiate back to tachyzoites. The resulting disseminated infection is of greatest concern when it enters the brain where encephalitis can ensue.

According to Hohlfeld, P. *et al.*,[19] toxoplasma infection during pregnancy can cause congenital infection and manifest as mental retardation and blindness in the infant. The infant is infected transplacentally after the parasites invade the placenta. Once acquired, the latent encysted organism will persist for life in the host. Like congenital syphilis and perinatally acquired HIV infection, congenital toxoplasmosis is usually not apparent at birth. Boyer, K.M.[20] reported that 70 to 90% of the infants who appear normal at birth would develop significant clinical illness by young adulthood. These

infants develop choreoretinits that can lead to blindness, obstructive hydrocephalus, and intracranial calcifications that are associated with mental retardation, seizure activity, and motor and developmental delays.

In Europe, pregnant women are screened monthly as part of standard prenatal care as early as 18 weeks' gestation, by using polymerase chain reaction (PCR) amplification of the B1 gene of *Toxoplasma gondii* in a sample of amniotic fluid. Whether the fetus has actual organ damage is determined by serial ultrasound examinations.[20]

Early diagnosis is important because the disease is most severe in the fetus when the mother is acutely infected in the first trimester. However, the disease in the mother may be easily overlooked sometimes, as it is often asymptomatic. It may also be overlooked because the maternal physical findings, *e.g.,* fever, lymphadenopathy (swelling of one or more lymph nodes), headache, myalgia (muscle pain), stiff neck, and anorexia (decreased sensation of appetite), can easily be attributed to other more common infections.[13]

Others (*Treponema pallidum*, Varicella-Zoster Virus (VZV), Parvovirus B19, and Human Immunodeficiency Virus (HIV))

Treponema pallidum

According to Rawston,S, [21] congenital syphilis is caused by the transplacental transmission of the spirochete, *Treponema pallidum,* which has a 100% vertical transmission rate. Syphilis in the mother is characterized by three different stages: the primary stage, which is characterized by the appearance of the syphilitic chancre and lymphadenitis and the secondary

stage, which is the result of hematogenous dissemination. The newborn infant with congenital syphilis is considered to be in the secondary stage. During the tertiary stage which is either asymptomatic (late latent) or symptomatic (tertiary stage) neurological, cardiovascular, and gummatous lesions (granulomas of the skin and musculoskeletal system) are seen.

Syphilis is currently at its lowest incidence since reporting first began in 1941 and the decline in incidence of congenital syphilis is attributable mainly to mandatory serologic screening during pregnancy. [22]

Congenital syphilis which has classifications as early disease (seen in children before two years) and late disease (seen after two years) is more likely to be transmitted by women who are in the primary or secondary stages of the disease rather than in the latent phase. Azimi, P [23] reported 40% fetal or perinatal deaths in the patients without treatment. If not detected or/treated these live-born infants will display symptoms within weeks or months of birth.

Early manifestations of congenital infection involve multiple body systems.[24] Infants may display hemorrhagic nasal discharge, hepatosplenomegaly (simultaneous enlargement of both the liver and the spleen), jaundice, increased liver enzymes, lymphadenopathy (swelling of one or more lymph nodes), hemolytic anemia, thrombocytopenia (presence of relatively few platelets in blood), Osteochondritis dissecans (a loose piece of bone and cartilage separates from the end of the bone because of a loss of blood supply and insufficient amounts of calcium) and periostitis (the inflammation of the periosteum), mucocutanous rash, central nervous system (CNS) abnormalities, failure to thrive, choreoretinits (inflammation of the choroid and retina of the eye), nephritis (inflammation of the kidney), and

nephrotic syndrome. Late manifestations result primarily from chronic inflammation of bone, teeth, and CNS.

Serologic tests are the main means of diagnosis. VDRL (Venereal Disease Research Laboratory) and RPR (rapid plasma reagin) tests detect antibodies against cardiolipin. While not specific for syphilis, these "nontreponemal" tests are useful in its diagnosis because the quantitative results of these tests correlate with disease activity. Thus, they are practical for screening purposes[21]

Varicella-Zoster Virus (VZV)

Varicella infection in the pregnant woman can cause severe consequences for both the mother and infant. Infection of the fetus in early pregnancy results in congenital varicella syndrome; transplacental infection at delivery results in neonatal varicella. Interestingly, recurrent VZV infection does not cause congenital varicella syndrome or neonatal varicella.[13] But because most adults at present are immune as a result of childhood illness, the incidence of maternal varicella is low in United States.[25] The clinical experience in Japan, which is now being substantiated by long-term studies in the United States, indicates that the immunity persists for prolonged periods after childhood immunization.[26]

Arvin, A[27] reported that about 25% of fetuses become infected when the mother has varicella but not every infected fetus is clinically affected. Infants infected later in pregnancy have less frequent and less severe involvement.

The anomalies associated with congenital varicella syndrome involve many organ systems. There are unusual cutaneous defects, with cicatrical skin

scars, atrophy of extremities, and evidence of damage to the central nervous system.[28]

Parvovirus B19

Most commonly, parvovirus B19 causes the childhood viral exanthem erythema infectiosum (Fifth disease or "Slapped cheek disease") that was first associated with the virus in 1983. By the age of 15 years, 50% of adolescents have detectable IgG antibodies to the virus.[29] Transmission is airborne or by droplet spread. Most infections in adulthood are asymptomatic or mild causing a subtle rash and arthralgia. Maternal infection during pregnancy can result in miscarriage or the development of nonimmune hydrops fetalis. The pathogenesis in the fetus is cardiac failure as a result of profound anemia caused by virus-induced arrest of red cell production. The risk of transplacental infection is about 30% and the risk of fetal loss about 9%, primarily in the second trimester. Chronic congenital infection with perinatal sequelae is rare.[30] The best way to diagnose B19 infection in the fetus is the demonstration of viral DNA in amniotic fluid, fetal blood, or tissues by PCR method.

Rubella Virus

A benign self-limited viral illness, rubella, is characterized by an exanthem and posterior cervical lymphadenopathy. Much of its significant morbidity is secondary to the effects of the illness when contracted by the fetus in utero. The congenital rubella syndrome that was so common before 1969 included growth retardation, deafness, congenital heart disease, and mental retardation. Since the availability of mass immunization, the reported incidence of rubella has dropped from 57,686 cases in 1969 to 200 to 400 cases from 1992 to 1998.[31] Although protection of women of childbearing age

is the goal of all immunization strategies, serological surveys in the United States continue to document 10 to 20% susceptibility in this population.

The gestational age at the time of infection determines the intensity of fetal involvement. Up to 20% of maternal infections occurring in the first eight weeks' of gestation result in miscarriage, spontaneous abortion, or stillbirth. Those fetuses infected before 11 weeks have multiple organ damage while those after 11 to 12 weeks are more likely to have only deafness and/or retinopathy. Fetal damage rarely occurs after 16 weeks' of gestation.[32]

Clinical manifestations can be evident at birth but more commonly result in a 'normal' newborn with late-onset sequelae. Early clinical manifestations can be transient or progressive. Transient early clinical manifestations of congenital rubella include generalized lymphadenopathy, hepatosplenomegaly, intrauterine growth restriction, hepatitis, jaundice, thrombocytopenic purpura (low platelet count), with petechiae (small red or purple spot on the body, caused by a minor hemorrhage) and 'blueberry muffin' lesions. These transient manifestations resolve in days or weeks usually without long-term sequelae. The most common permanent problems seen are sensorineural deafness, cataracts, peripheral pulmonary stenosis, mental retardation, central language defects, diabetes mellitus type 1, and hypogammaglobulinemia (a type of immune deficiency, where the number of gamma globulins is greatly reduced).[33]

The infant should be evaluated for congenital rubella if there is a maternal history of rubella during the pregnancy or neonatal manifestations suggestive of a congenital infection, such as thrombocytopenic purpura or cataracts. Diagnosis of congenital rubella requires virologic or serologic confirmation. The virus can be isolated for up to one year or more from the

nasopharynx, cerebrospinal fluid (CSF), urine, and buffy coat of the blood. Serologic confirmation is difficult. Although rubella-specific IgM can be measured in cord blood or neonatal serum, it is often associated with false positive and false negative results. It is recommended that serial measurements of IgG specific antibodies be done at three and six months to document persisting high antibody levels. Presence of rubella-specific hemagglutination inhibition (HAI) or enzyme immunoassay antibodies after nine months of age is diagnostic of congenital infection.[33]

The prevention of congenital rubella obviously is dependent upon adequate early immunization, resulting in a high prevalence of immunity in women of childbearing age. Women should be screened for rubella immunity at the beginning of pregnancy and immunization is recommended for seronegative women immediately after delivery.[34] Although inadvertent immunization of pregnant women has not resulted in fetal abnormality, postpartum immunization is considered safe.

Cytomegalovirus (CMV)

Currently CMV is the most common cause of congenital infection in the United States.[35] Infected infants of 10 to 20 % may suffer from sensorineural hearing loss, ocular damage, or impairment of cognitive and motor function. CMV is common in all socioeconomic groups but congenital infection with significant impairment is seen at highest rates in populations in which pregnant women have the highest risk of acquiring primary infection. In addition to the transplacental route, CMV can be transmitted at delivery via the maternal genital tract, during the postpartum period in breast milk, and in transfused blood products. CMV is easily spread in daycare centers and in families with young children. The organism can cause significant illness by

endogenous reactivation among immunosuppressed individuals, including transplant recipients.

According to Pass, R[36] approximately 40% of maternal primary infections are transmitted to the fetus. The likelihood of transmission is similar early as well as late in gestation. However, first trimester primary maternal infection is more likely to cause neonatal infection, which is evident at birth, and more likely to result in severe sequelae such as deafness and mental retardation. Transmission of CMV from the mother to fetus can occur even if the mother was infected long before conception. However, maternal infections that result from reactivation of the virus usually cause only asymptomatic viral shedding in the infant.

Congenitally infected infants are often divided into two groups: those with findings that are apparent in the neonatal period and those with signs of CNS damage that become apparent later in childhood. Those symptomatic at birth are most compromised. In addition to intrauterine growth restriction, over 70% have evidence of CNS involvement: microcephaly, lethargy, hypotonia, optic atrophy, decreased hearing, and intracranial calcifications. Such infants have a mortality rate of 12% by six months of age. Of infants who are asymptomatic at birth, 10 to 20% eventually will have CNS involvement. Congenital CMV is said to be the second leading cause of mental retardation in the United States and is currently the leading cause of sensorineural deafness. Hearing loss secondary to congenital CMV is progressive in childhood; even those with normal hearing at birth can develop hearing loss later.[37]

Transmission of the virus requires direct contact with bodily fluids. Hand washing and other preventive hygienic measures can decrease spread in

daycare centers and at home. Pre-pregnancy titers can also identify at-risk women. In the future, a CMV vaccine currently being evaluated in young adults may be of use.[38]

Herpes simplex Virus

Though herpes simplex infections were recognized by the ancient Greeks, the association between herpes simplex type 2 as a cause of neonatal disease and genital herpes was not made until the 1960s.[39] The recent development of antiviral therapy enables the reduction of mortality and morbidity. As herpes simplex is most often acquired during delivery rather than during gestation, it is more preventable and treatable compared with CMV or rubella. Like the varicella-zoster virus, the herpes simplex virus can persist in the latent state, resurfacing at any time during the individual's life span.

While there have been fluctuations in incidence of the disease, it is estimated that there are approximately 30 cases/100,000 live births in the United States. It is interesting that neonatal infection occurs far less frequently than might be expected given the high prevalence (1/5) of seropositivity to HSV-2 in childbearing women.[40] The infant attack rate among women with primary infection is 33 to 50%, while those with recurrent disease only show an attack rate of 1 to 3%.[41] Unfortunately, only 15 to 20% of women whose infants develop neonatal herpes infection have a history of symptomatic disease and only 25% have relevant symptoms at delivery.[40]

Perinatal infection manifests itself during the first month of life with 9% of infections occurring on day one and 40% by the end of the first week of life. There are three major categories seen: (1) localized skin, eye and mouth

infection; (2) encephalitis with or without skin, eye, and mouth disease; and (3) disseminated infection.[42] Infants with disseminated disease have the worst prognosis. Many are born to mothers with a new herpes infection who have not developed or passively transferred antibodies against the virus to the infant. Multiple organs are involved and initial signs and symptoms include irritability, seizures, respiratory distress, jaundice, bleeding diathesis, and shock.

Arvin, A. & Whitley, R[40] pointed out that the diagnosis of disseminated disease is difficult because symptoms are vague, nonspecific, and similar to those of bacterial sepsis or enteroviral infections. Nearly one-third of all infants with neonatal herpes have only encephalitis as the initial manifestation. As with disseminated disease, not all of these infants display the characteristic vesicular exanthem. Infection localized to the skin, eye, and/or mouth may seem benign but, in the absence of treatment, this onset is often associated with the subsequent development of the more serious disease manifestations. Skin vesicles are noted in 90% and keratoconjunctivitis (inflammation ("itis") of the cornea and conjunctiva) may be evident with eye involvement. Long-term neurological impairment is frequent in children who have recovered from encephalitis.

Other Maternal Infections

Bacterial vaginosis

Bacterial vaginosis, previously known as nonspecific vaginitis or Gardnerella vaginitis, is the most common cause of vaginal discharge. It may be the cause of up to one half of cases of vaginitis [43] in all women and the cause of from 10 to 30 percent of cases in pregnant women.[44] This clinical

syndrome is now recognized as a polymicrobial superficial vaginal infection involving a loss of the normal lactobacilli and an overgrowth of anaerobes. While commonly found in increased numbers in women with bacterial vaginosis, *Gardnerella vaginalis* is not invariably present. *G. vaginalis* has been reported in from 16 to 42 percent of women with no signs or symptoms of vaginitis.[45] Morbidity associated with bacterial vaginosis in pregnant women are amniotic fluid infection, clinical chorioamnionitis, postpartum endometritis, premature rupture of the membranes, preterm delivery and low birth weight.[46]

Urinary Tract Infection (UTI)

The organisms that cause UTIs during pregnancy are the same as those found in nonpregnant patients. If untreated, 40% of the asymptomatic bacteriura (ASB), will develop a symptomatic UTI. *Escherichia coli* accounts for 80 to 90 percent of infections. Other gram-negative rods such as *Proteus mirabilis* and *Klebsiella pneumoniae* are also common. Gram-positive organisms such as group B streptococcus and *Staphylococcus saprophyticus* are less common causes of UTI. Group B streptococcus has important implications in the management of pregnancy. Less common organisms that may cause UTI include enterococci, *Gardnerella vaginalis* and *Ureaplasma ureolyticum.*[47] Fetal risks associated with a UTI include abortion, preterm labour, low birth weight (LBW), fetal infection and perinatal death. Selective screening of pregnant women for ASB is recommended at the first antenatal visit.[48]

Other Maternal Factors

Out of several pregnancy related maternal complications, a few important ones worth mentioning here are hypertension, pre-eclampsia and

oligohydramnions etc. Hypertensive disorders of pregnancy result in 12% of maternal deaths globally, and up to 40% of maternal deaths in some countries. These conditions can also influence the health of the fetus or newborn and are responsible for up to 13% of stillbirths and 20% of early neonatal deaths in some areas of the world. The World Health Organization (WHO) estimates that 15% of women will have some degree of hypertension during pregnancy. Fortunately, most of these cases are benign and do not require treatment or result in complications. In some cases, however, the woman has a hypertensive disorder of pregnancy such as preeclampsia (hypertensive disorder of pregnancy with associated protein loss in the urine) and eclampsia, (serious complication of pregnancy characterised by convulsions) which can lead to serious complications or death. [49].

Pregnancy Induced Hypertension is also reported to have role in causing cerebral, cardiac, and renal complications, stillbirths and abruptio placentae in the mother. In the fetus, intrauterine growth retardation and hypoxia (lack of oxygen in tissues) due to superimposed pregnancy-induced hypertension are common. Managing hypertension during pregnancy is one of the most controversial areas of therapy in obstetric practice.[50]

Oligohydramnios and polyhydramnios are found to be causing problems for mother and baby. The amniotic fluid that surrounds the baby in the uterus is a clear-colored liquid cushion and protects the baby, provides it with fluids and is crucial in normal development. The baby breathes this fluid into its lungs and swallows it; this helps promote the healthy growth of the lungs and gastrointestinal tract. Amniotic fluid also helps the baby move around, aiding in normal development of muscle and bone. The amount of amniotic fluid increases until about 28-32 weeks of pregnancy. The level stays about the same until about 38 to 40 weeks, i.e. during full term

pregnancy. After that, the level begins to decrease. In some pregnancies, there may be too little or too much amniotic fluid. These conditions are referred to as oligohydramnios and polyhydraminos respectively. Both can sometimes cause problems for mother and baby, or be a sign of other problems. In the majority of cases, however, the baby is born healthy. The level of the amniotic fluid is measured in terms of amniotic fluid index (AFI). This is done using ultrasonography. If the amniotic fluid depth is less than 5 centimeters (cm), it is called oligohydramnios. If the depth measures greater than 25 cm, the condition is called polyhydramnios.

About 8 percent of pregnant women all over the world have too little amniotic fluid. Oligohydramnios can develop at any time during pregnancy, though it is most common in the last trimester. Oligohydramnios that occurs in the first half of pregnancy is more likely to have serious consequences than if it occurs in the last trimester. Too little amniotic fluid early in pregnancy can compress fetal organs and cause birth defects, such as lung and limb defects. Oligohydramnios that develops in the first half of pregnancy also increases the risk of miscarriage, preterm birth and stillbirth. When oligohydramnios occurs in the second half of pregnancy, it may be associated with poor fetal growth. Women with oligohydramnios are more likely than unaffected women to need a cesarean delivery.

Relevance of the Study

Although pregnancy-related complications continue to take a huge toll on the lives of women and newborns, and despite a series of programmatic initiatives, there is little evidence that maternity has become significantly safer over the last 20 years. The links between pregnancy-related care and maternal mortality are well recognized. All pregnancies should be evaluated well at correct stage since many risk factors are involved in pregnancy. High risk pregnancies should receive extra attention and is to be referred to perinatal centers before delivery, thereby significantly decreasing neonatal morbidity and mortality rates. Proper antenatal care ensures at the end of pregnancy, a healthy mother and a healthy baby, but the coverage of antenatal care in India remains inadequate.

There are several factors, both maternal and others that influence pregnancy at various stages. The maternal health and the uterine environment have a good say in deciding the successful birth of a healthy infant. Infections by intrauterine pathogens have been found as significant in causing various pregnancy related complications all over the world, in various degrees. Similar studies conducted in India are reported as very few. In Kerala, especially in the northern region, it is doubtful whether such an attempt has ever been made, other than the individual screening for one or the other of these pathogens like Rubella etc, when the pregnant mother sometimes is

suspected to have exposed to it, in order to rule out the doubt. Hence this study focuses on the incidence of intrauterine infections by TORCH pathogens and their role in causing common pregnancy associated complications like miscarriage, IUGR, IUD and congenital malformations among the pregnant women of Malabar area of Kerala state, where childhood marriages and teenage pregnancy have been represented as a common phenomenon. Parameters like PIH, Oligohydramnios, parity, gravida, age, diabetes, other factors that may contribute to pregnancy complications were also considered to eliminate their possible role.

Knowledge of the role of the intrauterine pathogens in causing pregnancy related complications is expected to contribute to the future pregnancy care in the area.

PREVALENCE OF TORCH INFECTIONS IN PREGNANCY RELATED COMPLICATIONS IN THE MALABAR REGION OF KERALA

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Review of Literature

Complicated Pregnancy

Most pregnant women experience only minor complications of pregnancy, but for a minority more serious problems occur. In extreme cases, these can result in the death of the baby and, very rarely in developed countries, that of the mother. Problems range from failure of the embryo to implant in the womb, leading to miscarriage, to rupture of the placenta and pre-eclampsia (pregnancy induced hypertension). The fetus can also develop problems in the womb, including genetic defects. [40]

Varying rates of maternal mortality ranging from 280-1360 per 100,000 live births were reported by different workers from northern parts of India. Kumar, R [51] reported major causes of maternal mortality as antepartum and postpartum hemorrhage (18.2%), puerperal sepsis (16.4%), severe anemia (16.4%), abortion (9.1%) and obstructed labor (7.3%). The largest number of deaths was in poor socioeconomic class as per this study. The majority of the deaths occurred in the 20-29 age group.

In another study conducted in the southern part of India, the mortality rate was still higher (1.67%) [52]. A report from Pondicherry shows that the septic abortions were the cause of 30.2% of the maternal mortality [53]. Ectopic pregnancy where the fertilised egg becomes embedded outside the womb has been increasing steadily in the UK in recent years. The reasons for ectopic

pregnancy are unclear, but research suggests damage to fallopian tubes caused by infections, such as sexually transmitted diseases, could be a factor.[52]

Miscarriage

In a study to establish the risk of spontaneous abortion and the obstetric factors predisposing to it Regan in 1989 observed that knowledge of the patient's reproductive history is essential for the clinical assessment of her risk of spontaneous abortion. As the most important predictive factor for spontaneous abortion is a previous abortion, the outcome of a woman's first pregnancy has profound consequences for all subsequent pregnancies.[54]

In 1996 a study on couples practicing natural family planning for conception or contraception, information on clinical evidence of infection was gathered beginning with week 5 of gestation. Frequencies of urinary, vaginal and other infections in subjects experiencing pregnancy loss were 11.1, 9.5 and 8.7% respectively, not significantly different from rates in subjects having liveborns which were 10.1, 10.2 and 10.3% respectively. Thus, no association between clinical infection and early, first trimester pregnancy loss was observed.[55]

Miscarriage was a significant risk factor for the acquisition of Hepatitis C virus (HCV) infection from the first miscarriage up to the fifth, the risk increasing with increasing number of miscarriages, according to Al-Kubaisy.[56] Sera from 3491 pregnant women were screened for the presence of anti HCV antibodies. Anti-HCV seroprevalence (3.21%) was significantly positively correlated with the number of miscarriages. A higher proportion of women with a history of miscarriage harbored HCV-Ab compared to those with no miscarriage.

Bujko [57] conducted a retrospective clinical investigation of 45 women in the first and second trimester of pregnancy aiming to demonstrate the role of genital HSV infection on spontaneous abortion. The results showed that the highest incidence of latent HSV type 2 infection (64%) occurred in women who had one or more spontaneous abortions, whereas this type of infection was found in only 5% of pregnant women of the control group. The incidence of asymptomatic cervical HSV type 2 infection was also considerably higher in patients with a history of spontaneous abortions.

Herpes simplex virus type 2 antigen was detected in nonpregnant and pregnant endometria, placentae, umbilical cords, and neonatal tissues. Placental HSV positivity was significantly correlated with spontaneous (39 %) versus therapeutic (14 %) abortions and with blighted ova (67 %). No significant correlation was found between HSV positivity and a clinical history of oral or genital HSV infection in either the patient or the male partner.[58]

Five hundred and twenty-three pregnant women were screened in a study to determine whether a relationship existed between spontaneous abortion, and the presence of antibody to *Toxoplasma gondii.* The percentage of women with this antibody and a past history of spontaneous miscarriage was not statistically different from the percentage of women without antibody and a past history of spontaneous abortion.[59] In another study by Lolis, D., *et al.,* [60] no correlation was found between antibody titers and IgG, IgM or IgA levels. This study concludes that toxoplasmosis should be considered as the cause of abortion when a patient's antibody titer exceeds 1:256.

Intrauterine Growth Retardation (IUGR)

It was in 1919 it was first suggested [61] that all newborns weighing less than 2,500 g should be classified as 'premature.' While in 1961 World Health Organization (WHO) acknowledged that many infants defined as 'premature' were not born early but were simply of 'low birth weight'.[62] The current WHO criterion for low birth weight is a weight less than 2,500g or below the 10th percentile for gestational age[6,63-65]. Low birth weight includes two pathologic conditions and one normal condition. The normal condition refers to the healthy but constitutionally small baby. The pathologic conditions include preterm delivery and intrauterine growth retardation (IUGR). Synonymous terms found in the literature to describe infants with IUGR include intrauterine growth restriction and fetal growth retardation. In the United States, IUGR is linked to an increase of six to 10 times in perinatal mortality.[63]

Fetal growth restriction is the second leading cause of perinatal morbidity and mortality, followed only by prematurity.[61] The incidence of IUGR is estimated to be approximately 5 percent in the general obstetric population.

While assessing the perinatal outcome by weight, infants who weigh less than 2,500 g (5 lb, 8 oz) at term have a perinatal mortality rate that is 5 to 30 times greater than that of infants whose birth weights are at the 50th percentile. The mortality rate is 70 to 100 times higher in infants who weigh less than 1,500 g (3 lb, 5 oz).[64] Perinatal asphyxia involving multiple organ systems is one of the most significant problems in growth-restricted infants.[66]

Fetal growth is dependent on genetic, placental and maternal factors. The fetus is thought to have an inherent growth potential that, under normal circumstances, yields a healthy newborn of appropriate size. There are many possible causes of IUGR which includes both intrinsic and environmental factors.[64]

Table 1: Conditions Associated with Intrauterine Growth Retardation [64]

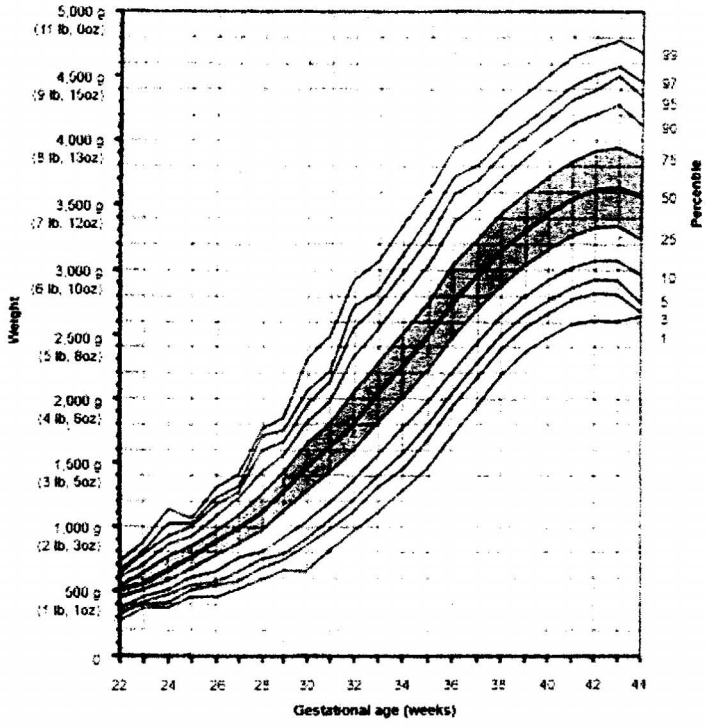
Medical	**Maternal**	**Infectious**
Chronic hypertension	Smoking	Syphilis
Preeclampsia early in gestation	Alcohol use	Cytomegalovirus
Diabetes mellitus	Cocaine use	Toxoplasmosis
Systemic lupus erythematosus	Warfarin (Coumadin, Panwarfin)	Rubella
Chronic renal disease	Phenytoin (Dilantin)	Hepatitis B
Inflammatory bowel disease	Malnutrition	HSV-1 or HSV-2
Severe hypoxic lung disease	Prior history of pregnancy with intratuterine growth retardation	HIV-1
	Residing at altitude above 5,000 feet	**Congenital**
		Trisomy 21
		Trisomy 18
		Trisomy 13
		Turner's syndrome

IUGR may result in significant fetal morbidity and mortality if not properly diagnosed. The condition is most commonly caused by inadequate maternal-fetal circulation, with a resultant decrease in fetal growth. Less common causes include intrauterine infections such as cytomegalovirus and rubella, and congenital anomalies such as trisomy 21 and trisomy 18.[65]

Timely diagnosis and management of IUGR is one of the major achievements in contemporary obstetrics. If the growth-restricted fetus is

identified and appropriate management instituted, perinatal mortality can be reduced,[67,68] underscoring the need for assessment of fetal growth at each prenatal visit.

Fetal weight percentiles throughout gestation [64]



The causes of IUGR are multiple, involving many different factors. Maternal environment is the most important determinant of newborn weight. In addition to a direct relationship with the degree of maternal plasma volume expansion, many clinical factors associated with IUGR include multiple gestation; fetal, genetic, and chromosomal anomalies (Down's syndrome and Turner's syndrome); infections such as TORCH syndrome (acronym for toxoplasmosis, rubella, cytomegalic disease, and herpes). Besides these various maternal disorders including anemia, severe chronic asthma, chronic renal disease, heart disease and hypertension and maternal stress factors, like narcotic addiction, cigarette smoking and chronic alcoholism, are associated

with IUGR along with placental anomalies, poor nutritional status of the mother etc.[69] During World War II, a population of women in Leningrad who underwent prolonged malnutrition delivered infants with an average birth weight of 400 to 600 g (14 to 21 oz) less than expected.[70]

Maternal causes of IUGR account for most uteroplacental cases. Chronic hypertension is the most common cause of IUGR. Moreover, the infants of hypertensive mothers have a three-fold increase in perinatal mortality compared with infants with IUGR who are born of normotensive mothers.[71]

Preeclampsia causes placental damage that result in uteroplacental insufficiency. The pathogenic mechanism is thought to be a failure of trophoblastic invasion by maternal spiral arterioles by 20 to 22 weeks of gestation.[61] This failure causes luminal narrowing and medial degeneration, leading to diminished blood flow to the developing infant. Consequently, these infants fail to grow normally.

In a later study of Guatemalan Indians, Lechtig, A *et al.*,[72] found that protein malnutrition occurring before 26 weeks of gestation resulted in IUGR. The study concludes that moderate protein-calorie malnutrition during pregnancy leads to lower placental weight which may be the mechanism by which maternal malnutrition is associated with high prevalence of low-birth-weight babies in these populations. The current consensus is that a maternal weight gain of less than 10 kg (22 lb) by 40 weeks of gestation is clearly a risk factor for IUGR.

Maternal smoking may be the cause of 30 to 40 percent of U.S. cases of IUGR. Dougherty, C.R. & Jones, A.D [73] in 1982 found a dose-dependent decrease in fetal weight with an increasing number of cigarettes smoked each

day (a 7.4 g decrease for each cigarette smoked per day). The results also show that the sex of the baby, parity, maternal smoking during the pregnancy, maternal height, weight, marital status, and race, and gestation were all important and significant factors affecting IUGR. Another study [74] found that women who smoked 11 or more cigarettes daily had infants weighing 330 g (11.5 oz) less than predicted and measuring 1.2 cm shorter than control subjects. Early use of alcohol by the pregnant mother may lead to fetal alcohol syndrome, while second- or third-trimester use may result in IUGR. As little as one to two drinks per day have been shown to result in a growth delayed child.[75] Not surprisingly, maternal cocaine use has been linked to IUGR, as well as to reduced head circumference. Other drugs associated with IUGR include steroids, warfarin and phenytoin.

IUGR occurs 10 times more frequently in twin deliveries than in single gestations. The incidence of IUGR in twins is about 15 to 25 percent.[70] Reasons for IUGR in twin pregnancies include poor placental implantation, placental crowding and twin-to-twin transfusion.

Infectious causes of fetal growth delay account for about 10 percent of all cases of IUGR. These causes include the "TORCH" group: *Toxoplasma gondii*, Rubella virus, Cytomegalovirus and *Herpes simplex* virus types 1 and 2. Other potential pathogens include Hepatitis A and Hepatitis B, Parvovirus B19, Human immunodeficiency virus and *Treponema pallidum.* [76]

Shi, D.Z in 1992 investigated the relationship between intrauterine infection and IUGR by measuring the specific antibodies of Toxoplasma and Cytomegalovirus in the umbilical serum samples from 30 cases of IUGR with 26 normal newborn infants as controls and followed-up the 14 positive cases for 17-25 months. The results showed that in 30 IUGR cases 14 were found to

have Toxoplasma and CMV infection, with an incidence significantly higher than that in the control group. One third of the infected patients were found to have retarded growth afterbirth and 63.63% of them appeared to have an increase in brainstem auditory evoked potentials (BAEP). This suggested that regular follow-up is indicated in these cases.[77]

Neonatal infections are frequent complications of extremely low-birth-weight (ELBW) infants receiving intensive care. A study conducted by Stoll, B.J[78] suggests that neonatal infections among ELBW infants are associated with poor neurodevelopmental and growth outcomes in early childhood. Hodgman in 2003 conducted a study to determine the causes of neonatal death for extremely-low-birth-weight infants. Out of 263 ELBW infants born alive, 104 (40%) died and 44 (42%) were autopsied. Infection was the leading cause of death in 57% in the autopsied babies. Sixteen (64%) of these deaths occurred within the first 48 h and were classified as being due to congenital infections. Infection as a cause of death peaked at 22 weeks. Other causes of death were lethal anomalies (20%), respiratory distress and its complications (9%) and immaturity, intraventricular hemorrhage and other conditions (14%)[79]. He concluded that congenital infections are leading cause of neonatal deaths in ELBW babies.

In addition to seroprevalence and transmission rate, the clinical symptoms of postnatal CMV infection in infants with a very low birth weight (<1500 g) were assessed in a 3-year prospective study. Of 170 infants, no CMV transmission was found in the 80 infants of seronegative mothers and in the 3 infants of seropositive mothers. Transmission occurred in 33 of the 87 CMV-exposed infants. Low birth weight and early postnatal virus transmission were risk factors for symptomatic infection. Very low birth

weight infants of CMV-seropositive mothers are at high risk of acquiring a symptomatic CMV infection postnatally via breast milk.[80]

Due to constraints in conducting specific tests for diagnosis of perinatal infection, Chaturvedi in 1989 [81] carried out a study to find out the value of cord serum IgM levels as a screening procedure for intrauterine infection in full term intrauterine growth retarded babies. In the study population both mean cord serum IgM (26.8 mg/dl) and mean maternal serum IgM (142.42 mg/dl) were raised as compared to the mean cord serum IgM (13.76 mg/dl) and mean maternal serum IgM (100.16 mg/dl) of the control group. However, statistically the rise was significant only between the maternal and cord serum IgM levels. But all the same, cord serum IgM levels exceeding 20 mg/dl and 30 mg/dl were found in 51.43 and 22.8% of full term IUGR neonates, respectively whereas among the control neonates only 20.0% had levels exceeding 20 mg/dl and none had levels above 30 mg/dl suggesting possible intrauterine antigenic challenge to perinatal infection in higher proportion of IUGR babies. Cord serum IgM levels were also seen to increase with increase in birth weight.

Many infants with IUGR are screened for TORCH infections. Khan, N.A. and Kazzi, S.N[17] are of opinion that the yield and costs of such a practice is not be justifiable. Seventy-five out of 182 infants (41%) with IUGR had a workup for TORCH infection. Maternal conditions associated with IUGR included: pregnancy-induced hypertension (19%), tobacco use (43%), alcohol abuse (21%), illicit drug use (24%), chronic hypertension, diabetic vasculopathy or collagen vascular disease (12%), and multiple gestation (3%). Placental pathology was available in 53/75 cases. Thirty-six of fifty-three (67%) placentae had abnormalities associated with IUGR. None

of the infants had positive IgM titers for Toxoplasma, Rubella, CMV, or HSV.

Intrauterine Fetal Death (IUD)

In a report dealing with autopsy studies in 93 still births at the Korle Bu Teaching Hospital, Accra, fetal infections were found as responsible for 13% of deaths and maternal pathologic conditions for 10.8%. The commonest maternal condition causing stillbirth was severe pre-eclampsia. Death was due to fatal birth trauma in 5.4% of cases with lethal congenital malformations and placental growth retardation causing 3.2% of deaths each [82].

In another study conducted to investigate the role of intrauterine infection as a cause for unexplained stillbirth, 117 stillbirth cases and 126 controls were studied from a region in the south Sweden. Chorioamnionitis (an inflammation of the chorion and amnion which causes of brain injuries in newborns) was found as a common diagnosis with 82% stillbirths and 68% 'healthy' deliveries. Extension of the inflammation to decidua basalis was seven times more common among stillbirths than among controls. The most common bacteria found at cultures were *Escherichia coli*, coagulase negative Staphylococcus, *Enterococcus faecalis* and group B Streptococcus. The risk for stillbirth was doubled if both inflammation and bacteria were present. These findings were similar to the results from studies in developing countries except for the higher incidence of stillbirths in such countries. Thus, a large part of otherwise unexplained stillbirths might be due to ascending infections.[83]

In a study conducted in Zimbabwe,[84] with a view to elucidate whether microbial infections are involved in the etiology of Intrauterine Fetal Death,

104 cases of stillbirth of unknown etiology and 96 age- and parity-matched referents with live births were analyzed with respect to microbial infection by cultures and by serology for bacteria, viruses and *Toxoplasma gondii.* Tests for *Treponema pallidum* and *Toxoplasma gondii* were more frequently positive in cases than in referents. This study also reports that there was no increased risk for Intrauterine Fetal Death in women with human immunodeficiency virus, cytomegalovirus, *Herpes simplex* virus or rubella virus. Their findings indicate that infections remain an important cause of intrauterine fetal death in Zimbabwe.

In Sweden, between 6% and 20% of fetal deaths are ascribed to infectious diseases, and a substantial number of these are associated with maternal viral infections. Tissue examination using PCR for parvovirus B19 DNA, cytomegalovirus DNA, and enterovirus RNA and serologic studies for viral infection were done by Petersson, K *et al.,*.[85] The incidence of intrauterine fetal death during the study period was 0.52%. The investigators recommend that PCR studies of placental and/or fetal tissue for parvovirus B19, CMV, and enterovirus be included in the routine analysis of fetal deaths.

Another study was conducted to estimate the incidence of the infections with paravovirus B19, CMV and enterovirus by the same authors. They concluded that Parvovirus B19, CMV and enterovirus may be considered as etiologic agents in cases of fetal death [86].

Congenital Disorders

Cataract is responsible for about 10% blindness among children in India and etiology of the condition is not well defined especially for childhood cataracts of this country. In a study that was performed to survey

the causes of childhood cataracts and to identify the preventable factors in four western states of India, out of 172 children, 88.4% had non-traumatic cataract and 11.6% had traumatic cataracts. Among non-traumatic cataracts, 4.6% were due to congenital rubella syndrome.[87]

In another study [88] conducted among 366 children of non-traumatic cataract to identify the causes of childhood cataract in south India with emphasis on factors that might be potentially preventable, 25% were hereditary, 15% were due to congenital rubella syndrome (CRS), and 51% were undetermined. In children under 1 year of age 25% were due to rubella and cataract of nuclear morphology had a 75% positive predictive value for CRS. Nearly half of non-traumatic cataract in south India is due to potentially preventable causes (CRS and autosomal dominant disease)[89]. Another study described the role of CMV infection, which is a well recognized cause of congenital hydrocephalus, in the associated flexion deformities of the thumbs and great toes.

Intrauterine infection is an important cause of some birth defects worldwide. The most common pathogens include rubella virus, CMV, Toxoplasma, etc. The infections caused by Rubella Virus, CMV, Toxoplasma, etc. are common, yet they are proved to be fatal during the pregnant period, especially during the first trimester. These infections may cause sterility, abortion, stillbirth, low birth weight, and affect multiple organs that may induce loss of hearing and vision, even fetal deformity and the long-term effects. These pathogens' infections may influence the microenvironment of placenta, including levels of enzymes and cytokines, and affect chondriosome that may induce the progress of birth defect. Early diagnosis of infections during pregnancy should be strengthened. There are still many things to be settled, such as the molecular mechanisms of birth defects, the effective

vaccines to certain pathogens. Birth defect researches in terms of etiology and the development of applicable and sensitive pathogen detection technology and methods are imperative.[90]

The relationship of TORCH infections with congenital heart disease (CHD) was explored in a study. The workers conducted comparison to detection TORCH pathogenic gene in 42 cases of CHD and 38 controls by polymerase chain reaction. The positive rate of CMV, HSV2 and Toxoplasma in 66 cases of CHD were as 26.2%, 4.7% and 16.7%, respectively, while those of 38 cases in control groups were 21.1%, 2.6% and 2.6%, respectively. The infection condition of Toxoplasma was significantly different, while those of CMV and HSV were not statistically different between CHD and control groups. This is the first report of the presence of TORCH genes in CHD cardiac tissue by PCR. It might lay the foundation for studying the relationship of TORCH infection with CHD in molecular level.[91]

Children between 0-6 years of age from six villages of Ambala District were screened for congenital malformations. Of 1371 children, malformations were observed in 30. Twenty children had major malformations and six had multiple anomalies. Cardiovascular malformations were the commonest (37%) followed by musculoskeletal (30%), gastrointestinal (23%), central nervous system (13%) and genitourinary anomalies (6.6%). An etiological factor like maternal rubella infection or drug exposure during early pregnancy could be ascertained in only 3 cases.[92]

A case is reported of a term newborn with intra uterine growth retardation and numerous malformations such as complex heart disease, abnormalities of distal limbs, cleft palate. Death of the infant occurred after two days. The diagnosis of rubella embryopathy was confirmed by the criteria

a high level of rubella antibodies in mother and newborn, and an isolation of rubella virus from the infant's urine. Diagnosis of rubella after reinfection was documented by a high level of antibodies in the mother three years before this pregnancy. Other observations reported in literature confirm the extreme rarity of congenital rubella after reinfection.[93]

The incidence of rubella infection and prevalence of IgG and IgM against the pathogen in pre-pregnancy and pregnancy within 3 months in China were determined in a study. Rubella IgG and IgM were measured by enzyme-linked immunosorbent assay. The overall number of birth defects was 102 (16.35%) with 62 males (18.55%) and 40 females (13.9%). The types of birth defects were neural tube defect, 44 cases (7.07%); limb defects, 13 (2.09%); cleft lip and palate, 5 (0.8%); and Down syndrome, 3 (0.48%). Low birth weight occurred in 31 cases (4.98%). The death rate from birth defects was 48 cases (47.06%). The positive rates of IgM and IgG of rubella infection of prepregnant women were, respectively, 5.02% and 83.78%; in pregnant women they were, respectively, 2.41% and 86.33%. The seropositive rate of rubella infection of pre-pregnancy was 88.80%; that of pregnancy was 88.74%.[94]

A study was conducted to identify congenital rubella syndrome cases in Ghana,[95] where rubella immunization is not available. Eighteen infants born within a 5-month period met the congenital rubella syndrome case definitions, coinciding with a 9-fold increase in presentation of infantile congenital cataract. The congenital rubella syndrome rate for this otherwise unrecorded rubella epidemic was conservatively estimated to be 0.8 per 1000 live births. A postepidemic rubella immunity rate of 92.6% was documented among 405 pregnant women; susceptibility was significantly associated with younger age and ethnicity.

Many viruses, even the common cold, are capable of producing sensorineural hearing loss.[96] According to Witters, I *et al.*,[97] CMV is a frequent cause of congenital infections in humans occurring in 0.4 to 2.3% of life births. Although preexisting maternal antibodies are generally protective, transplacental transmission of CMV during pregnancy may occur after recurrent maternal infection. Severe bilateral hearing loss in an infant affected as a result of a CMV reactivation in pregnancy was reported.

Congenital cytomegalovirus infection is a major cause of sensorineural hearing loss (SNHL) and neurologic impairment in children. Although the majority of children with symptomatic congenital CMV infection develop hearing loss, many symptomatic infants have normal hearing. In a follow-up study [98] conducted, the amount of infectious CMV in urine was quantified in 21 children who were born between 1994 and 1998. The children who developed hearing loss had higher urine CMV titers during infancy than those with normal hearing.

According to Williamson, W. D. *et al.*,[99] CMV infection is a major public health problem because 30,000 to 40,000 neonates with the infection are born each year in the United States. Although 90% of the congenitally infected infants are asymptomatic at birth, these infants are at risk for audiologic, neurologic, and developmental sequelae. The frequency of Sensorineural hearing loss was similar for infected infants born to mothers with recurrent CMV infections during pregnancy and for those born to mothers who experienced primary CMV infections. Children with SNHL often have no identified cause of the loss; thus, it is likely that many of these children had asymptomatic congenital CMV infection.

In a survey to identify the etiology of hearing impairment among Saudi children, serological tests for *Herpes simplex* virus infection were performed on 1054 children. They found positive IgM antibody against *Herpes simplex* virus, type 1 (HSV1) in 8 per cent, and positive IgM antibody against *Herpes simplex* virus type 2 (HSV2) in 0.8 per cent (ages ranged between 12 months and 14 years). 56 % with HSV1 were found to have bilateral sensorineural hearing loss. Only one case of the eight infected children with HSV2 was found to have bilateral sensorineural hearing loss of moderate degree. Known causes of hearing impairment were excluded together with hearing impairment due to multiple TORCH agents. The high prevalence of hearing impairment among children due to *Herpes simplex* virus infection is described.[100]

Factors Influencing the Complications

Maternal Infections

Infections in pregnancy may complicate its course and harm the fetus or newborn after vertical transmission. Treatment of asymptomatic bacteriuria is mandatory in pregnant women given the high risk of secondary pyelonephritis. Intraamniotic infection usually arises by the ascending route and is associated with premature rupture of membranes. Systemic infections due to viral, protozoal and bacterial pathogens may be transmitted transplacentally and cause embryopathies, fetopathies or neonatal infections. Depending on the responsible agent the negative impact on the course of pregnancy and on the fetus' or neonate's health can be prevented or reduced by prophylactic or therapeutic interventions.[101]

Preterm birth is a common cause of neonatal morbidity and mortality. Many asymptomatic genital infections have been associated with preterm birth, but attempts to determine a causal relationship between specific infections and preterm birth have been disappointing. Although there is a strong association between the presence of bacterial vaginosis and

Trichomonas vaginalis in pregnancy and preterm birth, treatment trials have failed to show a benefit of treatment of these organisms. Treatment of asymptomatic bacterial vaginosis or *T. vaginalis* to prevent preterm birth is not warranted.[102]

A comprehensive review of urinary tract infections (UTIs) during pregnancy is presented by Le, J., Briggs *et al.,*[103]. Pyelonephritis is the most common severe bacterial urinary tract infection that can lead to perinatal and maternal complications including premature delivery, infants with low birth weight, fetal mortality, preeclampsia, pregnancy-induced hypertension, anemia, thrombocytopenia, and transient renal insufficiency. Enterobacteriaceae account for 90% of UTIs.[103] Heavy colonization with group B streptococcus (GBS) has been also associated with increased risk of preterm birth and neonatal sepsis.[104] Genital chlamydial infections are associated with ectopic pregnancy. In Sweden, Egger, M *et al.,*[105] noticed declining rates of genital chlamydial infections that has probably led to a fall in the rate of ectopic pregnancies.

The microbial agents involved in adverse pregnancy outcome are broad and includes viral, bacterial and protozoal infections. Infertility, ectopic pregnancy, pelvic inflammatory disease, chorioamnionitis, premature rupture of membranes, preterm birth and puerperal sepsis are some of complications seen in women as a result of infection with sexually transmitted pathogens. In the fetus or neonate, complications include abnormalities of the major organ systems.[106]

Infections with HSV type I and type II and varicella/zoster-virus (VZV) are lifelong. During pregnancy, infections with HSV and VZV may induce severe maternal illness that occasionally runs a lethal course. With

viremia placental transmission of the virus may occur infecting the fetus and possibly causing spontaneous abortion, stillbirth and congenital malformations. The occurrence of such malformations is best documented for the "fetal varicella syndrome". Maternal varicella and genital *Herpes simplex* within the perinatal period represent a tremendous risk for the newborn to be infected during delivery; such infection may cause life threatening diseases that have a lethal outcome in more than 50% of affected children.[107]

Rappersberger, K.[107] described the life cycle of HSV/VZV in infected individuals and the peculiar clinical features of maternal infections during pregnancy. Chicken-pox is an uncommon disease in women during pregnancy. If, however, maternal varicella infects the fetus, Intrauterine Fetal Death or severe diseases like congenital or fetal varicella syndrome may ensue depending on the time of maternal infection. On the basis of clinical observations and virological testing Sauerbrei A [108], reported the development of neonatal varicella as a consequence of maternal chickenpox within 12 days before delivery.

Parvovirus B19 is the viral agent that causes the childhood exanthum erythema infectiosum, or fifth disease. Approximately 50% of pregnant women are seropositive for this agent and thus immune to primary infection. However, acute infection may develop in seronegative pregnant women exposed to B19 which can be associated with miscarriage and hydrops fetalis.[109]

Harger, J.H *et al.,*[110] in 1998 assessed the risk of maternal parvovirus B19 infection from exposure to various sources and the fetal morbidity of those infections. Demographic and occupational information of these pregnant women were obtained. They concluded that the risk of maternal B19

infection in pregnancy could not be predicted by a gravida's occupation, but it was significantly higher when the source of exposure was her own child.

Prenatal infections

Kaur *et al.,*[111] detected the seroprevalence of IgM antibodies to *Toxoplasma gondii*, rubella virus and cytomegalovirus and IgG antibodies to *Herpes simplex* virus type 1 and 2. Out of the 120 women studied, 112 (93.4%) had evidence of one or more infections. Prevalence of IgG antibodies to HSV was 70%. Seropositivities for toxoplasmosis, rubella and CMV respectively were 11.6, 8.3 and 20.8%. This data demonstrate high frequency of primary infections during pregnancy which support the conclusion that routine prenatal TORCH screening is justified.

The presence for specific IgM antibodies in neonates is diagnostic of congenital infection. In adults, IgM antibody results should be interpreted along with the clinical findings and history of the patient. Fung and Hilton [2] are of opinion that with a single serum specimen, specific IgM antibody detection may be helpful in differentiating between a recent versus past infection.

A study on the prevalence of seropositivity to TORCH agents was carried out by Canessa, A. *et al.,*[112] in pregnant women aged 15-45 years. An overall prevalence of 40.7% to *Toxoplasma gondii*, of 90.1% to Rubella virus, of 80.8% to Cytomegalovirus, of 82.3% and of 69% to *Herpes simplex* virus, respectively type 1 and type 2 was found. Cytomegalovirus infection was prevalent in women from low socioeconomic background. *Herpes simplex type* 1 infection was higher in women living in quarters of high density

population, whereas antibody prevalence to Rubella virus was higher in women from high socioeconomic setting.

The antibody levels against TORCH agents in sera samples from pregnant women when tested by Ustacelebi, S. *et al.*,[113] by ELISA, IgG antibodies against *Toxoplasma gondii* gave 47.8% seropositivity, Rubella-IgG gave 89.8% and Cytomegalovirus 87.5%. Complement fixation test was employed for the detection of *Herpes simplex* virus type 1 antibodies and 87.5% of sera tested were given positive result in various titers.

The seroprevalence rates of IgG to common TORCH agents in pregnant Saudi women were determined by Ghazi, H.O *et al.*,[114] using indirect enzyme-linked immunosorbent assay. A total of 926 samples of sera were tested for antibodies to TORCH agents known to cause serious congenital infections and human immunodeficiency virus (HIV-1 and HIV-2). Toxoplasma IgG antibodies were detected in 35.6%, CMV total IgG in 92.1%, rubella -IgG in 93.3%, HSV-1 IgG in 90.9%, HSV-2 in 27.1%, and VZV -IgG antibodies in 74.4%. A 0% seroprevalence rate for HIV-1 and -2 was found.

Seroprevalences observed in Finland were as 96.2% for VZV, 56.3% for CMV, 54.3% for HSV, 46.8% for HSV-1, 9.3% for HSV-2 and 58.6% for parvovirus B19. No infants with anti-CMV -IgM antibodies were born to CMV immunised women. Seroprevalence and the risk of viral infections during pregnancy cannot be extrapolated from one pregnant population to another.[115]

Enzyme-Linked immunosorbent assay (ELISA) combined with polymerase chain reaction (PCR) technique were used in a study by Cao, Y, to detect TORCH infection in pregnant women with histories of abnormal

pregnancies and normal pregnant women that acted as the controls. The fetal cord blood samples were also detected. In study group, the rates of previous TORCH infection were as TOX -16.67%, RUV -16.29%, CMV -46.29% and HSV-II -29.63%. The rates of active infection were as TOX- 38.89%, RUV-59.26%, CMV- 57.40% and HSV-II 46.29%. The rates of recurrent infection were as TOX- 11.11%, RUV -38.89%, CMV -38.89% and HSV-II 22.22%. These three kinds of infection rates in study group were significantly higher than that of control group. The incidence of maternal-fetal vertical transmission in study group was 73.08%. The study suggested the absolute necessity to screen TORCH infection for women who had the histories of abnormal pregnancies in order to prevent birth defects and perinatal complications. ELISA combined with PCR technique is a valuable method for the diagnosis of TORCH infection.[116]

A cross-sectional, sero-epidemiological survey of the prevalence of antibodies to TORCH agents during various stages of gestation revealed an overall rate of 13-15 % having antibodies to *Toxoplasma gondii*; 85-87 %, to rubella; 79-81 %, to *Herpes simplex* virus and 100 %, to cytomegalovirus. Although a tendency was noted towards an increase of antibody detection to each TORCH agent as gestation progressed, a statistically significant increase in antibodies titer and specific IgM antibody was found with regard to CMV. Their results suggested an increase in CMV infection or reactivation during pregnancy whereas an increase in the other TORCH infections was not obvious.[117]

Odland, J.O *et al.*,[118] assessed the prevalence of different viral infections in relation to late abortions, stillbirths, and congenital malformations in sera from Russian pregnant women and recurrent aborters. There was little difference in total antibodies to cytomegalovirus or B19

between the groups, while the normal pregnant women had a significantly higher prevalence of rubella antibodies. These results indicate that fewer women remain susceptible to primary CMV infection in pregnancy in Russia compared to Western Europe and North America. Natural immunization against rubella virus was lower than in other, unvaccinated female populations. Based on these observations, vaccination strategies for rubella are now initiated in the Russian Federation.

In a study conducted among pregnant women at Londrina State University, Parana, the rates of seropositivity to various infections were as follows: American trypanosomiasis -0.9%, syphilis -1.6%, toxoplasmosis -67% (IgG) and 1.8% (IgM), rubella -89% (IgG) and 1.2% (IgM), hepatitis B surface antigen -0.8%, hepatitis C virus -0.8% and human immunodeficiency virus infection -0.6%.[119]

In another study, the seroprevalence to *Toxoplasma gondii* (41.1%), rubella virus (88.2%), cytomegalovirus (86.0%), and *Herpes simplex* virus (80.0%) has been evaluated in fertile women living in Catania (Sicily).[120]

Toxoplasma gondii

Toxoplasma gondii infection in the immunocompetent adult usually causes no serious clinical symptoms, but congenital infection can lead to abortion or severe disease in the newborn infant. Early diagnosis should be made as soon as possible particularly in pregnant women and newborn babies since early treatment can minimize fetal sequelae. Hussein, A.H *et al.,*[121] conducted a study to evaluate IgM-ELISA and PCR methods in diagnosis of recent *Toxoplasma gondii* infection. PCR detected very recently infected cases than IgM-ELISA.

In a study, Attia, R.A *et al.,*[122] noted a significant difference between aborting women and the controls as regards IgG & IgM antibodies. IgG antibodies, showed statistical significant difference between those with no history of abortions or I-2 abortions versus those with 3 or more abortions. The infection was more among aborting women in rural (than urban) areas who suffered poor hygienic measures and awareness about the mode of transmission of Toxoplasma infection.

After a serological follow-up of the 110 infants during the first year of life or until the diagnosis of congenital toxoplasmosis (CT) could be ruled out, Robert-Gangneux, F. *et al.,*[123] concluded that despite the use of advanced methods, some cases of congenital toxoplasmosis cannot be detected early, which underlines the importance of careful follow-up of newborns who are at risk.

In 1978 and 1985 Austria and France implemented nationwide programmes to detect and immediately treat all toxoplasma infections during pregnancy. Women of unknown immune status are tested during the first trimester of pregnancy. French seronegative women are advised on good hygiene to avoid infection and retested monthly to detect seroconversion.[124] Parents can opt for termination if there is evidence of fetal macroscopic lesions.[125,126] In France, an estimated 44% of pregnant women are regularly checked for seroconversion[127] and between 5625 and 8850 women are treated during pregnancy every year to prevent congenital toxoplasmosis.

Other countries have decided against routine repeated screening in serologically negative women during pregnancy. In the United States, experts judged that such a programme was not warranted because of the low frequency of maternal infection and low chance of infection in the newborn.[128]

A UK working group of experts concluded in 1991 that "screening for acute toxoplasmosis in pregnancy should not be offered routinely.

Decavalas, G *et al.*,[129] screened two hundred seventeen parturients and eighty six recent aborters for IgM and IgG toxoplasma antibodies. Age, profession, educational level, residence (urban/rural), presence of cat and other domestic animals were recorded for each subject. None of the subjects was IgM-positive. Prevalence of IgG positivity was 52.3% in the parturients and 50.2% in the recently aborted women. None of the personal or social characteristics investigated could be related to IgG positivity. However, the frequency of toxoplasma antibodies was found to be higher in recent aborters from rural areas where contact with soil is common regardless of whether cats are kept as pets or not. This study confirms other investigators' conclusion on the importance of soil contact as a risk factor for infection.

In another study, Toxoplasma IgM antibodies in serum samples of 54 women who had maternal problems like abortion, preterm, fetal exitus, and delivery with pediatric anomalies have been investigated with ELISA. The cord sera of babies of these mothers were also investigated for Toxo-IgM. 11.1% of the mothers were positive for IgM whereas none of the cord blood samples were positive. They concluded that active, latent or reactive toxoplasmosis have to be discriminated and for this purpose detection of Toxo-IgM levels is useful.[130] semiquantitative

Treponema pallidum and others

In a study conducted by Lindstrand, A. *et al.*,[131] to elucidate whether recent syphilis infection is significantly more prevalent among women with mid-trimester miscarriage than among antenatal care attenders in midtrimester

pregnancy. Among antenatal care attenders 18.3%, and among women with midtrimester miscarriage 32.5%, had syphilis confirmed with the *Treponema pallidum* haemagglutination test. Their findings suggest a potential association between syphilis seropositivity and midtrimester miscarriage and it justifies more extensive studies to establish whether or not recent syphilis infection is a risk factor for midtrimester miscarriage.

The presence of parvovirus B19 infection as a possible cause of fetal loss in the third trimester was studied by Skjoldebrand-Sparre [132] in a prospective study of women experiencing third-trimester intrauterine fetal death (IUFD). The authors concluded that 7.5% of IUFDs in the third trimester may have been caused by parvovirus B19 infection, without signs of fetal hydrops. This finding indicates that B19 PCR should be included in the routine investigation of IUFD.

Rubella Virus

Data obtained in the third National Health and Nutrition Examination Survey conducted during 1988-1994 showed an overall rubella seropositivity rates in United States as 92% in persons aged 6-11 years, 83% in persons aged 12-19 years, 85% in 20-29 years, 89% in 30-39 years, and ≥93% in persons aged ≥ 40 years. It is also recommended that the elimination of rubella and chronic rubella syndrome in the United States will require international efforts, including vaccination of preschool- and school-age children and all susceptible young adults.[133]

Singla, N *et al.,*[134] observed IgM seropositivity in 28% of prepubertal age group and 6.84% in reproductive age group. 48 % of the cases of reproductive age group showed adverse pregnancy outcome and they showed

a higher percentage of IgM seropositivity (10.38%) as compared to those with normal obstetric performance (3.55%). IgG seronegativity was found in 28.68% in reproductive age suggesting their susceptibility to acquire primary rubella infection. 0.32% females were seropositive for both IgG and IgM indicating reinfection.

The effect of rubella virus infection in pregnant women or fetus was investigated in a study using IgG and IgM ELISA. Rubella IgG was detected positive in 76.1% of cases and Rubella IgM in 7.4% cases. 14.1% of cases were found to be double-negative of Rubella IgG and IgM while 2.4% of cases were double-positive to Rubella IgG and IgM. A part of the population under study was susceptive to Rubella and about 7.0% was infected by RV during pregnancy. Among these infected women, intrauterine infection may have occurred and caused varied degree of hurt to fetus or resulted in serious congenital rubella syndrome (CRS). These findings suggest that Rubella IgG and IgM should be monitored repeatedly during pregnancy in order to prevent the development of CRS and assure aristogenesis.[135]

Cytomegalovirus (CMV)

Harrison, C.J. & Myers, M.G. [136] suggested that the timing of initial maternal viremia and immune responses, the stage of fetal development, and the length of in utero exposure to CMV are important factors in subsequent disease expression and rates of congenital infection.

Prevalence of seroimmunity to CMV infection was tested in s study [137] by detecting ELISA IgG antibodies in 230 pregnant subjects from low socioeconomic backgrounds. Seroimmunity increased from 64.3% to 92.0% between the ages 15-20 and 35-40.

In order to assess congenital CMV infection in Korea, 575 pregnant women visiting the prenatal clinic at Severance Hospital, Seoul, Korea were studied. CMV -IgG antibody was present in 96% and IgM antibody in 0.7% of the pregnant women by the third trimester. 0.9% of cord sera were positive for CMV -IgM antibody. These results indicate that Korean pregnant women were highly immunized against CMV by the third trimester. The incidence of maternal primary infection during pregnancy seems to be rare and therefore most congenital infections in Korea might be following by maternal reactivation or reinfection.[138]

The role of the sexual transmission of CMV as a cause of congenital infection was investigated by Numazaki, K., *et al.*,[139] Understanding the epidemiology of CMV is a key element in the development of strategies for the prevention of infection. The transmission of CMV by sexual contact may be important in the pathogenesis of congenital infection. Entirely new approaches to the prevention and treatment of congenital CMV infection are necessary, including antiviral interventions and the development of a vaccine strategy.

Herpes simplex Virus (HSV)

Jordan, J. & Rytel, M.W [140] and Katz, D., *et al.*,[141] suggested that ELISA is a specific, sensitive and simple test which confirms the *Herpes simplex* virus infection history of patients.

In a study, IgG and IgM antibodies against HSV-1 and HSV-2 were investigated by ELISA in the sera of the mothers who had different kinds of obstetrical problems like abortions, stillbirth, prematurity, postmaturity, IUGR and in the newborns' cord sera who had congenital anomalies like

anencephaly, cataract and dolichocephaly. In these mothers HSV-1 IgG positivity ratio was 71/73 (97.3%). There was no significant difference in the age group distribution of HSV-1 IgG. The HSV-1 IgG positivity ratios in mother sera were the same as in cord sera. It was found that HSV-1 IgG antibodies passed transplacentally. HSV-1 IgM was found positive in 7 mothers (9.6%) of the study group. This data was the sign of active or reactive infection. In these mothers, 65/73 (89%) HSV-2 IgG and 6/73 (8%) HSV-2 IgM seropositivity were defined. In the cord sera these ratios were 65/73 (89%) and 2/73 (2.7%).[142]

Cross Infections

In an investigation by Gong, Z., *et al.,*[143] on TORCH epidemic in Wuhan region, the infection in the normal populations was inspected through a whole year by detecting the specific IgM antibodies to TORCH in sera. The cross infections of the four pathogens were common and the cross-reaction rate of the surveyed year was 2.6%.

Other Factors

Oligohydramnios

Oligohydramnios represents a physiopathologic process, associated to a high rate of pregnancy complications and increased fetal morbidity and mortality. Oligohydramnios can be defined by an amniotic fluid index < 5th percentile for gestational age or an amniotic fluid index < or = 5.0 cm regardless of gestational age. [144] Some other authors believe that the semiquantitative assessment of amniotic fluid volume can effectively screen for IUGR and thereby delineate a population, regardless of gestational age, that is at risk for perinatal morbidity and mortality.[145]

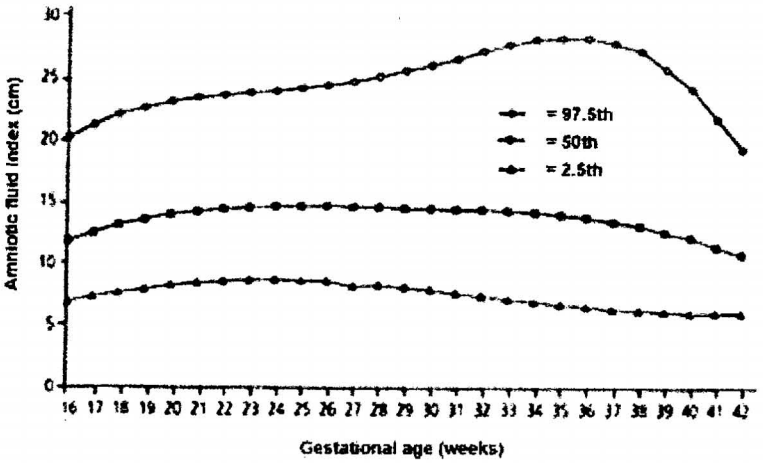
In a study [144] using sonographic criteria patients with reduced amounts of amniotic fluid showed signs and symptoms of gestosis, delivered more often before the 36th week of pregnancy and growth-retarded children than women with standard amounts of amniotic fluid. In oligohydramnios, 13% of the children had severe fetal malformation, whereas, with an amount of amniotic fluid in the lower standard range, only 5.5% showed severe fetal malformation.

A similar study [146] was conducted to determine the effectiveness of amniotic fluid ultrasound evaluation as a useful parameter to identify pregnancies at risk for fetal distress. IUGR observed in 39.56%. Significant correlation was observed with oligohydramnios, IUGR and cardiotocographic anomalies.

The oligohydramnios associated with congenital anomalies in 225,669 consecutive pregnancies were studied by Stoll C *et al.*,[147] in 1998. The prevalence of this association was 0.01%. They examined genetic and environmental factors for the origin of oligohydramnios associated with congenital malformations and found a correlation of 32.6% in prenatal cases and 12% in stillborn infants. Fifty-nine percent of the cases had more than one malformation, 13.8% had a chromosomal aberration, and 27.6% had multiple malformations that do not constitute a syndrome. The more frequent malformations associated with oligohydramnios were urinary, musculoskeletal, digestive and cardiac. Threatened abortions and diabetes mellitus were significantly more frequent among mothers of the children with congenital malformations associated with oligohydramnios than among the controls.

In an ultrasonopgraphic examination of oligohydramnios cases in the second and third trimester, Golan, A. *et al.*,[148] observed 22.1% cases of hypertension and 24.5% cases of IUGR. Intrauterine fetal death occurred in 5.5% of these pregnancies. The gross perinatal mortality was 16% and the corrected perinatal mortality was 10.7%. The overall rate of fetal malformations was 11% and that of lethal malformations 4.8%. The skeletal (7.6%) and urinary system (4.1%) were the predominant systems affected.

Percentiles for amniotic fluid index based on gestational age [64]



Pre-eclampsia /Pregnancy Induced Hypertension (PIH)

Pre-eclampsia or pregnancy-induced hypertension - is thought to occur in 10% of pregnancies and is the commonest cause of maternal death in the UK, killing around 10 women a year. It also leads to the death of some 1,000 babies a year. Symptoms include high blood pressure, fluid retention and protein in the urine. Blood pressure and urine are now regularly checked throughout pregnancy. Pre-eclampsia usually occurs towards the end of the pregnancy and the effects can be felt for some days after the birth. Some women are thought to have a genetic predisposition to it. Women are more

likely to suffer from the condition in their first pregnancy and most do not go on to experience it again in subsequent pregnancies. The causes of pre-eclampsia are unclear, but research suggests it may be linked to an immune reaction to the fetus or the placenta. If the condition is serious, women may be advised to rest or take drugs to lower their blood pressure and, in some cases, an early caesarean or induction may be performed. [149]

Pre-eclampsia has been described as a 'disease of first pregnancies' and many believe that its occurrence in a later pregnancy signals a fundamentally different entity. The results of a study to compare risk factors in first and subsequent pregnancies in Jerusalem in 1964-76 [149] lend no support to the hypothesis that there is a fundamental difference between pre-eclampsia in a first pregnancy compared with that occurring in a later pregnancy.

A decrease in placental blood volume was a possible factor behind IUGR in pregnancy-induced hypertension. Using an image analysis system, the cross-sectional areas and wall thickness of central blood vessels of the spiral artery, the so-called 'central artery' was measured in a study. It was thought that one of the more basic factors behind IUGR in pregnancy-induced hypertension might possibly be narrowings and spasms of the maternal placental blood vessels.[150]

Diabetes

Glucose can freely cross the placenta to the baby during pregnancy but insulin does not. The baby stores the extra glucose and may grow more rapidly than babies of women without diabetes. High blood glucose levels in the mother will result in high blood glucose levels in the baby but insulin crosses the placenta in only small, non-harmful amounts. The first 8 weeks

are when a baby's major organs develop so it's particularly important to gain tight control of blood glucose levels in those early stages. Rarely, congenital (structural) abnormalities do occur in the early stages of pregnancy, sometimes before pregnancy status. In otherwise healthy diabetic women, who have tight control of blood glucose levels, the risk of miscarriage is no greater than that of the general population - approximately one out of every nine or ten early-stage pregnancies. Women with diabetes may have large babies as a result of their high blood glucose levels. This effect can be reduced by keeping blood glucose levels as close to normal as possible. Obviously, a big baby makes delivery more difficult and for this reason, Cesareans are slightly more common for women with diabetes.[151]

Forsbach, G *et al.*,[152] in 1998 reviewed the Mexican studies published in past 15 years on diabetes mellitus (DM) and pregnancy. They found five descriptive studies on DM and pregnancy and six on the detection of gestational diabetes (GDM) in normal pregnant women using the oral glucose tolerance test (OGTT). Maternal complications were toxemia in 18%; polyhydramnios is 10% and urinary infection in 6%. Perinatal mortality was 7%, congenital malformations in 6%, macrosomia in 25% and prematurity in 8%. They concluded that Type 2 DM is the more frequent type of diabetes associated to pregnancy in Mexican groups; the systematic screening for GDM in normal pregnant women yields 4 to 11% of positivity.

Parity

The CMV positivity among 12,159 white women born in the British Isles was independently associated with increasing parity, older age, lower social class, and being single at antenatal booking. The findings are consistent

with the hypothesis that, in the UK, child to mother transmission of infection plays a significant part in the acquisition of CMV infection in adult life.[153]

Data from the National Congenital Rubella Surveillance Programme showed that 44% of children with congenital rubella reported to the programme were born to primiparae. This high proportion is thought to be due to the fact that there was a two-fold increase in the rate of abortion for rubella in pregnancy for women with two or more children. This higher incidence of congenital rubella in firstborns emphasises the need for rubella vaccination prior to a woman's first pregnancy.[154]

One study examined the unique role of primiparity in the etiology of IUGR in a series of 25,614 singleton births in the southern part of Israel. The rates of IUGR were 3.5% among primiparae and 1.7% in multiparae. Maternal age was associated with IUGR only among primiparae but not in multiparae. Of the obstetric factors examined, the following were found to be significantly associated with IUGR: hypertension, prior infertility, oligohydramnios, gross fetal congenital anomalies, and being a female fetus. The authors suggested that primiparity constitutes an independent risk factor for IUGR.[155] In another study, Gratacap-Cavallier, B. et al [156] presented data on CMV seroprevalence in pregnant women in France. The overall rate of seropositivity, using a specific IgG ELISA test, was 51.5 %. They found the place of birth in France as a major predictive factor of CMV antibody status followed by age and parity. In this study, they showed an independent effect of parity on CMV seroprevalence.

Gravida

An interview [157] of 48 primigravidae at their 3-4 months of pregnancy was conducted in a study to find out their knowledge of fetal development, awareness of hazards to development and sources of information. Although there was reasonable knowledge about normal fetal development, the women were not generally aware of the repercussions of maternal rubella or rationale for many routine tests at antenatal clinics.

Age: Teenage pregnancy

Data from South Australia indicate that in 1979 approximately 1/3 of abortions were performed on teenagers. Consideration of the risks of teenage pregnancy must include the risks of abortion. The main risk to the teenage mother's health is an increased likelihood of preeclampsia. At highest risk are those under age 16. Other studies have shown that if teenagers have adequate antenatal care they are not at high risk of obstetric complications than older women of similar race and socioeconomic background. The greatest medical risks to teenage childbearing are to the child. West Australian data have confirmed the findings of other studies that the infants of young teenagers in particular are at higher risk of a low birth weight or perinatal death than those of women in their 20s. The higher perinatal mortality in the infants of young teenagers is due in part to the higher percentage of infants of low birth weight.[158]

Kurup, A *et al.,*[159] when examined the obstetric and social implications of 150 pregnancies among the unmarried teenagers in Singapore, poor intrauterine growth appeared to be the most important adverse obstetric outcome in that mean birth weight was significantly reduced. They believe

this to be an important explanation for the five-fold increase in perinatal mortality seen in this group of mothers.

In a study in Nowrosjee Wadia Maternity Hospital, Bombay,[160] two hundred consecutive cases up to 19 years of age admitted for confinement were studied. Premature delivery is significantly higher in 15-17 age group (43%) compared to the 17-19 age group (14%). Also, only 4 girls (29%) in the age group of 15-17 years had full term normal delivery compared to 113 girls (61%) in the age group of 17-19 years, signifying that the outcome of pregnancy becomes worst in girls below the age of 17 years. Ten babies (71%) of mothers in the age group of 15-17 years were LBW as compared to 75 babies (44%) of mothers in the age group of 17-19 years signifying that the incidence of LBW babies is inversely proportional to maternal age. The authors also suggest the necessity of giving more attention to teenage pregnant girls for prevention and treatment of preeclampsia eclampsia, anaemia, prematurity and LBW.

Kurup, A [159] studied the differences in frequency and obstetric outcome of teenage pregnancy between the main ethnic groups in the Netherlands. As per their conclusion, teenage pregnancy in the Netherlands is much more common in minority ethnic groups than in the indigenous population, particularly among Islamic-Mediterraneans and Blacks. Obstetric outcomes vary considerably, these being best in Hindustani and poorest in black teenagers, and being worse in teenagers than in 20-24-year-old women. However, teenagers less often had assisted delivery.

Sarkar, C.S *et al.,*[161] recorded 4698 (18.68%) cases of labour in teenage mothers of a total of 25,142 deliveries during a period of 3 years. Predominance of primigravida (76.6%) and cases from rural areas (51.3%)

were recorded. Antenatal care was nil or inadequate in 48.6% cases. Eclampsia and pre-eclampsia affected teenage mothers (10.6%) were much more frequent than mothers of 20 years of age and above (5.2%). Incidence of 30% low birth weight baby, 20.1% prematurity and 16.4% perinatal mortality were also recoded by them.

A cross sectional observational study was done by Kumbi, S and Isehak [162], A to investigate and compare the difference in pregnancy outcome in teenage girls and in an older age group. The study concluded that the teenage pregnancy is associated with adverse outcome; and teenagers perform poorly in labour and delivery. The authors also recommend the initiation of a programme which should create a means of easily reaching the adolescent to promote family life education, contraception, counseling and education related to early marriage.

In another study by Gortzak-Uzan *et al.,*[163] all singleton first deliveries to mothers of age 16-24 years between 1990 and 1997 were considered to assess the perinatal outcome of teenage pregnancy in a large cohort and to determine risk factors for low birth weight (LBW) in teenage pregnancy. Teenage pregnancy was found to be associated with adverse outcome such as LBW, preterm delivery, small for gestational age and malformations. The risk for LBW was affected mainly by demographic factors (maternal ethnicity, lack of prenatal care) and medical factors (PIH, malformations).

Socioeconomic Factors

Low socioeconomic status is related to a higher risk profile of mothers and babies. Previous works had pointed out the increased risk of women from poor socioeconomic background to have more abortions, still birth and

intrauterine growth retardations[164]. Socioeconomic factors, including poverty, malnutrition, lack of education, and lack of access to prenatal care or emergency obstetrical care can increase a young woman's risk of pregnancy-related complications. Adverse pregnancy outcomes were observed in teenage women, older women, non-caucasian women and primigravid women. These poorer outcomes in older women and primigravidas mainly include higher risks of low birth-weight and prematurity of their babies. Teenage women, non-Caucasian women, and women of low socioeconomic status are less likely to have choice of obstetric care[165]. Pregnancy wastage rates among women from poorer communities are higher. In a study of 2537 rural and 2021 urban women from a low socioeconomic group in India, Gopalan and Naidu [166] observed a pregnancy wastage rate of 30 percent.

PREVALENCE OF TORCH INFECTIONS IN PREGNANCY RELATED COMPLICATIONS IN THE MALABAR REGION OF KERALA

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Materials and Methods

Study Groups

Patients attended the Institute of Maternity and Child Health, Calicut Medical College, Kerala, during the period from January 2001 to June 2004, were subjected for this study. Abortions of various categories were studied using medical, clinical, and serological parameters. The study groups included abortion cases (n=71) of various categories, full term pregnant women having babies with Intrauterine growth retardation (n=64), cases of Intrauterine fetal death (n=24) and congenital anomalies (n=62). The control groups included first trimester (n=30) and full term (n=84) normal pregnant women. Babies with birth weight less than 2500 g / less than 10^{th} percentile for their gestational age was treated as IUGR.

Proforma

The medical history and details of the cases like age, gravida (number of pregnancies), parity (number of successful deliveries), previous pregnancy loss, infections with HIV and hepatitis, Hb content of the mother, trimester of pregnancy, pregnancy induced hypertension, history of diabetes, oligohydramnios, glucose challenge test results, malformations of the infant, sex of the infant, weight of the infant etc. were collected and noted in a proforma. Tests for presence of HIV and HbSAg were done in the Microbiology laboratory of Calicut Medical College. Screening for the TORCH pathogens were done in the Department of Life Sciences and recorded in the proforma. Screening for both IgM and IgG against Toxoplasma, Rubella, CMV and HSV was done in mothers/ infants of all the categories. VDRL test was conducted for mother and duly recorded.

PROFORMA

Categories:	Miscarriage	- Mother
	Intrauterine Growth Retardation	- Mother & Infant
	Intrauterine Fetal Death	- Mother & Infant
	Congenital Malformations/Anomalies	- Mother & Infant
	First trimester controls	- Mother
	Full term Controls	- Mother & Infant

Date: OP/IP No: Unit: Ward:
Blood Sample No: M ..... C .....

MOTHER:

Age :
Trimester :
Socioeconomic status :
Contraceptive if any : Condoms/OC/ Injectables .....
GRAVIDA (No. of Pregnancy) :
Previous Miscarriage :
PARITY :
Past Medical illness :
Diabetes : Hypertension :
Renal diseases : Any others :
Duration of previous illness :
Medication if any :
Investigations:
Hb : Blood grouping/ Rh typing :
VDRL : HIV :
HbsAg : GCT :
Ultrasonogram:
Anomalies :
IUGR :
AFI (Amino Fluid Index) :
Present Obstetric History :

Obstetric Examination :

Miscarriage Category :

IUGR :

Hydramnios :

Oligohydramnios :

INFANT:

Age : Weight:

Sex :

Blood picture;

Hb :

TC :

DC :

ESR :

X-ray :

ECG :

EEG :

Ultrasonogram :

Congenital malformations :

Birth Defects :

Clinical features:

Growth retardation :

Cutaneous Malformations :

Jaundice :

Conjunctivitis :

Microencephalopathy :

Chororetinitis :

Deafness :

Heart Disease :

Brain Calcification :

Hepatomegaly :

Splenomegaly :

TORCH Screening

	IgM				IgG			
	Toxoplasma	**Rubella**	**CMV**	**HSV**	**Toxoplasma**	**Rubella**	**CMV**	**HSV**
Mother								
Infant								
Control Mother								
Control Infant								

Sample collection and analysis

In case of miscarriage and first trimester controls, maternal blood samples were collected at 8-12 gestational week (first trimester). In other categories (IUGR, IUD and congenital anomalies) and third trimester controls, maternal and cord blood samples were collected at the time of delivery (full term pregnancy). The samples were immediately transported to the microbiology laboratory of the Department of Life Sciences, University of Calicut using icepacks and serum samples were separated. The sera were stored in small screw caped vials at -20^{o} C until serological analysis. All samples were tested for the presence of IgM and IgG antibodies against *Toxoplasma gondii*, Rubella, Cytomegalovirus and *Herpes simplex* virus (1&2) using ELISA kits from Equipar diagnostics (Italy). The tests were done as per the directions given in the manual supplied along with the kits. The results were read at 450 nm in the ELISA reader (Sunrise model, Tecan, Austria). Syphilis was diagnosed using VDRL test.

IgM ELISA

Principle

In each kit for four organisms in the TORCH panel, respective antigen is fixed to the interior surface of microwells. The patient's serum is added and any antibody present to each organism will bind to these antigens. The microwells are washed to remove unbound serum proteins. Antihuman IgM conjugated with horseradish peroxidase enzyme is added and incubated. This will bind to any human IgM present. The microwells are washed to remove unbound conjugate and then chromogen /substrate is added. In the presence of peroxidase enzyme the colorless substrate is hydrolyzed to a colored end-

product. The color intensity is proportional to the amount of IgM present in the patient's serum.

Components of IgM ELISA kits

1. Antigen coated microwells. 8x12 microwell strips
2. Negative Control.
3. Low Positive standard.
4. High positive standard.
5. Serum diluent. Contain PBS/Tween, protein stabilizers and Rheumatoid factor absorbent solution
6. Wash buffer concentrate. Contain PBS/Tween. (Dilute 1:20 with distilled water before use)
7. Enzyme conjugate. Horseradish peroxidase conjugated goat anti human IgM (μ chain specific).
8. TMB substrate solution. Contain Tetramethylbenzidine.
9. Stop solution. Contain 1N HCL

Assay Procedure

1. Placed the required number of microwells in the microwell holder. Marked one end of each strip for orientation.
2. Diluted the samples 1: 100 with serum Diluent (10μl serum to 1ml serum diluent). The calibrators were not diluted since they are ready for use. Incubated the diluted samples at room temperature.
3. Pipetted 100μl negative control, low and high positive standards (cut-off) and serum specimens in subsequent wells.
4. Incubated at room temperature (23-25°C) for 15 minutes. In case of rubella this incubation step was for 30 minutes.

5. Washed microwells by inverting and flicking into a sink. Completely filled with wash buffer and repeated washes 3 times. Refilled with wash buffer and soaked for 5 minutes. The wells were emptied and blotted with absorbent paper. The wells were filled and aspired five times without soaking using an ELISA plate washer
6. Pipetted 100µl Enzyme Conjugate into each well.
7. Incubated at room temperature (23-25°C) for 15 minutes. In case of Rubella the time of incubation was 30 minutes.
8. Washed the microwells as in step 5.
9. Pipetted 100µl TMB substrate in each well.
10. Incubated at room temperature (23-25°C) for 10 minutes.
11. Pipetted 100µl Stop Solution into each well using the same pipetting sequence as in step 9.
12. Measured the colour intensity of the solution in each well using ELISA Plate Reader with a 450 nm filter.

Interpretation of results

The IgM ELISA test results were interpreted as negative, low positive or high positive.

IgG ELISA

Principle

In each kit for four organisms in the TORCH panel, antigens of each are fixed to the interior surface of separate microwells. The patient's serum is added and any antibody present to each organism will bind to these antigens. The microwells are washed to remove unbound serum proteins. Antibodies conjugated with horseradish peroxidase enzyme and directed against human

IgG are added and will in turn bind to any human IgG present. The microwells are washed to remove unbound conjugate and then chromogen /substrate is added. In the presence of peroxidase enzyme the colorless substrate is hydrolyzed to a colored end-product. The color intensity is proportional to the amount of IgG present in the patient's serum.

Components in IgG ELISA kit

1. Antigen coated microwells. 8X12 microwell strips.
2. Controls.

	Toxoplasma	**Rubella**	**CMV**	**HSV**
1.	0 IU/ml	0 IU/ml	Negative	Negative
2.	12.5 IU/ml	10 IU/ml	Low positive	Low positive
3.	75 IU/ml	25 IU/ml	High Positive	High Positive
4.	150 IU/ml	50 IU/ml		
5.	500 IU/ml	100 IU//ml		
6.	1000 IU/ml	1000 IU/ml		

3. Sample diluent. Contain PBS/Tween, and protein stabilizers
4. Wash buffer concentrate. Contain PBS/Tween. (Dilute 1:20 with distilled water before use).
5. Enzyme conjugate. Horseradish peroxidase conjugated goat anti human IgG.
6. TMB substrate solution. Contain Tetramethylbenzidine.
7. Stop solution. Contain 1N HCl solution.

Assay Procedure

1. Placed the required number of microwells in the microwell holder. Marked one end of each strip for orientation.
2. Diluted the samples 1: 100 with serum Diluent (10µl serum to 1ml serum diluent). The calibrators were not diluted since they are ready for use. Incubated the diluted samples at room temperature.
3. Pipetted 100µl negative control, low and high positive standards (cut-off) and serum specimens in subsequent wells.
4. Incubated at room temperature (23-25°C) for 15 minutes.
5. Washed microwells by inverting and flicking into a sink. Completely filled with wash buffer and repeat washes 3 times. Refilled with wash buffer and soaked for 5 minutes. The wells were emptied and blotted with absorbent paper. The wells were filled and aspired five times without soaking using an ELISA plate washer
6. Pipetted 100µl Enzyme Conjugate into each well.
7. Incubated at room temperature (23-25°C) for 15 minutes.
8. Washed microwells as in step 5.
9. Pipetted 100µl TMB substrate in each well.
10. Incubated at room temperature (23-25°C) for 5 minutes.
11. Pipetted 100µl Stop Solution into each well using the same pipetting sequence as in step 9.
12. Measured the colour intensity of the solution in each well using ELISA Plate Reader with a 450 nm filter.

Interpretation of results

In case of Toxoplasma and Rubella, the IgG levels were analyzed by comparing with six different titers of controls. The cut off value taken for

Toxoplasma and Rubella were 75 IU/ml and 25 IU/ml respectively. For CMV and HSV both low positive and high positive values were considered as positive.

VDRL Test

This is a serological test used for the diagnosis of syphilis. This detect the immunoglobulins produced in syphilitic patients which are called reagin, The test is a non specific serological test because the antigen used is an extract of bullock heart muscle and the essential component is cardiolipin in nature. Colloidal preparation of this antigen is precipitated into floccules when treated with reagin type of antibodies. So that reaction is a precipitation type of antigen- antibody reaction.

Quantitative and qualitative tests are used. Qualitative test is called VDRL test.

VDRL Screening- Quantitative test

VDRL antigen extract of bullock heart containing cardiolipin, lecithin. This is a soluble antigen manufactured by approved reference lab.

Requirements

- Phosphate buffer
- Pipette or dropper
- Standardized dilution (0.05 ml) cavity slides - glass slides with depressed well or slides with paraffin rings of around 2 cm diameter.
- VDRL shaker

- Low power microscope
- Water bath
- Patient serum

Preparation of antigen emulsion

0.4 ml of buffer was taken in flat bottom 1 ounce reagent bottle with glass stopper. Allowed the buffer to get uniformly distributed over the bottom surface. Cut opened the antigen vial and the contents taken in the pipette were added drop by drop into the bottle simultaneously mixing by rotating the bottle in circular movement. The speed of adding antigen is regulated in such a way that the total 0.5 ml is delivered within 6 seconds. Mixed antigen buffer mixture by rotating the bottle for a further 10 more sec. period. Now pipetted out 4.1 ml of buffer and added to the emulsion in the bottle. Closed the bottle with stopper. Mixed gently by inverting and reverting 30 times. Allowed the emulsion to remain at room temperature for 30 minutes for maturation and subsequently used for a maximum period of 8 hours. Under no condition the preparation was used on subsequent days.

Test

Inactivated the serum by heating to 50°C for 30 minutes in water bath. Diluted 0.05 ml in one of the cavities of VDRL cavity slide. Similar volume of positive and negative sera was taken in adjacent wells. Delivered 1 drop of freshly prepared VDRL antigen emulsion using a syringe and needle. The point of needle which has been standardised to deliver $1/60^{th}$ of an ml.

Mixed the reagent by rotating the slide with help of VDRL shaker adjusted to give a speed of 180 rpm. Examined for the flocculation under low magnification.

Interpretation of results

Large coarse clumps with very few or no individual needle shaped particles is indicative of positive result termed as reactive. Small clumps with fairly large number of needle shaped particles distributed singly is indicative of a weakly positive reaction reported as weakly reactive. Absence of floccules showing fine needle shaped particles distributed singly and uniformly is indicative of negative reaction.

Statistical Analysis

Statistical analysis was done using the SPSS software for microcomputers. The Pearson's Chi Square test and fisher's exact test were used to assess statistical significance. The association between various parameters under consideration was assessed and correlated.

PREVALENCE OF TORCH INFECTIONS IN PREGNANCY RELATED COMPLICATIONS IN THE MALABAR REGION OF KERALA

Thesis submitted to the
University of Calicut
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY
IN
MICROBIOLOGY

UNDER THE FACULTY OF SCIENCE

By

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Department of Life Sciences
University of Calicut
Kerala -673 635

2006

Results

A total of 221 complicated pregnancy and 114 normal pregnancy cases were included in this study. Abortion cases (n=71) of various categories, full term deliveries with IUGR babies (n=64), IUD (n=24), congenital anomalies (n=62) were included in the test category. Normal pregnant women at first trimester of pregnancy (n=30) served as the controls for the miscarriage cases and full term normal pregnant women (n=84) for the remaining categories.

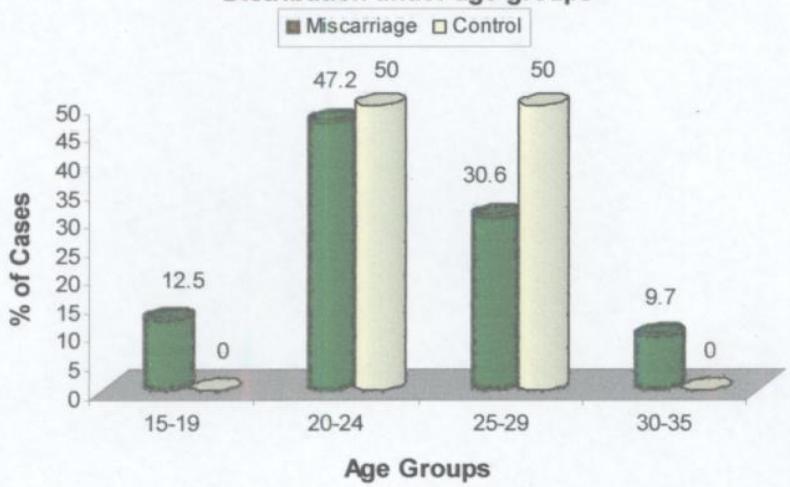
The patients' details including age, socioeconomic status, gravida, parity, previous pregnancy loss, presence of AIDS and hepatitis, Hb content of the mother, trimester stages of pregnancy, pregnancy induced hypertension, diabetes, oligohydramnios, malformations of the infant, weight of the infant etc. were recorded in the proforma. AFI values less than 5 cm or less than the 5th percentile is taken as an indication of oligohydramnios. GCT values less than or equal to 130 mg/dl is taken as normal. The main goal of this study was to screen the study groups for TORCH infections for correlating with complicated pregnancy. The serum samples from maternal and cord blood were analyzed for the presence of IgG and IgM immunoglobulins against TORCH agents using ELISA kits (Equipar diagnostics). The results were analyzed using SPSS (version 13) software for microcomputers. The Pearson's Chi Square test and Fishers Exact test were used to find out the significance of the test results.

Miscarriage

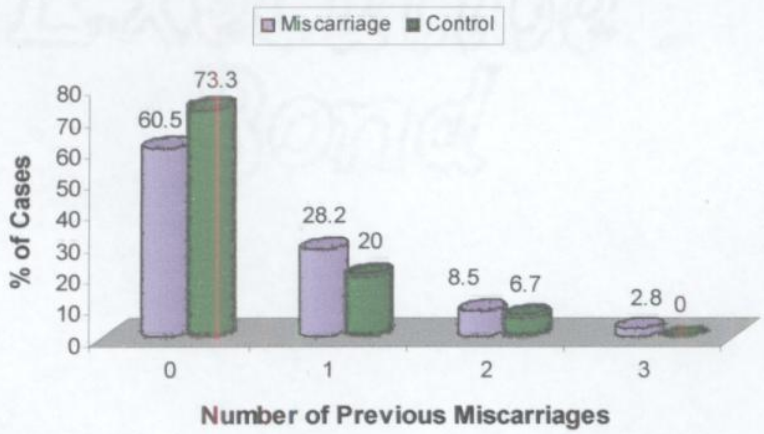
Seventy one miscarriage cases and thirty normal pregnant women were studied analyzing their medical, clinical, and serological data. The miscarriage cases in this study include Complete Abortion (17.18%), Incomplete Abortion (9.36%), Missed Abortion (54.74%), and Threatened Abortion (18.72%). Here 90.1 % of the study population was from the low socioeconomic group where as the rest (9.9%) came under the middle class group ($p<0.204$). The abortion categories were not found as significantly influenced by the socioeconomic status. Neither the test nor the control groups had Diabetes, AIDS, Syphilis and Hepatitis. Two mothers among the test group had renal diseases. Other complications noted in the miscarriage mothers were Hydrocephalus, Hydramnios, Omphalocoe, Thyroid enlargement, Intrauterine Fetal demise, Oedema at abdominal wall, Amenorrhea and Granulonephritis in nominal numbers. The age groups under which various categories were distributed and their prevalence are shown in Graph. Miscarriage. 1. The previous abortions, Parity and gravida status of the study groups are presented in the Graph. Miscarriage. 2, Graph. Miscarriage. 3 and Graph. Miscarriage. 4 respectively.

The IgM seropositivity against TORCH agents are presented in the Graph. Miscarriage. 5 and 7. TORCH cross infections presented in the Graph. Miscarriage. 6 and 8. The immune status of the general population was analyzed through IgG estimation and presented in Graph. Miscarriage. 9. Other parameters like socioeconomic status, pregnancy induced hypertension and Hb content of the patient were given in the Graph. Miscarriage. 10.

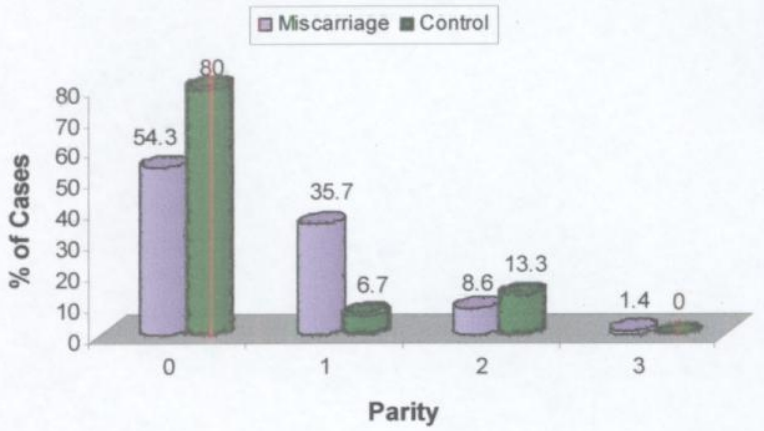
Graph. Miscarriage 1
Distribution under age groups



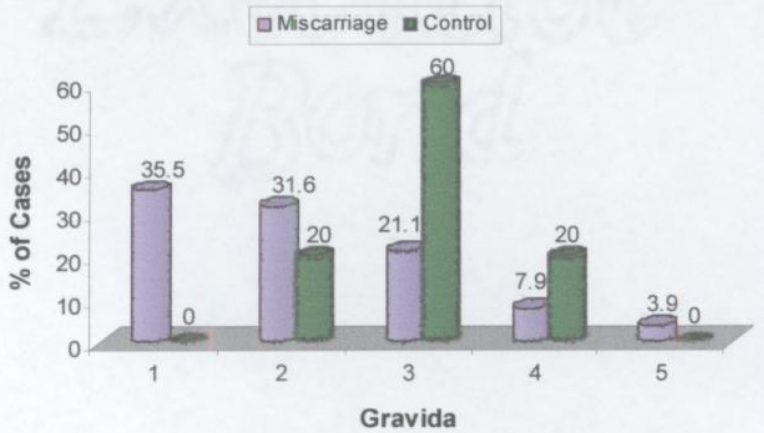
Graph. Miscarriage 2
Previous Miscarriages in the study groups



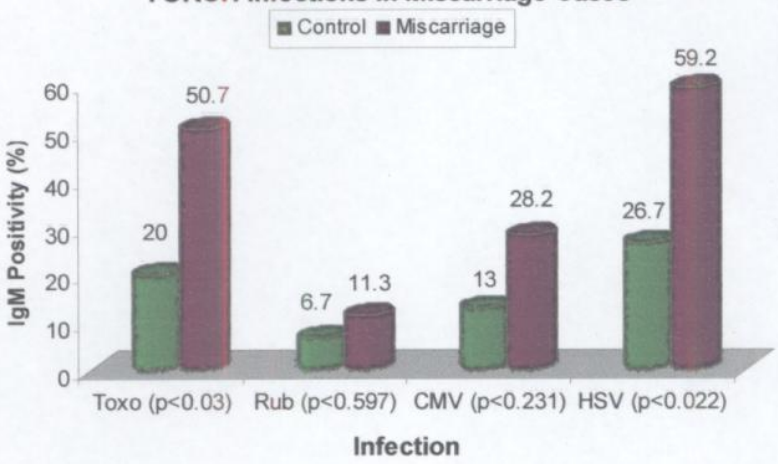
Graph. Miscarriage 3
Parity of the study groups



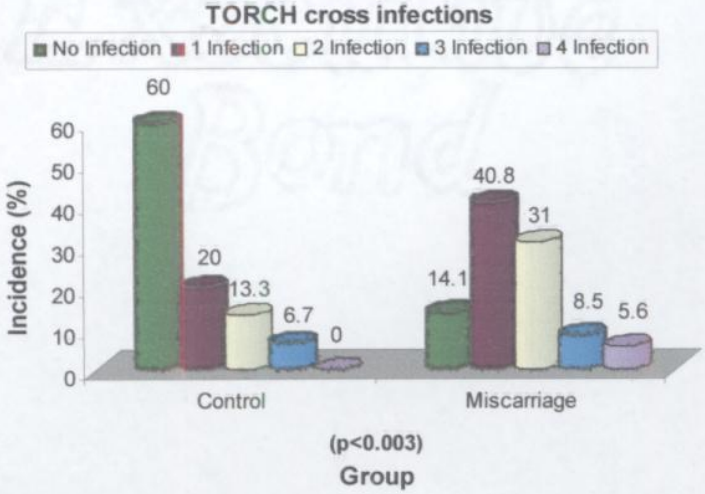
Graph. Miscarriage 4
Gravida of the study groups



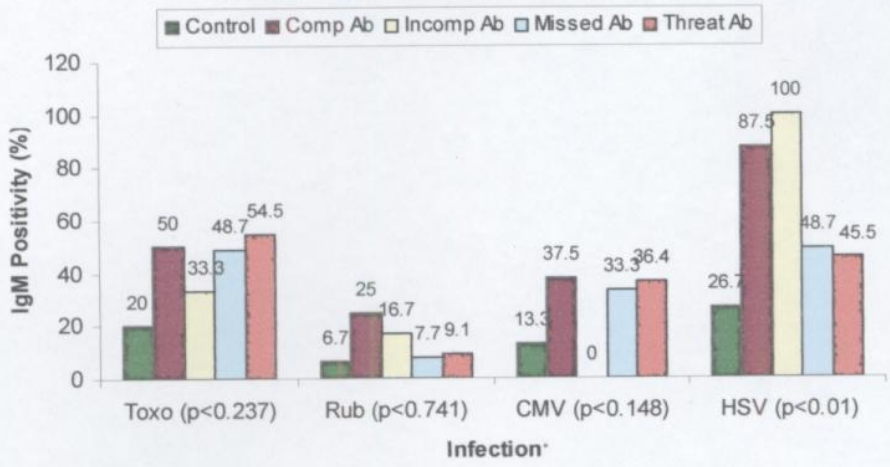
Graph. Miscarriage.5
TORCH infections in Miscarriage Cases



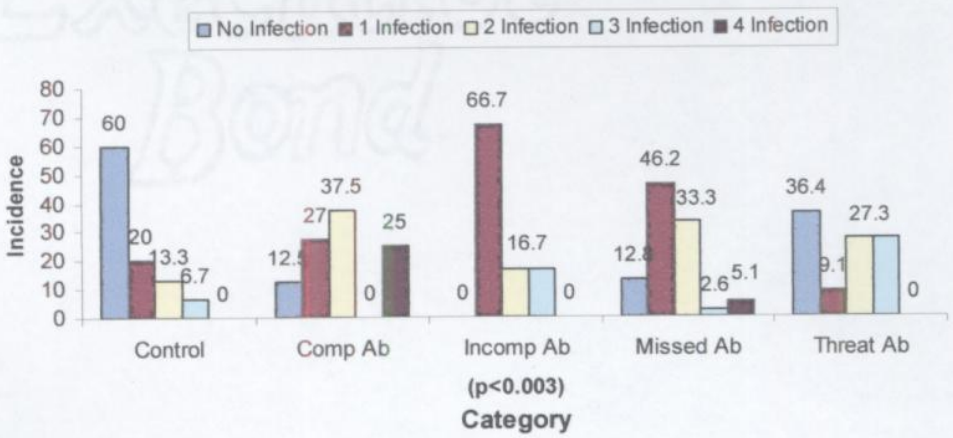
Graph. Miscarriage 6
TORCH cross infections



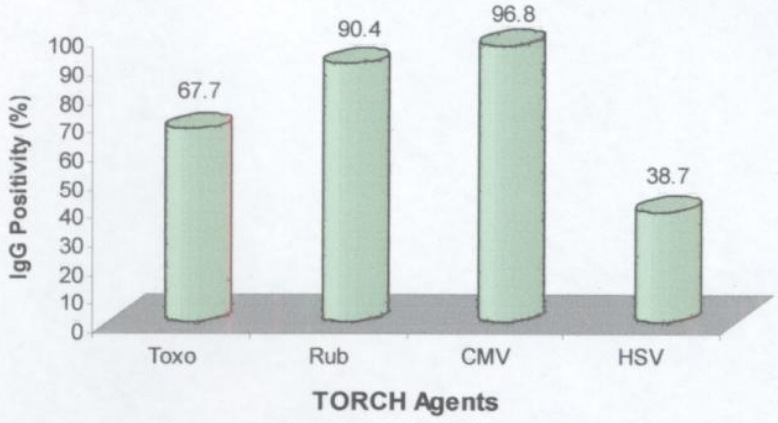
Graph. Miscarriage 7
TORCH infections in Abortion Categories



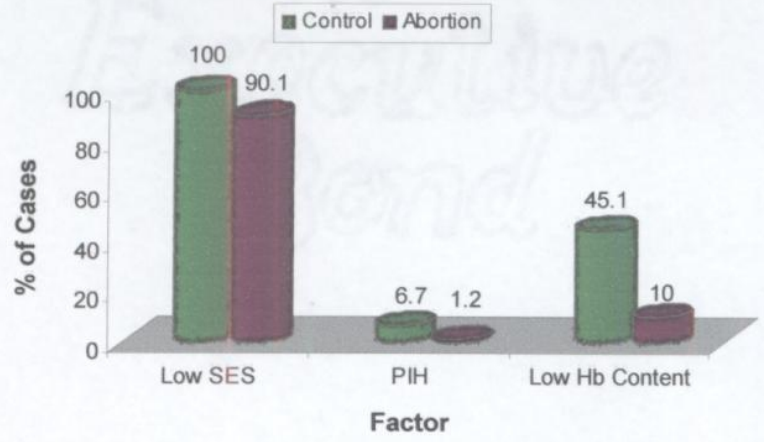
Graph. Miscarriage. 8
TORCH Infections in Abortion Categories



Graph. Miscarriage. 9
Immune Status of the General Population



Graph. Miscarriage. 10
Comparison of SES, PIH and Hb Content



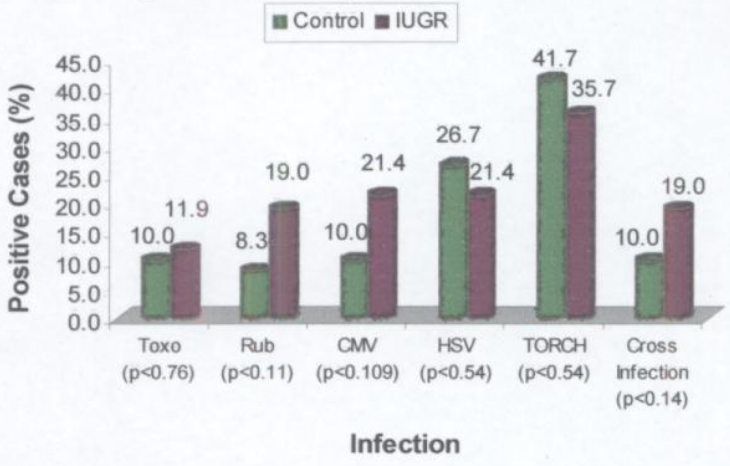
Intrauterine Growth Retardation

The study group included 64 full term pregnancy cases with IUGR and 84 normal pregnant as controls. Both maternal and cord blood samples were analyzed for IgM and IgG against TORCH pathogens using ELISA method. The mean age of the mothers having IUGR and control group was 23 and 24 years respectively. Male: female ratio of the infants of the two categories was 1:1.4 and 1:1 respectively.

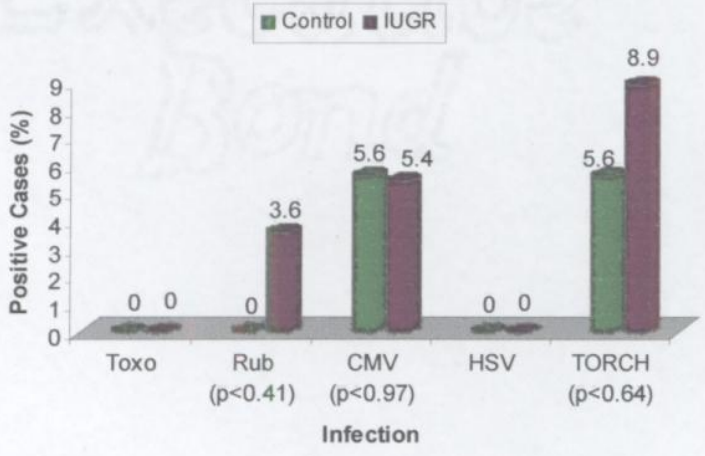
The complications noted in the IUGR mothers included renal disease, diabetes, microcephaly, choreoretinits, splenomegaly, cutaneous malformation, hepatomegaly, jaundice and asymmetric IUGR, but in very low percentages. None of the normal mothers suffered from any of these complications. Both the control and test group were free of Syphilis, HIV infection and Hepatitis.

The IgM seropositivity of maternal and cord blood sera are shown in the Graph. IUGR.1 and 2 respectively. IgG seropositivity are presented in the Graph. IUGR. 3 and 4 respectively. Age, gravida, previous abortions and parity of the study groups are given in Graph. IUGR 5 – 8. Other influencing factors like socioeconomic status, Hb content, PIH, GCT, Oligohydramnios and diabetes are shown in the Graph. IUGR 9.

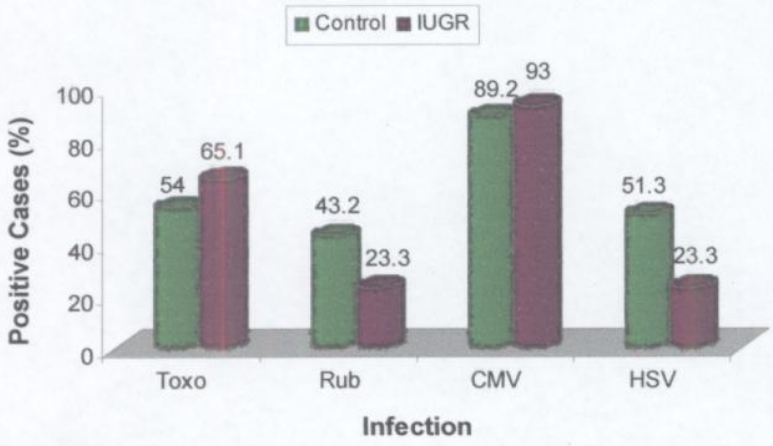
Graph .IUGR. 1
IgM Seropositivity of Maternal Serum



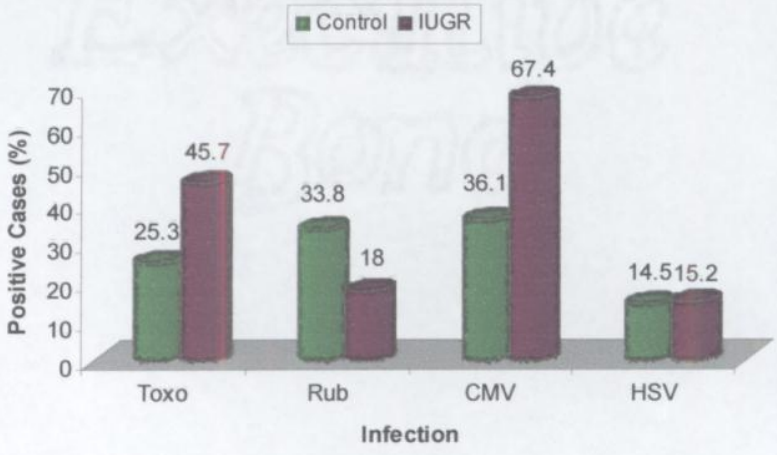
Graph. IUGR. 2
IgM Seropositivity of Cord Blood Serum



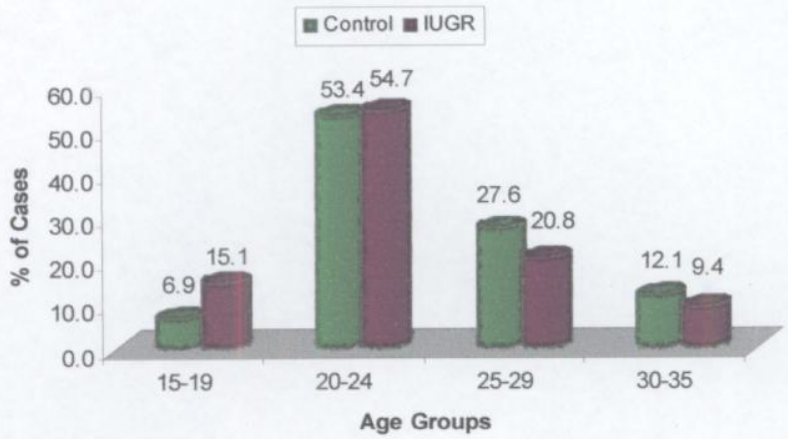
Graph. IUGR.3
IgG Seropositivity of Maternal Serum



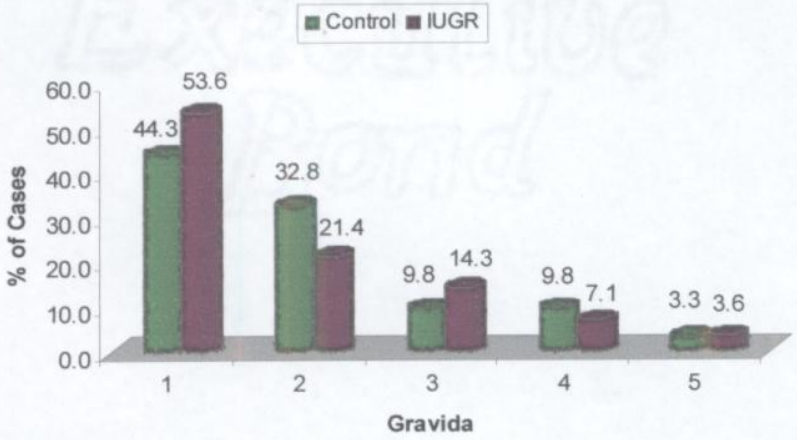
Graph. IUGR.4
IgG Seropositivity of Cord Blood Serum



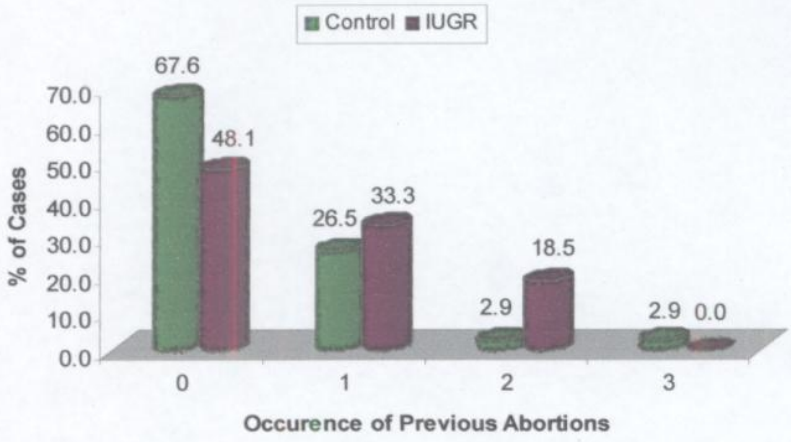
Graph. IUGR.5
Age of Study Groups



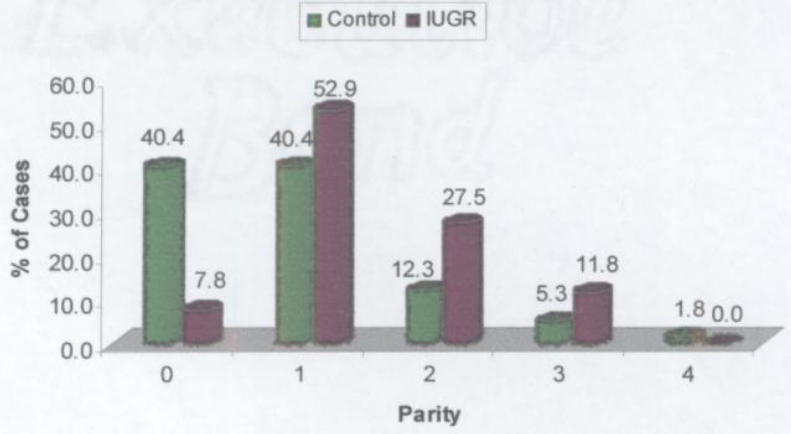
Graph. IUGR.6
Gravida of the Study Groups



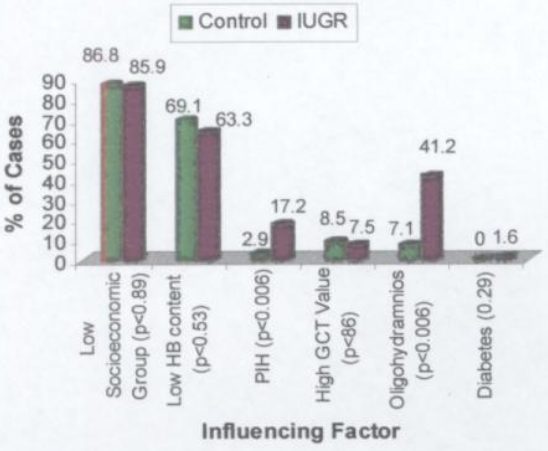
Graph.IUGR.7
Previous Abortions in the Study Groups



Graph.IUGR.8
Parity of the Study Groups



Graph. IUGR. 9
Other related Factors

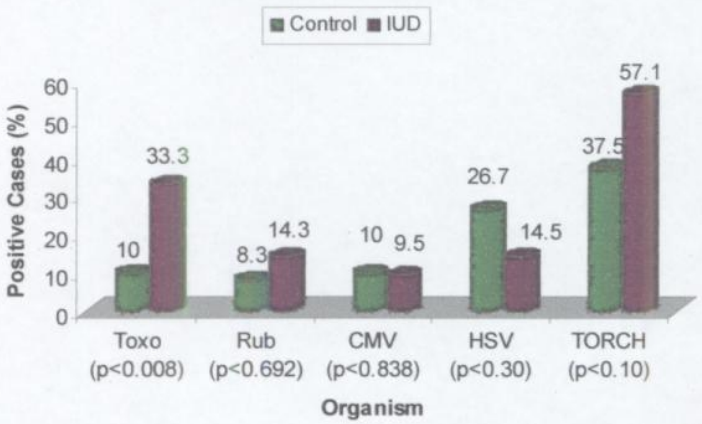


Intrauterine Fetal Death

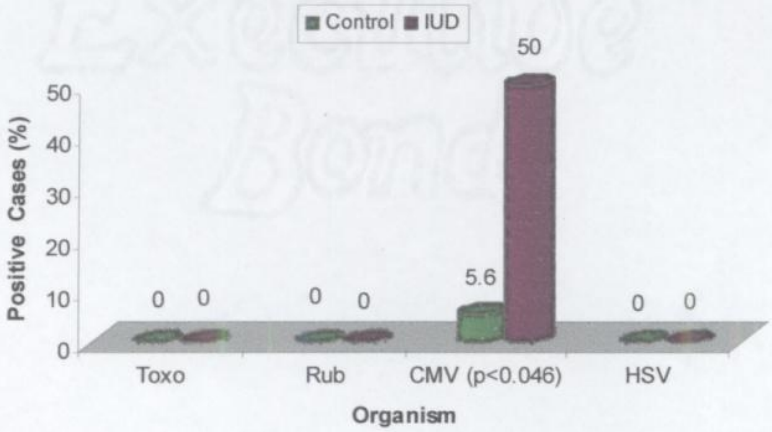
The study on Intrauterine Fetal Death was conducted on 24 IUD cases and 84 full term normal pregnancies as controls. The mean age ratio of the IUD cases and controls were as 24 : 23. Teenagers account for about 6.9% of the controls and 5% of the IUD cases. The age groups under which various categories were distributed and the prevalence is presented in the Graph. IUD. 5. Here 44.7% of the controls and 30% of the IUD cases were primigravida as presented in the Graph. IUD. 6. The parity of the study groups are presented in the Graph. IUD. 7. About 37.5% of the controls and 13.3% of the IUD cases were primipara while 50% of the controls and 42.9% of the IUD cases had previous pregnancy loss as shown in the Graph. IUD. 8. All the IUD cases and 86.9% of the controls were from low socioeconomic background. Other related parameters are presented in the Graph. IUD 9. Of the patients with low AFI values, 66.7% were among the IUD cases and 33.3% were from the control group.

The IgM analysis of the maternal sera showed statistically significant influence of *Toxoplasma gondii* in the IUD cases. The maternal IgM seropositivity levels are given in the Graph. IUD. 1. The transplacental infection rate was measured through IgM analysis of cord blood sera and only CMV showed significant influence (Graph. IUD.2). TORCH cross infections and IgG levels of mothers are represented in Graph. IUD. 3 and 4.

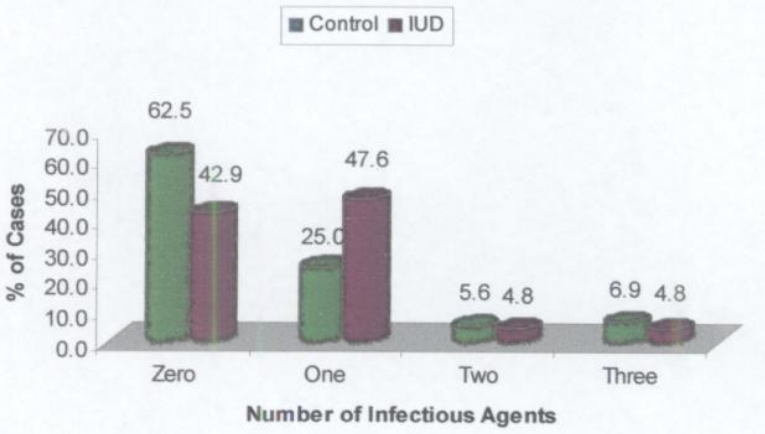
Graph. IUD. 1
IgM seropositivity in Maternal Sera



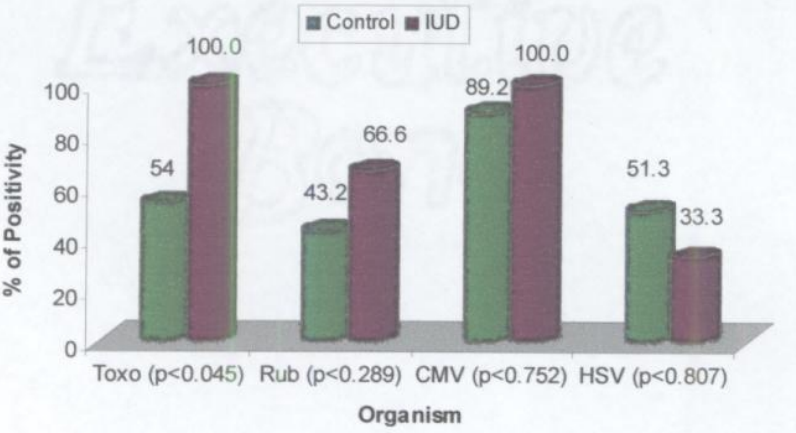
Graph. IUD.2
IgM Seropositivity in Cord Blood Sera



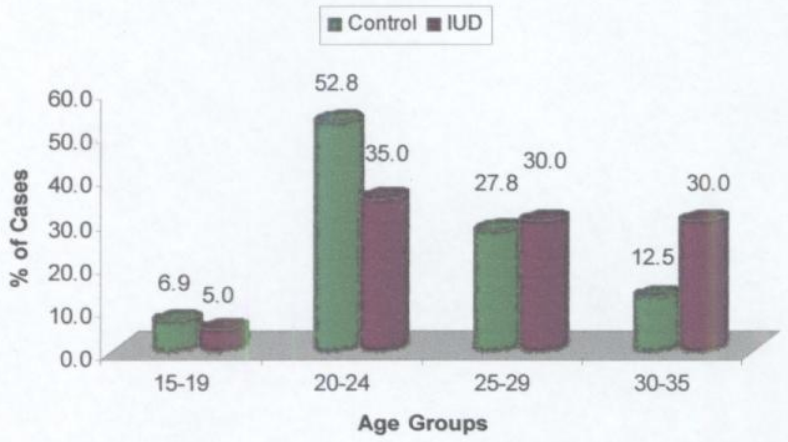
Graph. IUD. 3
TORCH Cross infections of Mothers



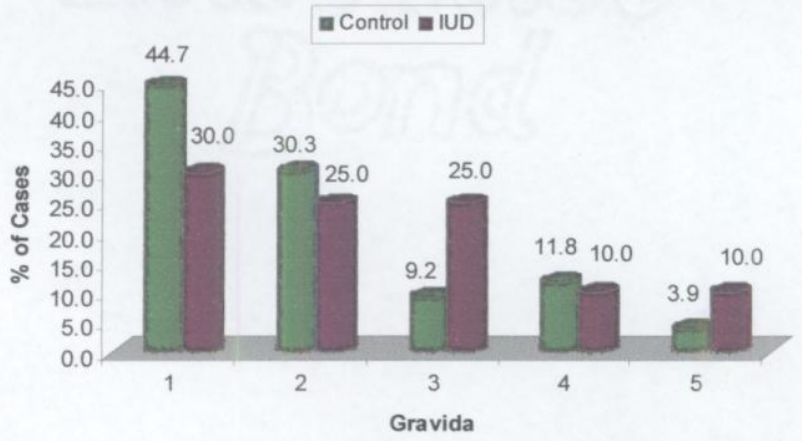
Graph. IUD. 4
TORCH IgG Seropositivity in IUD cases



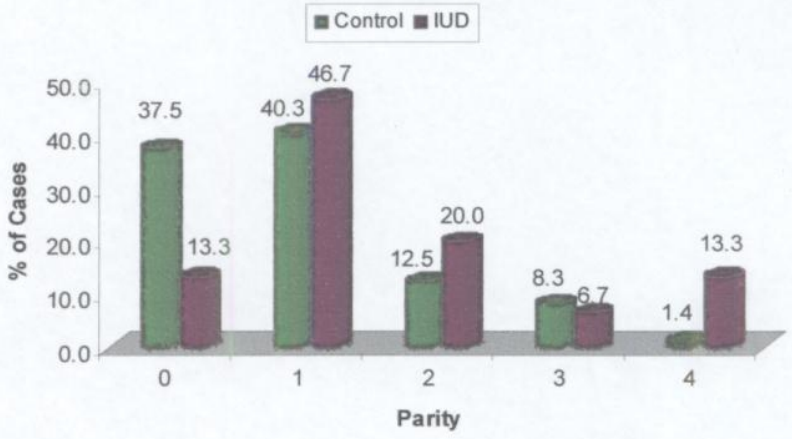
Graph. IUD. 5
Age Groups and IUD



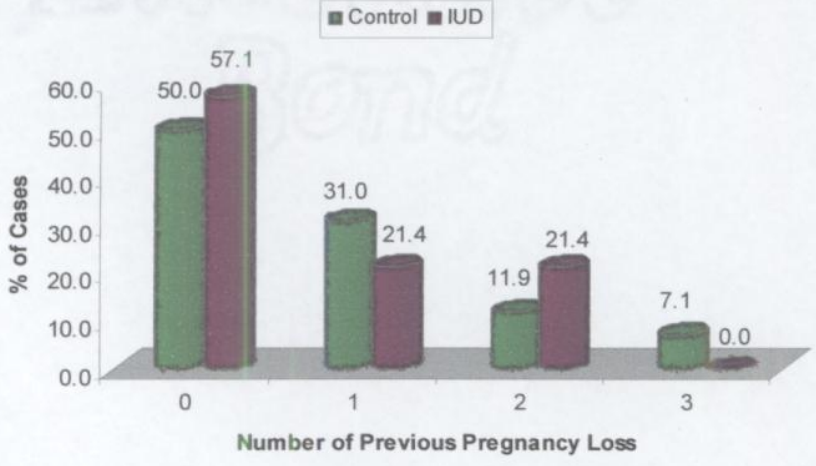
Graph. IUD. 6
Gravida of the Study Group



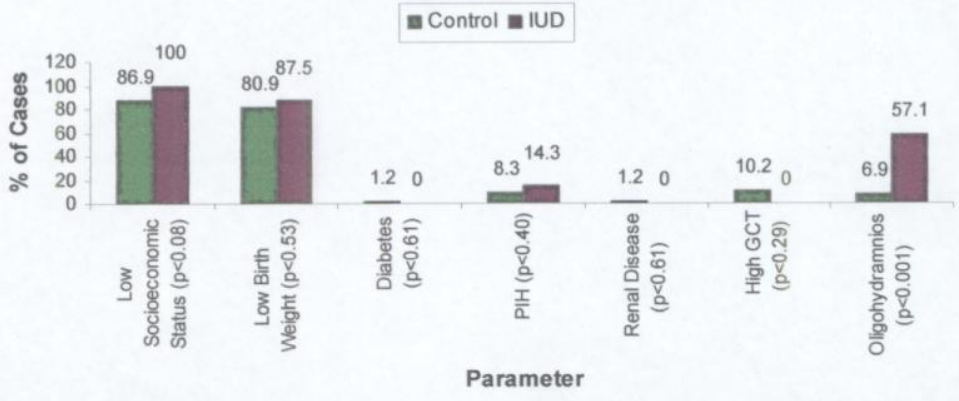
Graph. IUD. 7
Parity of the Study Groups



Graph. IUD. 8
Previous Pregnancy Loss



Graph. IUD. 9
Other related parameters



Congenital Anomalies

The study on congenital anomalies was done on 62 cases of anomalies in comparison with 84 normal pregnant women that acted as controls. Their medical, clinical, and serological data were analyzed to correlate congenital anomalies with various parameters under consideration. The low socioeconomic status of 95.5% of the test group was found as significant compared to the 86.9% of the control group. This observation seems to be slightly significant ($p<0.094$). Neither the test nor the control groups had diabetes, HIV infection, Syphilis and Hepatitis.

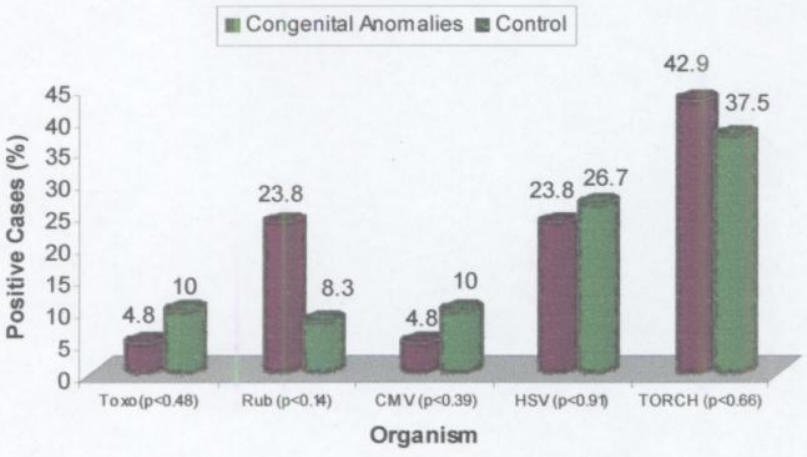
The complications observed in the study group included Microcephaly, Hydrocephalus, Microencephalopathy, Holoprosencephaly, Meningomyelocele, Anencephaly, Coarse facies, Cataract, Microopthalmia, Splenomegaly, Hepatomegaly, Nephrocalcinosis, Congenital Heart Disease, Hydrops fetalis, Eczema, Cleft lip, Cleft palate, Lung anomaly, Prominent renal pelvis, Polydactily, Syndactyly, Absence of diaphrum, Preauricular skin tags, Lack of upper limbs, Micrognathia and Hypospadiasis. The details are presented in the Table. Congenital Anomalies. 1.

The IgM seropositivity of the mothers and infants against TORCH agents are presented in the Graph. Congenital Anomalies 1 and 2 respectively. TORCH cross infections are presented in the Graph. Congenital Anomalies.3. The immunity status of both groups against TORCH pathogens were analyzed through IgG estimation and presented in Graph. Congenital Anomalies. 4. Gravida, Parity and previous miscarriages were presented in Graphs: Congenital Anomalies. 5-7. Other parameters like socioeconomic status, pregnancy induced hypertension and Hb content of the patient, sex of the infant, AFI and GCT content are given in the Graph. Congenital Anomalies.8. incidence under age groups are presented in the Graph. Congenital Anomalies. 9.

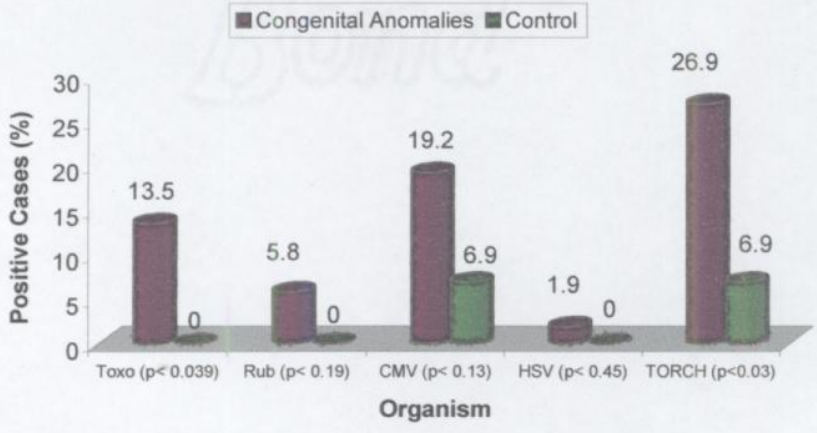
Table : Congenital Anomalies. 1

No.		List of Congenital Malformations Type of Malformation	Cases
1.	Head and nervous system defects	Microcephaly (a condition of abnormal smallness of the head usually associated with mental retardation)	5
2.		Hydrocephalus (abnormal increase in the amount of cerebrospinal fluid within the cranial cavity that is accompanied by expansion of the cerebral ventricles, enlargement of the skull and especially the forehead, and atrophy of the brain)	3
3.		Microencephalopathy (alterations of brain structure)	1
4.		Holoprosencephaly (Anterior midline brain, cranial, and facial malformations resulting from the failure of the embryonic prosencephalon to undergo segmentation and cleavage)	1
5.		Meningomyelocele (herniation of meningeal and spinal cord tissue through a bony defect in the vertebral column)	5
6.		Anencephaly (Neural Tube Defect)	1
7.		Coarse facies (Mental Retardation)	1
8.	Visual Defect	Cataract (a clouding of the lens of the eye or its surrounding transparent membrane that obstructs the passage of light)	3
9.		Microopthalmia (abnormal smallness of the eye usually occurring as a congenital anomaly)	1
10.	Other Defects	Splenomegaly (abnormal enlargement of the spleen)	7
11.		Hepatomegaly (Enlargement of Liver)	5
12.		Nephrocalcinosis (A condition characterized by precipitation of calcium phosphate in the tubules of the kidney, with resultant renal insufficiency)	1
		Congenital Heart Disease	1
13.		Hydrops Fetalis (Edema of the entire body due to abnormal accumulation of serous fluid in the tissues)	1
14.		Eczema (Dermatitis)	1
15.		Cleft lip, Cleft Palate	7
16.		Lung anomaly	1
17.		Prominent Renal Pelvis	1
18.		Polydactily, Syndactyly	2
19.		No Diaphrum	1
20.		Preauricular skin tags	1
21.		Fibroid Complicating Pregnancy	1
22.		No Upper Limbs	1
23.	Malformations	Micrognathia (shrinkage of the lower jaw)	2
24.		Hypospadiasis (Defect of Urethral Tube)	2

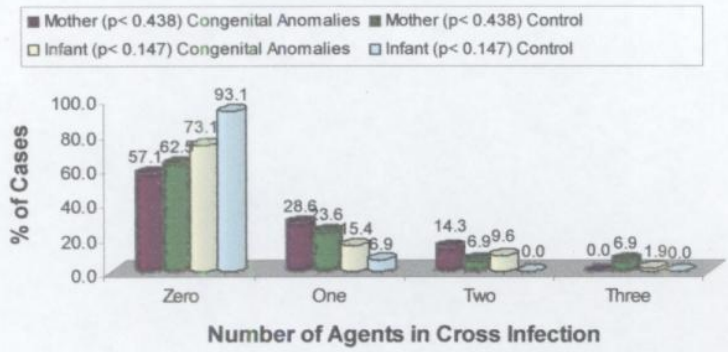
Graph. Congenital Anomalies .1
IgM Seropositivity of Maternal Serum



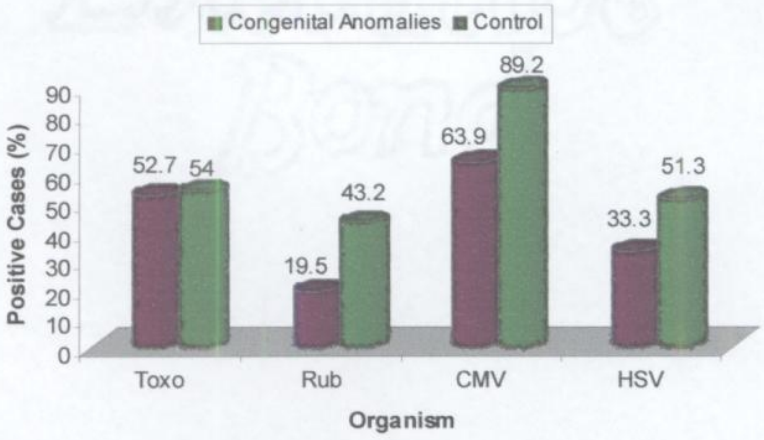
Graph. Congenital Anomalies .2
IgM Seropositivity of C ord Blood Serum
(Transplacental Infection)



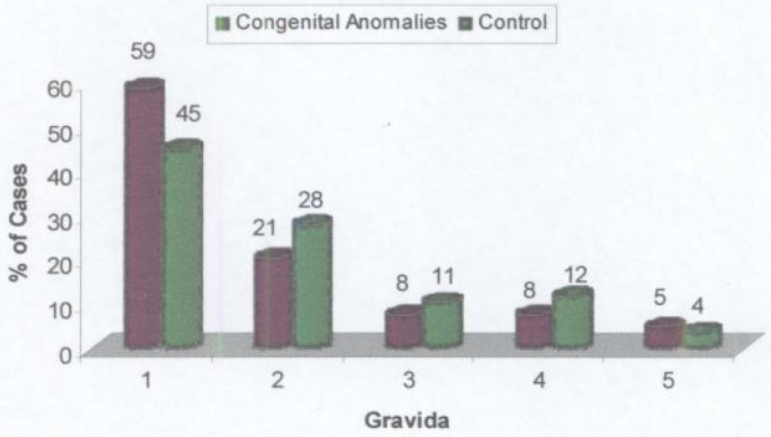
Graph: Congenital Anomalies.3
Cross Infections



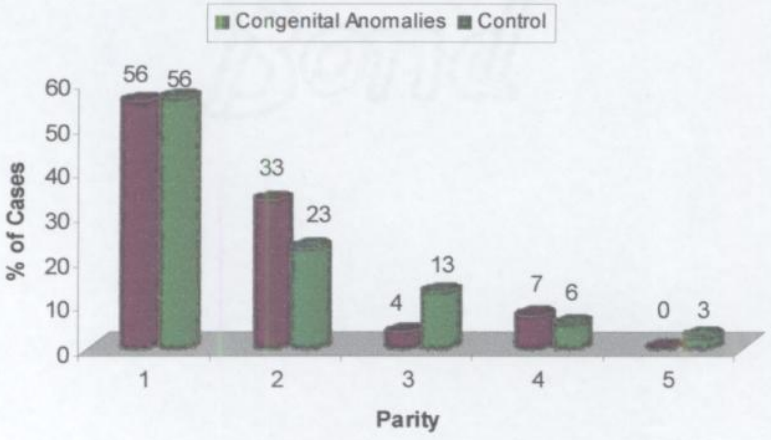
Graph. Congenital Anomalies .4
IgG Seropositivity of Mother



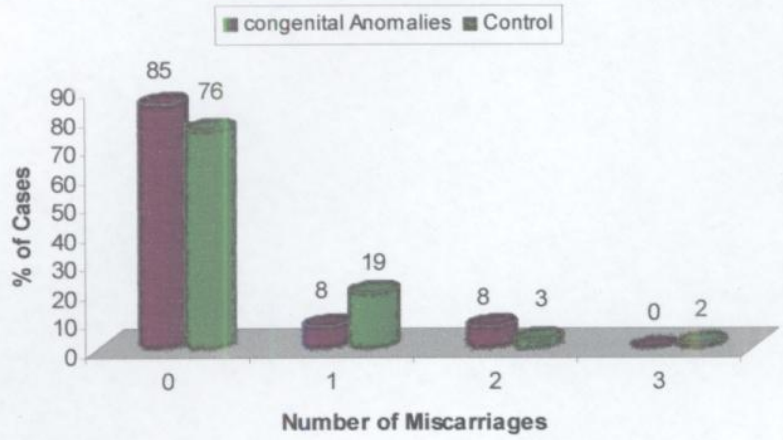
Graph. Congenital Anomalies. 5
Gravida of the Study Groups



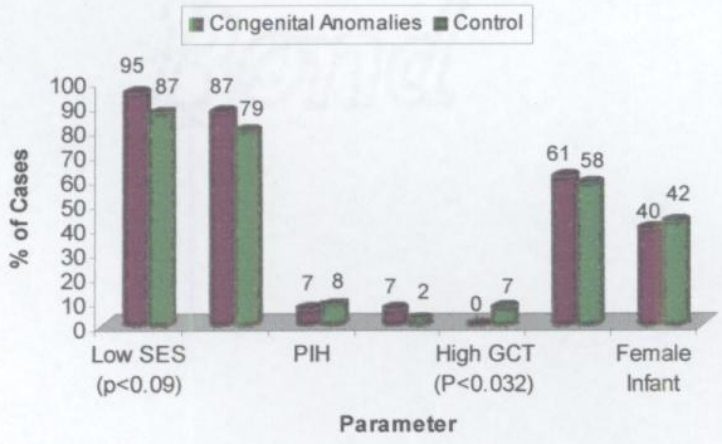
Graph. Congenital Anomalies. 6
Parity of the Study Groups



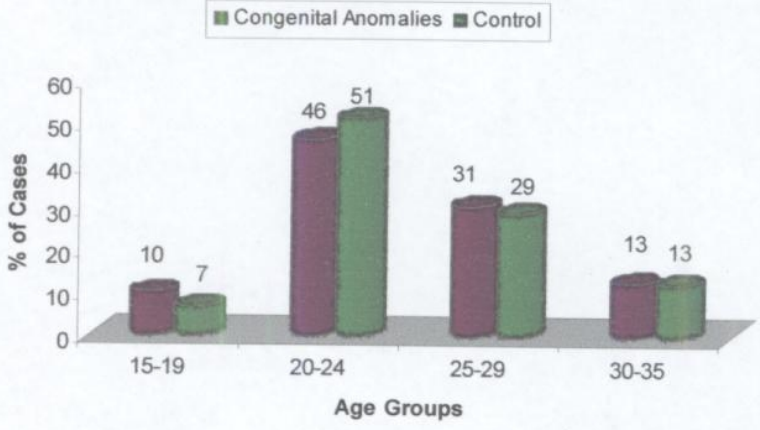
Graph. Congenital Anomalies. 7
Previous Miscarriages in the Study Groups



Graph. Congenital Anomalies. 8
Other Related Parameters

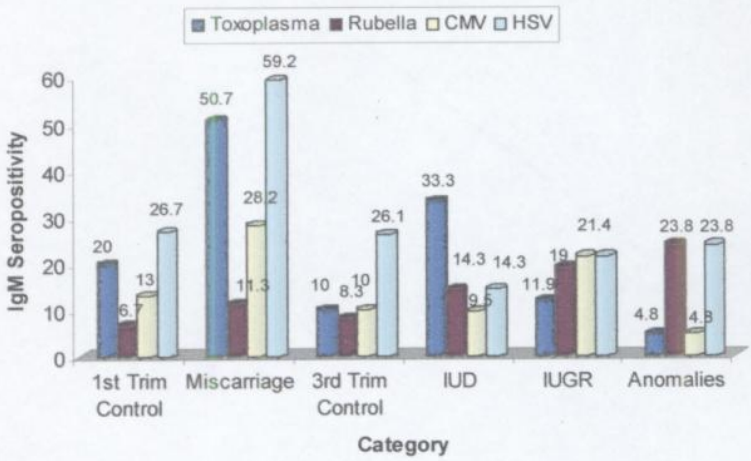


Graph. Congenital Anomalies. 9
Age Groups and Congenital Anomalies

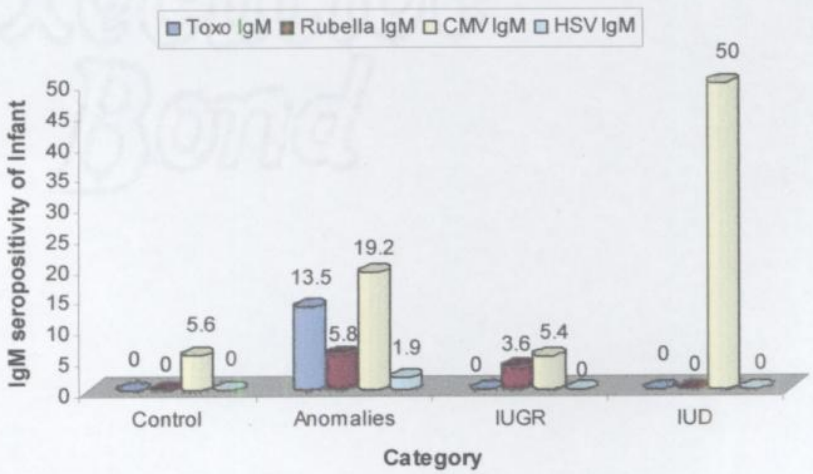


Comparison of TORCH IgG and IGM in the study groups

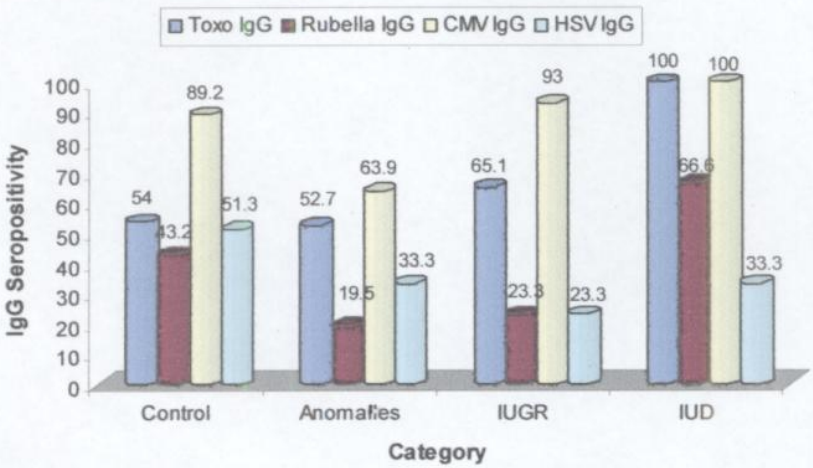
Graph: TORCH. 1 IgM Status of Mothers



Graph: TORCH.2 Transplacental Infections



Graph: TORCH 3. IgG Seropositivity



PREVALENCE OF TORCH INFECTIONS IN PREGNANCY RELATED COMPLICATIONS IN THE MALABAR REGION OF KERALA

Thesis submitted to the
University of Calicut
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY
IN
MICROBIOLOGY

UNDER THE FACULTY OF SCIENCE

By

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2006

Discussion

Miscarriage

The mean age of the miscarriage cases in this study was 23.8 years and this is very close to that of normal pregnant women, which was 23.9 years. Miscarriage was observed among 12.5% of the teenagers of this study. This is found as high compared to the rate of 5.5% observed in other parts of India.[167]. Increased rate of pregnancy related complications like abortion, IUGR and pre-eclampsia in teenage mothers were reported in the previous studies[158,160,161]. May be due to similar observations, Kumbi,S and Isehak,A recommended for the initiation of a programme which should easily reach the adolescent to promote family life education, contraception, counseling and education related to early marriage [162].

In majority (90.1%), abortion occurred in their first trimester of pregnancy. Of these, 14.81 % mothers were in the age of 15-19 years or teenagers; the rest falling in the other age groups. The overall teenage pregnancy rate of 11% (Graph. Miscarriage. 1) observed in this study group (including test and control) comes within the range 8-18.68 [161,167] reported from various parts of the world.

A history of previous abortion has been noticed among 39.5% of the aborted mothers and this is significant compared to that observed in only 26.7% of the controls. Of the 39.5%, most (28.2%) had undergone abortion only once previously, while 8.5% and 2.8% had it twice or thrice respectively. According to Reagen,[54] previous abortion is the most important predictive factor for spontaneous abortion and a woman's first pregnancy outcome has profound consequences on all subsequent pregnancies. Many authors[157,168] had previously described the necessity of systematic evaluation and management of couples with early pregnancy wastage. Knowledge of the patient's reproductive history is essential for the clinical assessment of her risk of spontaneous abortion. These workers also suggested routine screening for maternal infections, sympathetic counseling, and educating the primigravida about the pregnancy related infections and its complications.

The gravida and parity status of the groups under this study, which included 71 abortion cases and 30 controls, are presented in Graph. Miscarriage. 3& 4. Here 54.3% of the abortion cases were nullipara while 35.7%, 8.6 % and 1.4 % respectively were of parity 1, 2, and 3. Among the controls, the incidence of the corresponding parity was as 80%, 6.7%, 13.3% and 0% respectively. The abortion cases were very much higher (35.7%) to the controls (6.7%) among the primipara in this study. The data from the National Congenital Rubella Surveillance Programme showed that 44% of children with congenital rubella were born to primiparae. It is on this basis they emphasized the need for rubella vaccination prior to a woman's first pregnancy.[154] In another study, Gratacap-Cavallier, B. et al [156] in France had reported that the CMV seroprevalence was independently influenced by parity and age. Among the miscarriage cases, 35.5% were of primigravida and 31.6%, 21.1%, 7.9% and 3.9% respectively were of gravida 2, 3, 4 and 5. As presented in the Graph. Miscarriage. 4, none of the control belonged to the

primigravida group ($p<0.039$). The unawareness of the primigravidae about the maternal complications due to infections has been reported by Eiser and Eiser[157].

The socioeconomic status, hemoglobin content and pregnancy induced hypertension (Graph. Miscarriage. 10), though have been studied, were not seen as influencing miscarriage cases of this study group, though Canessa, A. *et al.*,[112] had observed an increased prevalence of cytomegalovirus infection in women from low socioeconomic background. Neither the miscarriage cases nor the controls had diabetes and this parameter seems to be unrelated to miscarriage.

Majority (48.63%) of the miscarriage cases were of missed abortion category. Prevalence of other categories was as threatened abortion- 16.63%, complete abortion -15.26%, spontaneous abortion -11.16% and incomplete abortion- 8.31%.

The role of the intrauterine pathogens –*Toxoplasma gondii*, Rubella virus, Cytomegalovirus and *Herpes simplex* virus in causing miscarriage was studied by detecting the IgM immunoglobulins specific to these pathogens. The immunity status of general population and the study groups to these pathogens were also analyzed through the IgG estimations.

Toxoplasma gondii

Toxoplasma IgM was observed in 50.7% of the miscarriage cases and this is statistically significant ($p<0.03$), compared to it's prevalence in 20% of the normal. This rate is almost near to the 53.1% *Toxoplasma* infection observed by Zargar, A. H. *et al.*,[169] among the miscarriage cases in Srinagar.

the normal. This rate is almost near to the 53.1% *Toxoplasma* infection observed by Zargar, A. H. *et al.,*[169] among the miscarriage cases in Srinagar. Repeatation.

Prevalence of *Toxoplasma* specific IgM was observed as differing among various categories of miscarriages of this study, as incomplete -33.3%, complete - 50.0%, threatened - 54.5% and missed abortion- 48.7%. Cengiz *et al.,*[130] is of opinion that to enable proper management of complicated pregnancy, detection of *Toxoplasma gondii* IgM level is necessary for which purpose active, latent or reactive toxoplasmosis have to be discriminated.

As per our study, 67.7% of the general population of Malabar region has Toxo -IgG; i.e. immunity against *Toxoplasma gondii,* leaving 32.3% susceptible to the infection. The infection observed in 50.7% is more or less in agreement with this susceptibility to Toxoplasma in miscarriage cases. The immunity level in 67.7% is high compared to the 35.6%[114], 47.5%[113] and 53.6% [170] observed against this pathogen in other populations.

In France, where 53.6% of IgG positivity was observed, nearly 1% of the pregnancies were only complicated due to toxoplasmosis. In our study group, toxoplasmosis can be ascribed as a cause to pregnancy complications, in 50.7%, which is very much higher. In France they adopt antenatal screening programs against the disease. Canessa, A. *et al.,*[112] reported an overall seroprevalence of 40.7% to *Toxoplasma gondii* among the pregnant women from Italy and suggested the absolute necessity to screen for TORCH infections in women having the history of abnormal pregnancies, in order to prevent birth defects and perinatal complications.

The comparatively higher rate of resistance against *Toxoplasma gondii* may depend upon the environment and life style of the people here. A previous observation[129] that, '*Toxoplasma* antibodies are found to be higher

in recent aborters from the rural areas, where contact with soil is common, regardless of whether cats are kept pets or not', can be true here also.

Rubella Virus

This study has revealed that 90.4% of our population have been pre-exposed and acquired resistance to Rubella virus as observed through the Rubella specific IgG estimation. This is in agreement with the corresponding rates of 93.3% [114], 89.9% [113] and 85.5% [170] observed in other populations in different countries. The Rubella IgM found in 11.3% of the miscarriage cases is almost in accordance with the 9.6 % susceptibility to this pathogen observed in our general population. But, compared to the incidence of Rubella IgM in 6.7% of the normal, the incidence of 11.3% is insignificant (P <0.597).

On category- wise analysis of miscarriage cases for Rubella -IgM, 25 % of the complete abortion cases had evidence of rubella infection. Incomplete, threatened and missed abortions had the incidence lower, as 16.7%, 9.1% and 7.7% respectively. Singler *et al.,*[134] have reported rubella reinfection in 0.32% females showing seropositivity for both IgG and IgM at a time.

Cytomegalovirus (CMV)

As with Rubella, CMV infection was found as not contributing much to the miscarriage in this study. When 28.2% of the aborted mothers had CMV-IgM, 13% of normal pregnant women showed IgM seropositivity to CMV (Graph. Miscarriage. 5). But this difference is not statistically significant.

Among the miscarriage categories, incidence of CMV -IgM was as, complete abortion -37.5%, threatened abortion- 36.4%, and missed abortion - 33.33% while incomplete abortion category was completely seronegative for this pathogen. Only 3.2% of the general population is susceptible to this intrauterine pathogen as per this study, since 96.8% were immune to it, observed through their IgG estimation. Other workers have also observed more or less the same level of immunity to the pathogen as 92.1%[114], 87.8%[113] and 97.2%[170] among their people.

As per this study, prevalence of CMV and Rubella infections in miscarriage was higher compared to the control, though the difference is not statistically significant.

***Herpes simplex* virus (HSV)**

As in case of *Toxoplasma gondii*, HSV infection also has a significant correlation with the incidence of miscarriage in this study. HSV specific IgM observed in 59.2% of the cases is found as significant (P<0.22) compared to the incidence in 26.7% of controls. The infection in 59.2% of miscarriages agrees well with the of 61.3% infection susceptibility to HSV observed in our general population. The HSV-IgG found in 38.7% of our people is much lower to the corresponding observations of 90.9% [114] and 87.5%[113] by previous workers. Also, the study revealed that all (100%) of the incomplete abortion cases, 87.5% of the complete, 48.7% of missed and 45.5% of threatened abortions were positive for HSV- IgM.

Toxoplasma and HSV were found to have a significant role in causing miscarriages in this study. Almost the same rate of influence was observed for these two pathogens on various categories like missed and threatened abortions while it was differing in complete and incomplete miscarriage. HSV

infection was noticed in all cases of incomplete abortion, while Rubella and CMV were not found as very prevalent. CMV infection was totally absent in incomplete abortion category. Mihaela M. *et al.*[171] have recently reported that CMV infections have no role in miscarriage, which is partially true as per this study.

Another important finding here was the presence of cross infections or condition where cases being positive to more than one TORCH pathogens at a time (Graph. Miscarriage. 6). When 40.8% of the abortion cases and 20 % of the normal have positivity against any one of these, the rate of multiple seropositivity against two, three and four were as 31%, 8.5% and 5.6% among the miscarriage, and as 13.3%, 6.7% and 0% among the control group, respectively. It is very important that 5.6% of the aborted mothers were infected with all the four pathogens. At the same time none of the control was infected to this extent. Gong Z *et al.*,[143] have reported cross infections with all the TORCH pathogens in only 2.6% in their study. A much higher rate (93.4%) of multiple seroconversion to TORCH pathogens among pregnant women has been reported by some other workers also. [11] Y. Aubard *et al.*,[172] reported double maternal seroconversion against *Toxoplasma gondii* and Cytomegalovirus. Double maternal seroconversion was observed in our study also in 31% of miscarriages, which is significant compared to the occurrence in 13.3% of the controls. As shown in the Graph. Miscarriage. 8, the TORCH cross infections shows significant variation ($p<0.003$) between the abortion categories.

For syphilis, on the basis of the seronegativity observed in all the members of the study group and controls, the necessity of the existing practice of routine serological antenatal screening can be reconsidered. Our suggestion is supported by the observations and arguments of Rodier *et al.*,[170]

who has suggested that the serological antenatal screening must be discussed before doing it routinely, and it should be based on the economic situation of the health system. Though prevalence of infections like AIDS, hepatitis etc., was studied in these patients, no significant relationship was observed for these factors with the incidence of abortions.

Intrauterine Growth Retardation

Sixty four full term deliveries with IUGR and Eighty four normal deliveries were studied to assess the role of TORCH infections in Intrauterine Growth Retardation. The mean age of the mothers having IUGR and control was 23 and 24 years respectively. Male to female ratio of the infants of IUGR and control categories was 1:1.4 and 1:1 respectively.

In this whole study group, comprising IUGR and controls, 10.8% of the pregnancy was among teenage girls. When the previously observed Indian rate of teenage pregnancy was only 6.3%,[167] it is about 15.1% among the IUGR cases and 6.9% among the controls, together forming 10.8% in the whole study group. Teenage pregnancy rates reported from other parts of the world ranged from 8-18.68 [161,167]

Incidence of IUGR was found to be decreasing with increase in gravida status of the mother, as Primigravida –53.6%, gravida 2- 21.4%, gravida 3 – 14.3%, gravida 4 –7.1% and gravida 5 –3.6%. (Graph. IUGR.6). Poor pregnancy outcomes in primigravid women and teenagers were pointed out by Jonas *et al.*,[165] in 1992. Also 76.6% preponderance of primigravida with inadequate antenatal care was reported by previous workers.[161] They also

point out the ignorance of teenage mothers about the pregnancy care that may complicate pregnancy outcomes.

Here 51.9 % of the IUGR and 32.4 % of the control mothers had had previous abortion (Graph. IUGR.7). Observation by Regan [54] that previous pregnancy loss is a predictive factor for the outcome of subsequent pregnancies, is true here to some extent.

Both maternal and cord blood samples were analyzed for IgM and IgG against TORCH pathogens using ELISA method. Maternal IgG screening results as presented in Graph. IUGR. 3 show seropositivity to *Toxoplasma gondii*, Rubella virus, CMV and HSV as 65.1%, 23.3%, 93% and 23.3 % respectively in IUGR cases and as 54%, 43.2%, 89.2% and 51.3% respectively in controls. Here we should recall the IgG prevalence of the general population in the Malabar region, which is 67.7%, 90.4%, 96.8% and 38.7%. In both IUGR cases and their controls the IgG level to these pathogens was low compared to that of the general population, except in case of HSV in the control group where the level is a little high. The cord blood IgG levels as given in Graph. IUGR 4 also showed similar pattern. It is quite natural, since IgG is a transplacentally transferable immunoglobulin and it's presence in mother's serum will be reflected in the cord blood.

IgG seronegativity was previously used with predictive value for the susceptibility to acquire primary rubella infection.[134] But many previous reporters have documented congenital rubella after reinfection.[93,134] The studies in Korean pregnant women suggest that the incidence of maternal primary infection during pregnancy seems to be rare and therefore most congenital infections in Korea might be following by maternal reactivation or reinfection.[138] HSV reactivation and latent infection is also well documented.

So IgG predictive value for susceptibility shows some degree of flexibility in case of these virus infections.

But in this study, when a good number of IUGR cases and control group are seronegative and there by susceptible to infection by TORCH agents, no statistically significant level of serum IgM to TORCH agents was observed in the IUGR mothers. (Graph. IUGR.1 and 2). Rubella and CMV seem to have some role with a respective prevalence of specific IgM in 19% and 21.4% cases, which is not significant compared to controls. Khan, N.A. and S.N. Kazzi [17] have reported of TORCH infections in 41% of the IUGR mothers they studied, while their infants were free of it. But Rubella specific IgM was present in 3.6% neonates of our study. Though Chaturvedi, P. and P.C. Desai [81] had pointed out the influence of neonatal infections in causing IUGR, the incidence observed here is not statistically significant, when compared to the control neonates.

Incidence of other related parameters like socioeconomic status, Hb content, PIH, GCT, Oligohydramnios and diabetes are given in the Graph.IUGR 9. The socioeconomic status of the study groups was almost same in the controls (86.8%) and IUGR cases (85.9%). Low birth weight that accounts for the neonatal mortality in low socioeconomic groups was reported from Leningrad [70]. But our observations do not support this. A slightly lower value of Hemoglobin content was found in 63.3 % of the IUGR cases compared to controls in this study. Malnutrition as a cause of IUGR was reported among Guatemalan Indians [72]. Glucose challenge test values and diabetes prevalence were not significantly different among the test and control.

While considering the other parameters influencing IUGR, it was noticed that pregnancy induced hypertension and oligohydramnios have roles mention worthy. Pregnancy induced hypertension occurred in 17.2% of the IUGR cases while it was observed only in 2.9% of the controls. This is statistically significant ($p<0.006$). Some previous workers had also reported PIH as one of the factors influencing IUGR.[69] A prevalence of 19% PIH has been reported by Khan, N.A. and S.N. Kazzi in mothers with IUGR.[17] The decrease in placental blood volume was a possible factor behind intrauterine growth retardation due to pregnancy-induced hypertension.[150] The infants of hypertensive mothers had a three fold increased risk of perinatal mortality[71].

We have also noticed that IUGR is significantly related to Amniotic Fluid Index. Incidence of 41.2 % of oligohydramnios in the test group is significant ($p<0.006$) compared to the 7.1 % of the control group. A similar study showed a significant correlation between oligohydramnios and IUGR in which 39.56% of the oligohydramnios cases had IUGR.[146] Observations of Rabe *et al.*,[144] on reduced amounts of amniotic fluid showed that children with oligohydramnios had severe fetal malformation.

In this study, 12.5% of the IUGR cases had one or more of the anomalies/complications like renal disease, diabetes, microcephaly, choreoretinits, splenomegaly, cutaneous malformation, hepatomegaly, jaundice and asymmetric IUGR, though in very few numbers. None in the control group suffered from any of these complications. Various workers had reported complications like congenital anomalies, [147] prenatal mortality [173] associated with IUGR and oligohydramnios.

The GCT values had no significant relation with the IUGR, as per this study. About 8.5% of the controls and 7.5% of tests group had GCT values

above 130 mg/dl. Both the control and test group were free of Syphilis, AIDS and Hepatitis.

In conclusion, as per this study, infection with TORCH agents, though has been observed in IUGR mothers, is not found as significant in causing intrauterine growth retardation. Some other factors like Pregnancy Induced Hypertension, Oligohydramnios and age are found to be influencing IUGR. The role of various factors including maternal, genetic, placental and environmental factors in causing IUGR was reported by various workers [64]. More than 10% of the IUGR cases were thought to be caused by infectious causes [77]. Though there are supporters [69,78,79] for the view that TORCH infections are one of the influencing factors of IUGR, our observations are not supportive to this.

Intrauterine Fetal Death

This part of the study was done on 24 cases of Intrauterine Fetal Death (IUD) in comparison with 84 full term normal pregnancies that acted as controls. The mean age ratio of the mothers of IUD cases and controls was as 24:23. That is, age of mothers is not a factor influencing IUD. Teenagers account for only about 5% of the IUD cases and 6.9% of the controls (Graph.IUD. 5). It seems that intra uterine death is not very common in teenage mothers of the Malabar area from where majority of the cases represented this study group. IUD prevalence in the other age groups like 20-24 years (35%), 25-29 years (30%) and 30-35 years (30%) were almost similar, while in controls 52.8% belonged to the age group, 20-24 years (Graph. IUD. 5). Occurrence of 30% of IUD cases in the 30-35 years group is higher compared to the 12.5% observed in the same age group of the controls.

Intrauterine fetal death was happening in male and female infants in the same proportion. When the male to female ratio in the IUD cases was 1:1 it was as 1.4:1 in controls. i.e., it seems that IUD is happening to male than female infants, as per this study.

Here 44.7 % of the controls and 30% of the IUD cases were primigravida as presented in the Graph. IUD.6. Like IUGR, IUD also decreased with increase in gravida status of the mother, as Primigravida -

30%, gravida 2 -25%, gravida 3 -25 %, gravida 4 -10 % and gravida 5 -10 %. (Graph. IUD.6). Jonas *et al.*,[165] had observed poor pregnancy outcomes in primigravid women and teenagers and 76.6% preponderance of primigravida with inadequate antenatal care was reported by previous workers.[161] They have attributed it to the ignorance of teenage mothers about the pregnancy care that may complicate pregnancy outcomes.

As represented in the Graph. IUD 7 about 37.5% of the controls and 13.3% of the IUD cases were primipara. Also previous pregnancy loss observed in 42.9% of the intrauterine fetal death cases were not relevant compared to the incidence in controls (Graph. IUD. 8). All (100%) the IUD cases and 86.9% of the controls were from low socioeconomic background. It can be assumed that parity, previous pregnancy loss etc. has no much influence in causing IUD in mothers. Low socioeconomic status of the mothers is found to be influencing IUD to some extent. Several previous studies are there to support that women from poor socioeconomic background are at high risk of pregnancy related complications. [164-166]

TORCH IgG levels of IUD mothers are presented in Graph. IUD.4. All members (100%) of the IUD category were positive for Toxoplasma and CMV -IgG whereas 66.6% and 33.3% respectively were seropositive to Rubella and HSV. As per this, no cases were at risk of seroconversion to Toxoplasma and CMV while 33.3% and 66.6% respectively have susceptibility to Rubella and HSV. But IgG seronegativity, though was previously used with predictive value for the susceptibility to acquire primary rubella infection,[134] since there are well documented reports of HSV reactivation and latent infection available, IgG value for predicting susceptibility shows some degree of flexibility in case of these virus infections.

The IgM study of the maternal sera of IUD cases shows that *Toxoplasma gondii* has got a significant role in causing intrauterine fetal death. Graph. IUD.1 shows a statistically significant ($p<0.008$) difference existing in the level of *Toxoplasma gondii* IgM between IUD cases (33.3%) and controls (10%). It is to be noticed that 33.3% of Toxoplasma IgM is observed in these mothers when all of them (100%) have Toxoplasma IgG in a detectable level. That is, role of IgG in predicting susceptibility to a pathogen is doubtful here. But the Cord blood analysis of IUD cases has shown seronegativity for Toxoplasma IgM which shows that the fetal death has happened here before producing a proper immune response to the infection. It is to be assumed that in 33.3% of these mothers IgM may be in the declining stage while IgG was taking over, because IUD may be the result of an infection caused probably in the last trimester. Intrauterine fetal death includes all fetal deaths occurring during pregnancy, after 28th week of gestation and also during labour.[174]

The other organisms like Rubella and HSV seem to have no significant role in causing IUD, their maternal IgM prevalence being 14.3% and 14.5% in IUD cases and 8.3% and 26.7% in controls respectively. Also, complete seronegativity was noticed for these pathogens in cord blood.

In mothers of intrauterine fetal death, CMV-IgM was not significantly observed and its prevalence was as 9.5% in IUD cases and 10% in controls (Graph. IUD.1). At the same time, cord blood analysis (Graph. IUD.2) revealed a statistically significant ($p<0.046$) level of IgM with a prevalence of 50% in intrauterine fetal death cases and 5.6% in controls. Probably an early infection of CMV in mother could have extended to the stage of intrauterine infection, which is supported by the presence of maternal IgG to CMV in IUD cases. Pass, R [36] had reported that in case of CMV, the likelihood of

transmission of maternal infection to fetus is similar early as well as late in gestation and also even if the mother was infected long before conception. Also in case of CMV the chance of reinfection and reactivation has also been reported. IgM antibody is not transplacentally transferable and it's presence in the cord blood is possible only by it's synthesis in the fetal body, by it's own immune system. This could be happened only in a later trimester [175] and hence is it detected in the cord blood.

The findings in our study is almost in agreement with the observations of Moyo *et al.,*[84] who had reported high frequency of positive cases for *Toxoplasma gondii* (along with *Treponema pallidum)* in their study on IUD. They also reported that IUD had no relation with infections by human immunodeficiency virus, cytomegalovirus, *Herpes simplex* virus or rubella virus, as our observation. But in our study CMV is found to have role on IUD.

It is very important that 57.1 % of intrauterine fetal death cases of this study were infected with one or the other of the TORCH agents while the corresponding rate in the control group was 37.5% (Graph. IUD.1). On further analysis (Graph. IUD.3) it can be seen that cross infection with TORCH pathogens had not played a significant role in causing IUD, since no significant number of IUD cases were found to be infected with two or more TORCH pathogens.

The parameters like low birth weight, diabetes of mother, pregnancy induced hypertension, renal diseases and high GCT values were found to have no influence on IUD in this study. All (100%) of the IUD cases and 86.9% of the controls ($p<0.08$) were of low socioeconomic group.

Oligohydramnios could be correlated with Intrauterine Fetal Death of the fetus from the low AFI values observed in 57.1% of the IUD cases and 6.9% of the controls. This observation was of high statistical significance ($p<0.001$). Golan, A. *et al.,*[148] reported an Intrauterine fetal death rate of 5.5% in pregnancies with oligohydramnios. The gross perinatal mortality in their study was 16%. Rabe *et al.,*[144] had observed that children with oligohydramnios had severe fetal malformations.

In conclusion, infections with *Toxoplasma gondii* and presence of oligohydramnios are found to have a role on IUD compared to infection with other TORCH agents and other factors like low birth weight, diabetes of mother, pregnancy induced hypertension, renal diseases, high GCT values, maternal age etc. as per this study. Low socioeconomic status seems to be influencing IUD to some extent. Precise diagnosis of the causes of IUD is a prerequisite as a basis for counseling, prevention and treatment of the patients.

Congenital Anomalies

This part of the study was conducted by analyzing 62 cases of congenital anomalies and comparing the findings with 84 normal full term deliveries. Their medical, clinical, and serological data were analyzed and recorded in order to correlate with congenital anomalies.

The complications observed in the study group included mainly Microcephaly, Hydrocephalus, Microencephalopathy, Holoprosencephaly, Meningomyelocele, Anencephaly, Coarse facies, Cataract, Microopthalmia, Splenomegaly, Hepatomegaly, Nephrocalcinosis, Congenital heart disease, Hydrops fetalis, Eczema (Dermatitis), Cleft lip, Cleft palate, Lung anomaly, Prominent renal pelvis, Polydactily, Syndactyly, lack of Diaphrum, Preauricular skin tags, Fibroid complicating pregnancy, lack of Upper Limbs, Micrognathia and Hypospadiasis. The details are presented in the Table: Congenital Anomalies. 1.

A study [92] conducted in six villages of Ambala District had screened for congenital malformations and reported their prevalence as Cardiovascular malformations (37%) musculoskeletal (30%), gastrointestinal (23%), central nervous system related (13%) and genitourinary anomalies (6.6%). Incidence of these and other anomalies in our study group was as Cardiovascular (1.7 %) musculoskeletal (32.14%), gastrointestinal (21.42%), central nervous

system related (30.36%), genitourinary (7.14%) and visual defects (7.14%). Higher levels of central nervous system disorders were observed in our study group. Several authors reported similar complications previously. [88,91,93]

Mothers of congenital malformation cases when categorized age wise (Graph. Congenital Anomalies. 9), there observed no difference in the mean age ratio between the anomaly cases and controls, and it was as 24: 25. Prevalence of congenital anomalies in mothers of age groups under study was as 20-24 years-46%, 25-29years-31% and 30-35 -13%. This incidence was almost similar to that observed in the respective age groups in controls, showing that there is no role for age of mothers in causing congenital anomalies. Ten percent mothers of the congenital anomalies and 7% control mothers were teenagers. Teenage pregnancy was reported to cause various complications including malformations.[163] Poor pregnancy outcomes in teenagers and primigravid women had also been pointed out by Jonas *et al.*,[165] and they have attributed the poor outcome to the ignorance of teenage mothers about the pregnancy care, which may complicate pregnancy outcomes.

Congenital anomalies were found in the same rate and pattern in male and female infants among the test and control groups. The male: female ratio of the infants in the congenital anomaly group was 1.5:1 whereas in controls it was 1.4:1. Almost comparable male to female ratio of 1.3:1 was reported in a previous study. [94]

Gravida, parity status and history of previous miscarriages are presented in Graphs. Congenital Anomalies. 5-7. Here, 56% each of both test and control groups were primipara. About 59% of the anomaly cases and 45% of the controls were of primigravida, which shows that gravida has no role in

causing congenital malformations. Both the anomaly cases and controls had history of previous miscarriages. It seems that gravida, parity and previous miscarriage have not influenced the study groups.

The immunity status of the cases under study was analyzed through IgG estimation and is presented in Graph. Congenital Anomalies. 4. The IgG seropositivity of the normal full term pregnant women were as Toxoplasma-54%, Rubella -43.2%, CMV–89.2% and HSV-51.3%. The corresponding IgG levels of the congenital anomaly cases were not significantly different from these (except to Rubella) and were as 52.7%, 19.5%, 63.9% and 33.3% respectively. In some previous studies carried out on congenital disorders, 17.3% showed IgG seropositivity to *Toxoplasma gondii* [176], 86.33% to Rubella virus [94], 56.3% to CMV [115], and 54.3% to HSV [115]. Toxoplasma IgG level of our anomaly cases is very high compared to these studies, while the pattern is the reverse for Rubella, where the level is high in the referred study compared to ours. For the other two pathogens, such a drastic difference was not there for IgG, and more or less same level was observed in both the studies.

Graph.Congenital Anomalies:1 shows that TORCH specific IgM levels were not different in patients and controls thus revealing it's insignificance here. It could be so, since IgM is an indication of recent or current infection and that occurring in the later stage of pregnancy cannot be a contributory factor to congenital anomaly. This is because organogenesis of the fetus will be over by the first eight weeks and the infection after this stage will not influence the already developed fetus.[151] So IgM manifested here, whether it is in congenital anomaly case or control, may have nothing to do with the malformations observed and this IgM can be the result of infection happened recently, in the later stage of pregnancy.

When a measure of TORCH -IgM of the mothers fail to detect the role of the pathogens in congenital malformations, it is very necessary to consider the IgM positivity of cord blood serum, since fetal IgM is a sure indication of intrauterine infection. [175]

A notable finding here was the IgM seropositivity observed against all the intrauterine pathogens in cord blood serum, to a level of Toxo-13.5%, Rubella-5.8%, CMV-19.2% and HSV-1.9%. In controls, complete seronegativity existed against all these pathogens, except CMV in which case it was 6.9% (Graph. Congenital Anomalies.2). It is to be assumed that intrauterine infection in these cases had taken place in an early stage of pregnancy, probably at the time of organogenesis, leading to various anomalies observed here. In response to this infection, IgM is produced by the fetal body when it's immune system got matured for this, which usually begins by about 20 weeks of age, according to Jayaram Paniker.[175] The author has also reported that IgM in the fetus or newborn indicates the intrauterine infection and it's detection is useful in the diagnosis of congenital infections such as toxoplasmosis, rubella HIV infection and syphilis. Reports of infections of first trimester influencing the developing fetus are available from previous studies[90] also. According to these workers, these pathogens' infections may influence the microenvironment of placenta, including levels of enzymes and cytokines, and affect chondriosome that may induce the progress of birth defect.

These observations are also supported by the presence of IgG observed in the mothers of congenital anomaly cases that would have produced in response to the early infection which affected the fetal organogenesis. It is true that almost the same level of IgG was observed in control mothers also. But in any case, all invasions of mothers with intrauterine pathogens may not

lead to intrauterine infection. Here presence of specific IgM in cord blood can be taken as a sign of infections extended to the intrauterine stage.

In case of *Toxoplasma gondii* the IgM prevalence in the cord blood was highly significant ($p<0.039$) than others (Graph. Congenital Anomalies.2). The seriousness of fetal infection with Toxoplasma is evident from the observation of Boyer, K.M.[20] that 70-90% of the infants who apprear normal at birth could develop significant clinical illness like organ damage, mental and neurological problems etc. by young adulthood. Morten Lebech *et al.*,[177] had reported the importance of IgM screening for Toxoplasmosis in neonates which alone can identify 70 – 80% of cases with congenital toxoplasmosis. A previous study on congenital toxoplasmosis by Lebech, M *et al.*[177], showed a transmission rate of 19.4% when clinical signs and symptoms were found in 15%.

IgM to Rubella, CMV and HSV were also noticed in the cord blood of the study group. Previous studies have shown relationship existing between Rubella [94] and CMV [98] infection with hearing loss of infants. In our study also various malformations were observed which can be attributed to intrauterine TORCH infections (Table: Congenital anomalies.1).

Details of TORCH cross infections observed in the group are presented in the Graph. Congenital Anomalies.3. Here 14.3% of the mothers of the congenital anomalies had infections with two or more TORCH agents while it was 13.8% in the controls. In infants with congenital anomalies, 12.5% were found as infected with two or more pathogens. In the control infants none were recorded with multiple infections. Though maternal cross infections seem to have no significant role in causing congenital anomalies, cross infections in the infant seems to have some role.

Neither the test nor the control groups had diabetes, infection with HIV, Syphilis and Hepatitis. The role of these parameters in causing congenital anomalies is not documented.

Other parameters like socioeconomic status, pregnancy induced hypertension and Hb content of the mothers under study, sex of the infant, AFI and GCT content were given in the Graph. Congenital Anomalies.8.

The socioeconomic status of 95% of the test group and 87% of the control was low. This observation seems to be slightly significant ($p<0.094$). There were no previous reports of socioeconomic status influencing congenital anomalies.

Observations of Rabe *et al.,* on reduced amounts of amniotic fluid showed that children with oligohydramnios had severe fetal malformation.[144] In our study this criteria didn't seem to be an influencing parameter.

In conclusion, transplacental infections with *Toxoplasma gondii* in a significant level and with other TORCH agents to a considerable level (though not statistically significant) were found to have role in causing congenital anomalies. Low socioeconomic status seems to have a minor role. Other factors like low birth weight, diabetes of mother, pregnancy induced hypertension, oligohydramnios, renal diseases, GCT values and maternal age were not found as significant in causing congenital anomalies.

PREVALENCE OF TORCH INFECTIONS IN PREGNANCY RELATED COMPLICATIONS IN THE MALABAR REGION OF KERALA

Thesis submitted to the

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IN

MICROBIOLOGY

UNDER THE FACULTY OF SCIENCE

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2006

Summary

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In conclusion, this study has established the general role of TORCH pathogens on miscarriage in first trimester pregnancy. When only 14.1% of the abortion cases were totally free of TORCH infections, 60% of the control group was free of it. The role of *Toxoplasma gondii* and *Herpes simplex* infection on miscarriage is well proved here. Rubella and Cytomegalovirus were not found to have influenced abortion, as per this study. The increased susceptibility of our general population to *Toxoplasma gondii* and HSV, compared to Rubella and CMV, further supports these observations. Various abortion categories are also influenced by these pathogens differently. It is observed that cross infections or multiple infections with two or more of the TORCH agents have a statistically significant ($p<0.003$) prevalence in first trimester miscarriage cases.

As per this study, though sign of infection with TORCH agents has been observed in IUGR mothers, it is not found as significant in causing intrauterine growth retardation, compared to other factors like Pregnancy Induced Hypertension, Oligohydramnios and Age.

Infections with *Toxoplasma gondii* and Cytomegalovirus are significant in causing IUD. Oligohydramnios and low socio economic status were also found to be influencing. Other factors like low birth weight, diabetes of mother, pregnancy induced hypertension, renal diseases, high GCT values, maternal age etc were not very relevant. Precise diagnosis of the cause of IUD is a prerequisite as a basis for counseling, prevention and treatment of the patients.

Transplacental infection with *Toxoplasma gondii* significantly and with other TORCH pathogens in a considerable level are found to have role in causing congenital malformations among the populations of Malabar region. Infection with other TORCH agents, low birth weight, diabetes of mother, pregnancy induced hypertension, oligohydramnios, renal diseases, high GCT values, maternal age etc. were not significant. Low socioeconomic status also seems to have role in causing congenital malformations.

The findings of this study can be summarised as;

- The Malabar population is more susceptible to *Toxoplasma gondii* and HSV compared to CMV and Rubella.
- These intrauterine pathogens have significant role in causing first trimester miscarriage.
- Significant relationship exists between TORCH cross infections and miscarriage.
- IGUR has not been influenced significantly by TORCH infections in this study.
- *Toxoplasma gondii* and CMV have significant role in causing Intrauterine Fetal Death.
- Congenital anomalies can be correlated to transplacental infections with *T. gondii* significantly and with other infections in a minor level. Low socioeconomic status also had influence on anomalies.
- IUD and Congenital anomalies were significantly related to transplacental infections and slightly to socio economic status.
- PIH influences IUGR and intrauterine fetal death whereas Oligohydramnios significantly influences IUGR.

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References

References

1. Sivanandan, T.V. Challenge of newborn babies' healthcare grows bigger. *The Hindu* (2005).
2. Information kit. *World Health Day , WHO* (1998).
3. Fact sheet. *Population Reference Bureau* (1998).
4. India's Progress Towards Reproductive Health Goals: ICPD + 5. India Country Paper, . *Ministry of Health and Family Welfare (MOHFW)* (1999).
5. Sundari, T.K. Can health education improve pregnancy outcome? : Report of a grassroots action-education campaign. . *The Journal of Family Welfare.* **39**, 1-12 (1993).
6. Giancarlo Mari, M.D. Intrauterine Growth Retardation *Hygeia Foundation, Inc. and institution for perinatal loss* **1** (2005).
7. Definitions of CONGENITAL MALFORMATION on the Web. *wikipedia, http://en.wikipedia.org/wiki/Congenital_malformation* (2005).
8. Pebley, A.R. Intrauterine mortality and maternal nutritional status in rural Bangladesh. *Population Studies* **39**, 425-31 (1985).
9. Nielsen, B.B., Liljestrand, J., Hedegaard, M., Thilsted, S.H. & Joseph, A. Reproductive pattern, perinatal mortality, and sex preference in rural Tamil Nadu, south India: community based, cross sectional study. *Bmj* **314**, 1521-4 (1997).

10. KK, H.P.V. Relationship between threatened abortion and congenital malformations. *Journal of Indian Association of Paediatric Surgeons.* **1**, 13-6 (1996).
11. High Risk Pregnancy and Pregnancy Complications, http://www.allaboutmoms.com. *all about moms* (2005).
12. Anne, T. & Elizabeth, R. Healthy MOTHERS and Healthy NEWBORNS: Policy Perspectives on Newborn Health The Vital Link. *Policy Perspectives on Newborn Health 2002* 1-6 (2002).
13. Boyer, S.G. & Boyer, K.M. Update on TORCH Infections in the Newborn Infant. *NBIN* **4**(2004).
14. Wilcox, A.J., Weinberg, C.R. & O'Connor, J.F. Incidence of early loss of pregnancy. . *N Engl J Med* **319**, 189-194 (1988).
15. Mims, C., Nash, A. & Stephen, J.M. Pathogenesis of Infectious Diseases. (2001).
16. Gwendolyn, L.G. Infections in pregnant women. *The Medical Journal of Australia* **176**, 229-236 (2002).
17. Khan, N.A. & Kazzi, S.N. Yield and costs of screening growth-retarded infants for torch infections. *Am J Perinatol* **17**, 131-5 (2000).
18. Garland, S.M. & Gilbert, G.L. Investigation of congenital infection--the TORCH screen is not a legitimate test. Paediatric Infectious Diseases Group of the Australasian Society for Infectious Diseases. *Med J Aust* **159**, 346-8 (1993).
19. Hohlfeld, P. et al. Fetal toxoplasmosis: outcome of pregnancy and infant follow-up after in utero treatment. *J Pediatr* **115**, 765-9 (1989).
20. Boyer, K.M. Toxoplasmosis: Current status of diagnosis, treatment and prevention. . *Semin Pediatr Infect Dis* **11**, 165-171 (2000).

21. Rawston, S. Treponema pallidum (Syphilis). In Principles and Practice of Pediatric Infectious Diseases, eds Long S, Pickering L and Prober C. Churchill-Livingston, New York, NY 954-965 (2003).

22. Centers for Disease Control and Prevention, Primary and secondary syphilis—United States 1998. *MMWR* **48**, 1299-1302 (1999).

23. Azimi, P. Syphilis (Treponema pallidum). *In Nelson Textbook of Pediatrics, eds Behrman R, Kliegman R and Jenson H. Saunders, Philadelphia, PA* 978-982. (2004).

24. Centers for Disease Control and Prevention. Sexually transmitted disease treatment guidelines 2002. . *MMWR* **51**, 1-80 (2002).

25. Brunell, P.A. Varicella in pregnancy, the fetus, and the newborn: problems in management. *J Infect Dis* **166 Suppl 1**, S42-7 (1992).

26. Johnson, C.E., Stancin, T. & Fattlar, D. A long-term prospective study of varicella vaccine in children. . *Pediatrics* **100**, 761-766 (1997).

27. Pastuszak, A.L. et al. Outcome after maternal varicella infection in the first 20 weeks of pregnancy. *N Engl J Med* **330**, 901-5 (1994).

28. Arvin, A. Varicella-zoster virus. . *In Principles and Practice of Pediatric Infectious Diseases, eds Long SS, Pickering LK and Prober CG. Churchill-Livingston*, 1041-1049 (2003).

29. Centers for Disease Control. Risks associated with human parvovirus B19 infection. . *MMWR* **38**, 81-97 (1989).

30. Miller, E., Fairley, C.K., Cohen, B.J. & Seng, C. Immediate and long term outcome of human parvovirus B19 infection in pregnancy. *Br J Obstet Gynaecol* **105**, 174-8 (1998).

31. Centers for Disease Control and Prevention. Rubella and congenital rubella syndrome-United States 1991-1997. . *MMWR* **46**, 350-354 (1997).

32. Best, J.M. & O'Shea, S. Rubella virus. . *In Diagnostic Procedures for Viral, Rickettsial and Chamydial Infections, eds Lennette EH and Schmidt NJ. American Public Health Association*, 731-795 (1989).

33. Maldonado, Y. Rubella. *In Nelson Textbook of Pediatrics, eds Behrman R, Kliegman R and Jenson H. Saunders*, 1032-1034 (2004).

34. Danovaro-Holliday, M.C., Gordon, E. & Woernle, C. Identifying risk factors for rubella susceptibility in a population at risk in the United States. . *Am J Public Health* **93**, 289-291 (2003).

35. Fowler, K.B., Stagno, S. & Pass, R.F. Maternal age and congenital cytomegalovirus infection: Screening of two diverse newborn populations: 1980–1990. . *J Infect Dis* **168**, 552. (1993).

36. Pass, R. Cytomegalovirus. . *In Principles and Practice of Pediatric Infectious Diseases, eds Long S, Pickering L and Prober C. Churchill-Livingston*, 1050-1059 (2003).

37. Hicks, T., Fowler, K. & Richardson, M. Congenital cytomegalovirus infection and neonatal auditory screening. . *J Pediatr* **123**, 779 (1993).

38. Pass, R.F., Duliere, A.M. & Boppana, S. A subunit cytomegalovirus vaccine based on recombinant envelope glycoprotein B and a new adjuvant. . *J Infect Dis* **180**, 970 (1999).

39. Nahmias, A. & Dowdle, W.R. Antigenic and biological differences in herpesvirus hominis. . *Prog Med Virol* **10**, 110-159 (1968).

40. Arvin, A. & Whitley, R. Herpes simplex virus infections. . *In Infectious Diseases of the Fetus and Newborn Infant, eds Remington J and Klein J. Saunders,* , 425-446 (2001).

41. Prober, C.G. et al. Low risk of herpes simplex virus infections in neonates exposed to the virus at the time of vaginal delivery to mothers with recurrent genital herpes simplex virus infections. *N Engl J Med* **316**, 240-4 (1987).

42. Kohl, S. Herpes simplex virus. . *In Nelson Textbook of Pediatrics, eds Behrman R, Kliegman R and Jenson H. Saunders, Philadelphia, PA*, 1051-1057 (2004).

43. Eschenbach, D.A. et al. Diagnosis and clinical manifestations of bacterial vaginosis. *Am J Obstet Gynecol* **158**, 819-28 (1988).

44. Colli, E., Bertulessi, C., Landoni, M. & Parazzini, F. Bacterial vaginosis in pregnancy and preterm birth: evidence from the literature. *J Int Med Res* **24**, 317-24 (1996).

45. Hill, G.B. The microbiology of bacterial vaginosis. *Am J Obstet Gynecol* **169**, 450-4 (1993).

46. MAJERONI, B.A. Bacterial Vaginosis: An Update. *American Family Physician* **Cover story**(1998).

47. John, E.D., JR & Lefevre, M.L. Urinary Tract Infections During Pregnancy. *American Family Physician* (2000).

48. Bulletins, N. Treatment of Common Medical Problems in Pregnancy Part II *Internet Clinical Information National Medicines Information Centre* **3**(1997).

49. Detection and Management of Hypertensive Disorders of Pregnancy to Prevent Complications. *Maternal and Neonatal Health* (2005).

50. Pregnancy Complicated By Disease. *THE MERCK MANUAL* **2**, Chapter 251 (2005).

51. Kumar, R., Sharma, A.K., Barik, S. & Kumar, V. Maternal mortality inquiry in a rural community of north India. *Int J Gynaecol Obstet* **29**, 313-9 (1989).

52. Rao, K.B. Maternal mortality in a teaching hospital in southern India. A 13-year study. *Obstet Gynecol* **46**, 397-400 (1975).

53. Rajaram, P., Agrawal, A. & Swain, S. Determinants of maternal mortality: a hospital based study from south India. *Indian J Matern Child Health* **6**, 7-10 (1995).

54. Regan, L., Braude, P.R. & Trembath, P.L. Influence of past reproductive performance on risk of spontaneous abortion. *Bmj* **299**, 541-5 (1989).

55. Simpson, J.L. et al. Further evidence that infection is an infrequent cause of first trimester spontaneous abortion. *Hum Reprod* **11**, 2058-60 (1996).

56. Al-Kubaisy, W.A., Niazi, A.D. & Kubba, K. History of miscarriage as a risk factor for hepatitis C virus infection in pregnant Iraqi women. *East Mediterr Health J* **8**, 239-44 (2002).

57. Bujko, M., Sulovic, V., Zivanovic, V., Dotlic, R. & Bardic, I. Herpes simplex virus infection in women with previous spontaneous abortion. *J Perinat Med* **16**, 193-6 (1988).

58. Robb, J.A., Benirschke, K. & Barmeyer, R. Intrauterine latent herpes simplex virus infection: I. Spontaneous abortion. *Hum Pathol* **17**, 1196-209 (1986).

59. Johnson, A.M., Roberts, H., Wetherall, B., McDonald, P.J. & Need, J.A. Relationship between spontaneous abortion and presence of antibody to Toxoplasma gondii. *Med J Aust* **1**, 579-80 (1979).

60. Lolis, D., Tzigounis, V., Michalas, S., Koumentakou, E. & Kaskarelis, D. Toxoplasma antibodies and spontaneous abortion. *Int J Gynaecol Obstet* **15**, 299-301 (1978).

61. Bernstein I & SG., G. Intrauterine growth restriction. In: Gabbe SG, Niebyl JR, Simpson JL, Annas GJ, eds. Obstetrics: normal and problem pregnancies. 3d ed. New York. Churchhill-Livingstone. 863-86 (1996).

62. PM., D. The search for perinatal definitions and standards. *Acta Paediatr Scand Suppl* **319** 7-16 (1985).

63. Doubilet, P.M. & Benson, C.B. Sonographic evaluation of intrauterine growth retardation. *AJR Am J Roentgenol* **164**, 709-17 (1995).

64. David Peleg, M.D., Colleen M. Kennedy, M.D. & Stephen K. Hunter, M.D., Ph.D. Intrauterine Growth Restriction: Identification and Management. *American Family Physician* **58**(1998).

65. Vandenbosche, R.C. & Kirchner, J.T. Intrauterine growth retardation. *Am Fam Physician* **58**, 1384-90, 1393-4 (1998).

66. Neerhof, M.G. Causes of intrauterine growth restriction. *Clin Perinatol* **22**, 375-85 (1995).

67. Manning FA & C., H. Intrauterine growth retardation: diagnosis, prognostication, and management based on ultrasound methods. In: Fleischer AC, et al., eds. The principles and practice of ultrasonography in obstetrics and gynecology. **4**, 331-48 (1991).

68. Craigo, S.D. The role of ultrasound in the diagnosis and management of intrauterine growth retardation. *Semin Perinatol* **18**, 292-304 (1994).

69. Prada, J.A. & Tsang, R.C. Biological mechanisms of environmentally induced causes of IUGR. *Eur J Clin Nutr* **52 Suppl 1**, S21-7; discussion S27-8 (1998).

70. McCormick, M.C. The contribution of low birth weight to infant mortality and childhood morbidity. *N Engl J Med* **312**, 82-90 (1985).

71. Piper, J.M. et al. Perinatal outcome in growth-restricted fetuses: do hypertensive and normotensive pregnancies differ? *Obstet Gynecol* **88**, 194-9 (1996).

72. Lechtig, A. et al. Effect of moderate maternal malnutrition on the placenta. *Am J Obstet Gynecol* **123**, 191-201 (1975).

73. Dougherty, C.R. & Jones, A.D. Th determinants of birth weight. *Am J Obstet Gynecol* **144**, 190-200 (1982).

74. Nilsen, S.T., Sagen, N., Kim, H.C. & Bergsjo, P. Smoking, hemoglobin levels, and birth weights in normal pregnancies. *Am J Obstet Gynecol* **148**, 752-8 (1984).

75. Mills, J.L., Graubard, B.I., Harley, E.E., Rhoads, G.G. & Berendes, H.W. Maternal alcohol consumption and birth weight. How much drinking during pregnancy is safe? *Jama* **252**, 1875-9 (1984).

76. Robert C Vandenbosche, M.D. & Jeffrey T Kirchner, D.O. Intrauterine Growth Retardation. *American Family Physician* **58**(1996).

77. Shi, D.Z. Research on the relation between intrauterine infection and intrauterine growth retardation. *Zhonghua Fu Chan Ke Za Zhi* **27**, 70-2, 123 (1992).

78. Stoll, B.J. et al. Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection. *Jama* **292**, 2357-65 (2004).

79. Hodgman, J.E., Barton, L., Pavlova, Z. & Fassett, M.J. Infection as a cause of death in the extremely-low-birth-weight infant. *J Matern Fetal Neonatal Med* **14**, 313-7 (2003).

80. Maschmann, J., Hamprecht, K., Dietz, K., Jahn, G. & Speer, C.P. Cytomegalovirus infection of extremely low-birth weight infants via breast milk. *Clin Infect Dis* **33**, 1998-2003 (2001).

81. Chaturvedi, P. & Desai, P.C. Cord and maternal serum IgM in IUGR babies. *Indian Pediatr* **26**, 660-4 (1989).

82. Wiredu, E.K. & Tettey, Y. Autopsy studies on still births in Korle Bu Teaching Hospital. II: Causes of death in 93 still births. *West Afr J Med* **17**, 148-52 (1998).

83. Tolockiene, E. et al. Intrauterine infection may be a major cause of stillbirth in Sweden. *Acta Obstet Gynecol Scand* **80**, 511-8 (2001).

84. Moyo, S.R. et al. Intrauterine death and infections during pregnancy. *Int J Gynaecol Obstet* **51**, 211-8 (1995).

85. Petersson, K., Norbeck, O., Westgren, M. & Broliden, K. Detection of parvovirus b19, cytomegalovirus, and enterovirus infections in cases of intrauterine fetal death. *Obstet Gynecol Surv* **60**, 284-6 (2005).

86. Petersson, K., Norbeck, O., Westgren, M. & Broliden, K. Detection of parvovirus B19, cytomegalovirus and enterovirus infections in cases of intrauterine fetal death. *J Perinat Med* **32**, 516-21 (2004).

87. Johar, S.R., Savalia, N.K., Vasavada, A.R. & Gupta, P.D. Epidemiology based etiological study of pediatric cataract in western India. *Indian J Med Sci* **58**, 115-21 (2004).

88. Fraser, S.H. et al. Hydrocephalus ex vacuo and clasp thumb deformity due to congenital cytomegalovirus infection. *J Paediatr Child Health* **30**, 450-2 (1994).

89. Eckstein, M., Vijayalakshmi, P., Killedar, M., Gilbert, C. & Foster, A. Aetiology of childhood cataract in south India. *Br J Ophthalmol* **80**, 628-32 (1996).

90. Zheng, X.Y. et al. Intrauterine infections and birth defects. *Biomed Environ Sci* **17**, 476-91 (2004).

91. Wang, X., Zhang, G.C. & Han, M. Detection of TORCH genom in the cardiac tissue of congenital heart disease. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi* **15**, 176-8 (2001).

92. Kumar, V., Singh, A.J. & Marwaha, R.K. An epidemiological study of congenital malformations in rural children. *Indian Pediatr* **31**, 909-14 (1994).

93. Sibille, G. et al. Reinfection after rubella and congenital polymalformation syndrome. *J Genet Hum* **34**, 305-12 (1986).

94. Cheng, N. et al. Prevalence of birth defects and rubella infection in pregnant women in Gansu, west China. A survey. *J Reprod Med* **48**, 869-74 (2003).

95. Lawn, J.E. et al. Unseen blindness, unheard deafness, and unrecorded death and disability: congenital rubella in Kumasi, Ghana. *Am J Public Health* **90**, 1555-61 (2000).

96. Linthicum, F.H., Jr. Viral causes of sensorineural hearing loss. *Otolaryngol Clin North Am* **11**, 29-33 (1978).

97. Witters, I., Van Ranst, M. & Fryns, J.P. Cytomegalovirus reactivation in pregnancy and subsequent isolated bilateral hearing loss in the infant. *Genet Couns* **11**, 375-8 (2000).

98. Rivera, L.B. et al. Predictors of hearing loss in children with symptomatic congenital cytomegalovirus infection. *Pediatrics* **110**, 762-7 (2002).

99. Williamson, W.D., Demmler, G.J., Percy, A.K. & Catlin, F.I. Progressive hearing loss in infants with asymptomatic congenital cytomegalovirus infection. *Pediatrics* **90**, 862-6 (1992).

100. al Muhaimeed, H. & Zakzouk, S.M. Hearing loss and herpes simplex. *J Trop Pediatr* **43**, 20-4 (1997).

101. Egger, M., Muhlemann, K., Aebi, C. & Tauber, M.G. [Infections in pregnancy]. *Ther Umsch* **56**, 577-82 (1999).

102. Carey, J.C. & Klebanoff, M.A. Bacterial vaginosis and other asymptomatic vaginal infections in pregnancy. *Curr Womens Health Rep* **1**, 14-9 (2001).

103. Le, J., Briggs, G.G., McKeown, A. & Bustillo, G. Urinary tract infections during pregnancy. *Ann Pharmacother* **38**, 1692-701 (2004).

104. Whitney, C.G. et al. The international infections in pregnancy study: group B streptococcal colonization in pregnant women. *J Matern Fetal Neonatal Med* **15**, 267-74 (2004).

105. Egger, M., Low, N., Smith, G.D., Lindblom, B. & Herrmann, B. Screening for chlamydial infections and the risk of ectopic pregnancy in a county in Sweden: ecological analysis. *Bmj* **316**, 1776-80 (1998).

106. Moodley, P. & Sturm, A.W. Sexually transmitted infections, adverse pregnancy outcome and neonatal infection. *Semin Neonatol* **5**, 255-69 (2000).

107. Rappersberger, K. Infections with herpes simplex and varicella zoster virus in pregnancy: clinical manifestations in mother, fetus and newborn--therapeutic options. *Hautarzt* **50**, 706-14 (1999).

108. Sauerbrei, A. Varicella-zoster virus infections in pregnancy. *Intervirology* **41**, 191-6 (1998).

109. Markenson, G.R. & Yancey, M.K. Parvovirus B19 infections in pregnancy. *Semin Perinatol* **22**, 309-17 (1998).

110. Harger, J.H., Adler, S.P., Koch, W.C. & Harger, G.F. Prospective evaluation of 618 pregnant women exposed to parvovirus B19: risks and symptoms. *Obstet Gynecol* **91**, 413-20 (1998).

111. Kaur, R., Gupta, N., Nair, D., Kakkar, M. & Mathur, M.D. Screening for TORCH infections in pregnant women: a report from Delhi. *Southeast Asian J Trop Med Public Health* **30**, 284-6 (1999).

112. Canessa, A. et al. Antibody prevalence to torch agents in pregnant women and relative risk of congenital infections in Italy (Liguria). *Biol Res Pregnancy Perinatol* **8**, 84-8 (1987).

113. Ustacelebi, S. et al. Detection of antibodies against TORCH agents during pregnancy. *Mikrobiyol Bul* **20**, 1-8 (1986).

114. Ghazi, H.O., Telmesani, A.M. & Mahomed, M.F. TORCH agents in pregnant Saudi women. *Med Princ Pract* **11**, 180-2 (2002).

115. Alanen, A., Kahala, K., Vahlberg, T., Koskela, P. & Vainionpaa, R. Seroprevalence, incidence of prenatal infections and reliability of maternal history of varicella zoster virus, cytomegalovirus, herpes simplex virus and parvovirus B19 infection in South-Western Finland. *Bjog* **112**, 50-6 (2005).

116. Cao, Y., Qiu, L. & Zhang, Q. [Study on the relationship between the history of abnormal pregnancy and TORCH infection in pregnant woman]. *Zhonghua Fu Chan Ke Za Zhi* **34**, 517-20 (1999).

117. Taechowisan, T., Sutthent, R., Louisirirotchanakul, S., Puthavathana, P. & Wasi, C. Immune status in congenital infections by TORCH agents in pregnant Thais. *Asian Pac J Allergy Immunol* **15**, 93-7 (1997).

118. Odland, J.O., Sergejeva, I.V., Ivaneev, M.D., Jensen, I.P. & Stray-Pedersen, B. Seropositivity of cytomegalovirus, parvovirus and rubella in pregnant women and recurrent aborters in Leningrad County, Russia. *Acta Obstet Gynecol Scand* **80**, 1025-9 (2001).

119. Reiche, E.M. et al. Prevalence of American trypanosomiasis, syphilis, toxoplasmosis, rubella, hepatitis B, hepatitis C, human immunodeficiency virus infection, assayed through serological tests among pregnant patients, from 1996 to 1998, at the Regional University Hospital Norte do Parana. *Rev Soc Bras Med Trop* **33**, 519-27 (2000).

120. Condorelli, F. et al. Seroprevalence to some TORCH agents in a Sicilian female population of fertile age. *Eur J Epidemiol* **9**, 341-3 (1993).

121. Hussein, A.H., Nagaty, I.M. & Fouad, M.A. Evaluation of IgM-ELISA versus PCR in diagnosis of recent Toxoplasma gondii infection. *J Egypt Soc Parasitol* **32**, 639-46 (2002).

122. Attia, R.A., el-Zayat, M.M., Rizk, H. & Motawea, S. Toxoplasma IgG. & IgM. antibodies. A case control study. *J Egypt Soc Parasitol* **25**, 877-82 (1995).

123. Robert-Gangneux, F. et al. Value of prenatal diagnosis and early postnatal diagnosis of congenital toxoplasmosis: retrospective study of 110 cases. *J Clin Microbiol* **37**, 2893-8 (1999).

124. Remington, J.S., McLeod, R. & Desmonts, G. Toxoplasmosis. In: Remington JS, Klein JO, eds. Infectious diseases of the fetus and newborn infant. **4th ed.** , 349-364 (1992).

125. Berrebi, A. et al. Termination of pregnancy for maternal toxoplasmosis. *Lancet* **344**, 36-9 (1994).

126. Wallon, M., Gandilhon, F., Peyron, F. & Mojon, M. Toxoplasmosis in pregnancy. *Lancet* **344**, 541 (1994).

127. Ancelle, T. [The return of trypanosomiasis: a new challenge from a forgotten disease]. *Med Trop (Mars)* **56**, 347-8 (1996).

128. Bader, T.J., Macones, G.A. & Asch, D.A. Prenatal screening for toxoplasmosis. *Obstet Gynecol* **90**, 457-64 (1997).

129. Decavalas, G., Papapetropoulou, M., Giannoulaki, E., Tzigounis, V. & Kondakis, X.G. Prevalence of Toxoplasma gondii antibodies in gravidas and recently aborted women and study of risk factors. *Eur J Epidemiol* **6**, 223-6 (1990).

130. Cengiz, A.T., Kiyan, M., Cengiz, L., Kara, F. & Ugurel, M.S. [Determination of Toxoplasma IgM by ELISA in maternal blood and cord blood of infants born with abnormalities or fetal death]. *Mikrobiyol Bul* **26**, 121-30 (1992).

131. Lindstrand, A. et al. Prevalence of syphilis infection in Mozambican women with second trimester miscarriage and women attending antenatal care in second trimester. *Genitourin Med* **69**, 431-3 (1993).

132. Skjoldebrand-Sparre, L. et al. Parvovirus B19 infection: association with third-trimester intrauterine fetal death. *Bjog* **107**, 476-80 (2000).

133. Dykewicz, C.A. et al. Rubella seropositivity in the United States, 1988-1994. *Clin Infect Dis* **33**, 1279-86 (2001).

134. Singla, N., Jindal, N. & Aggarwal, A. Primary rubella virus infection: prevalence and relationship to pregnancy wastage. *Indian J Pathol Microbiol* **46**, 688-9 (2003).

135. Zheng, F., Du, J. & Hu, Y. A study of rubella virus infection during pregnancy. *Zhonghua Fu Chan Ke Za Zhi* **37**, 391-4 (2002).

136. Harrison, C.J. & Myers, M.G. Relation of maternal CMV viremia and antibody response to the rate of congenital infection and intrauterine growth retardation. *J Med Virol* **31**, 222-8 (1990).

137. Parmigiani, S.V. et al. Accuracy of the serological ELISA test compared with the polymerase chain reaction for the diagnosis of cytomegalovirus infection in pregnancy. *Sao Paulo Med J* **121**, 97-101 (2003).

138. Sohn, Y.M., Park, K.I., Lee, C., Han, D.G. & Lee, W.Y. Congenital cytomegalovirus infection in Korean population with very high prevalence of maternal immunity. *J Korean Med Sci* **7**, 47-51 (1992).

139. Numazaki, K., Fujikawa, T. & Chiba, S. Relationship between seropositivity of husbands and primary cytomegalovirus infection during pregnancy. *J Infect Chemother* **6**, 104-6 (2000).

140. Jordan, J. & Rytel, M.W. Detection of herpes simplex virus (HSV) type-1 IgG and IgM antibodies by enzyme-linked immunosorbent assay (ELISA). *Am J Clin Pathol* **76**, 467-71 (1981).

141. Katz, D., Hilliard, J.K., Mirkovic, R.R. & Word, R.A. ELISA for detection of IgG and IgM antibodies to HSV-1 and HSV-2 in human sera. *J Virol Methods* **14**, 43-55 (1986).

142. Cengiz, L., Kiyan, M., Cengiz, A.T., Kara, F. & Ugurel, M.S. [Detection of herpes simplex virus 1 and 2 (HSV-1 and HSV-2) IgG and IgM by ELISA in cord blood and sera of mothers with pregnancy complications]. *Mikrobiyol Bul* **27**, 299-307 (1993).

143. Gong, Z., Luo, L. & Xiao, H. Preliminary study on ToRCH epidemic laws in Wuhan region. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi* **13**, 139-41 (1999).

144. Rabe, D., Leucht, W., Hendrik, H.J., Boos, R. & Schmidt, W. Sonographic evaluation of the quantity of amniotic fluid. II. Oligohydramnios--significance for the course of pregnancy and labor. *Geburtshilfe Frauenheilkd* **46**, 422-6 (1986).

145. Hill, L.M., Breckle, R., Wolfgram, K.R. & O'Brien, P.C. Oligohydramnios: ultrasonically detected incidence and subsequent fetal outcome. *Am J Obstet Gynecol* **147**, 407-10 (1983).

146. Corosu, R., Moretti, S., Lucchini, C. & Vizzaccaro, F. [Clinical considerations on oligohydramnios]. *Minerva Ginecol* **51**, 219-22 (1999).

147. Stoll, C., Alembik, Y., Roth, M.P. & Dott, B. Study of 224 cases of oligohydramnios and congenital malformations in a series of 225,669 consecutive births. *Community Genet* **1**, 71-7 (1998).

148. Golan, A. et al. Oligohydramnios: maternal complications and fetal outcome in 145 cases. *Gynecol Obstet Invest* **37**, 91-5 (1994).

149. Funai, E.F. et al. Risk factors for pre-eclampsia in nulliparous and parous women: the Jerusalem perinatal study. *Paediatr Perinat Epidemiol* **19**, 59-68 (2005).

150. Fuchi, I., Noda, K. & Matsubara, Y. Studies on pregnancy hypertension and IUGR-SFD: effects of drugs on the blood vessels in the placenta of pregnant SHRSP. *Clin Exp Pharmacol Physiol Suppl* **22**, S286-7 (1995).

151. Diabetes and pregnancy. *Juvenile Diabetes Research Foundation* (2003).

152. Forsbach, G., Vazquez-Lara, J., Alvarez-y-Garcia, C. & Vazquez-Rosales, J. Diabetes and pregnancy in Mexico. *Rev Invest Clin* **50**, 227-31 (1998).

153. Tookey, P.A., Ades, A.E. & Peckham, C.S. Cytomegalovirus prevalence in pregnant women: the influence of parity. *Arch Dis Child* **67**, 779-83 (1992).

154. Marshall, W.C. et al. Parity of women contracting rubella in pregnancy. Implications with respect to rubella vaccination. *Lancet* **1**, 1231-3 (1976).

155. Shoham-Vardi, I., Leiberman, J.R. & Kopernik, G. The association of primiparity with intrauterine growth retardation. *Eur J Obstet Gynecol Reprod Biol* **53**, 95-101 (1994).

156. Gratacap-Cavallier, B. et al. Cytomegalovirus seroprevalence in French pregnant women: parity and place of birth as major predictive factors. *Eur J Epidemiol* **14**, 147-52 (1998).

157. Eiser, C. & Eiser, J.R. Health education needs of primigravidae. *Child Care Health Dev* **11**, 53-60 (1985).

158. Straton, J.A. & Stanley, F.J. Medical risks of teenage pregnancy. *Aust Fam Physician* **12**, 474, 477-8, 480 (1983).

159. van Enk, W.J., Gorissen, W.H. & van Enk, A. Teenage pregnancy and ethnicity in The Netherlands: frequency and obstetric outcome. *Eur J Contracept Reprod Health Care* **5**, 77-84 (2000).

160. Bhalerao, A.R., Desai, S.V., Dastur, N.A. & Daftary, S.N. Outcome of teenage pregnancy. *J Postgrad Med* **36**, 136-9 (1990).

161. Sarkar, C.S., Giri, A.K. & Sarkar, B. Outcome of teenage pregnancy and labour: a retrospective study. *J Indian Med Assoc* **89**, 197-9 (1991).

162. Kumbi, S. & Isehak, A. Obstetric outcome of teenage pregnancy in northwestern Ethiopia. *East Afr Med J* **76**, 138-40 (1999).

163. Gortzak-Uzan, L., Hallak, M., Press, F., Katz, M. & Shoham-Vardi, I. Teenage pregnancy: risk factors for adverse perinatal outcome. *J Matern Fetal Med* **10**, 393-7 (2001).

164. Olsen, J. Male factors and socioeconomic indicators correlate with the risk of spontaneous abortion. *J Obstet Gynaecol* **19**, 49-53 (1999).

165. Jonas, O., Roder, D. & Chan, A. The association of maternal and socioeconomic characteristics in metropolitan Adelaide with medical, obstetric and labour complications and pregnancy outcomes. *Aust N Z J Obstet Gynaecol* **32**, 1-5 (1992).

166. Gopalan, C. & Naidu, A.N. Nutrition and fertility. *Lancet* **2**, 1077-9 (1972).

167. Harlap, S., Shino, P.H. & Romchoran, S. Life table of spontaneous abortion and the effects of age, parity and other variables. *In Porter IH, Hook EB, editors. Human embryonic and fetal death.* , 104-6 (1989).

168. Rock, J.A. & Zacur, H.A. The clinical management of repeated early pregnancy wastage. *Fertil Steril* **39**, 123-40 (1983).

169. Zargar, A.H. et al. Seroprevalence of toxoplasmosis in women with recurrent abortions/neonatal deaths and its treatment outcome. *Indian J Pathol Microbiol* **42**, 483-6 (1999).

170. Rodier, M.H. et al. Seroprevalence of Toxoplasma, Malaria, Rubella, Cytomegalovirus, HIV and treponemal infections among pregnant women in cotonou Republic of Benin. . *Acta Tropica* **59**, 271-7 (1995).

171. Mihaela, M., Koraljka, H., Nina, M., Srecko, C. & Magdalena, G. Possible role of bacterial and viral infections in misscarriages. *Fertil. Steril* **81**, 662-9. (2004).

172. Aubard, Y. et al. Double maternal seroconversion to cytomegalovirus and Toxoplasma gondii. *Eur J Obstet Gynecol Reprod Biol.* **80**, 275-8 (1998).

173. Wolff, F. & Schaefer, R. Oligohydramnios--perinatal complications and diseases in mother and child. *Geburtshilfe Frauenheilkd* **54**, 139-43 (1994).

174. Dutta, D.C. Text book of obstetrics. 343 (2001).

175. Ananthanarayanan, R. & Jayaram Paniker, C.K. Antibodies-Immunoglobulins. *Textbook of Microbiology*, 89 (2005).

176. Wong, A., Tan, K.H., Tee, C.S. & Yeo, G.S. Seroprevalence of cytomegalovirus, toxoplasma and parvovirus in pregnancy. *Singapore Med J* **41**, 151-5 (2000).

177. Lebech, M. et al. Feasibility of neonatal screening for toxoplasma infection in the absence of prenatal treatment. Danish Congenital Toxoplasmosis Study Group. *Lancet* **353**, 1834-7 (1999).

07-05 EXTENDED SUMMARY
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PREVALENCE OF TORCH INFECTIONS IN PREGNANCY RELATED COMPLICATIONS IN THE MALABAR REGION OF KERALA: A COMPREHENSIVE REPORT

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The influence of TORCH infections in pregnancy and associated complications like abortions, intrauterine growth retardation (IUGR), intrauterine death (IUD) and congenital anomalies, in the Malabar area of Kerala was assessed to establish a basic knowledge for future pregnancy care. Pregnancy cases attended at IMCH, Calicut Medical College, Kerala were studied the controls group being first trimester and full term normal pregnant women. The maternal and chord blood samples were analyzed for the presence of IgM and IgG immunoglobulins against TORCH infections using ELISA. IgG assay shows that the general population has high susceptibility to *Toxoplasma gondii* and HSV infections. To support this, we observed significant seroprevalence of IgM against these organisms in abortion cases. Cross infection with one or more agents of the TORCH was also observed in significant level. No considerably significant correlations could be established between IUGR and TORCH infections. Occurrence of IUGR was more in teenage. IUD and congenital anomalies were significantly related to trasplacental infections.

Introduction

Teenage pregnancy is a fairly common occurrence in India, due to many factors such as early marriages, girls reaching puberty at younger ages and high specific fertility rate in the adolescent age group, etc. Socio economic factors also contribute significantly to the pregnancy related complications. A vast majority of teenage pregnant girls belong to the lower/middle class families. Bhalerao *et al.* (1990) point out that pregnant teenagers are at greater risk and require additional care.

Some maternal infections, especially during the early gestation, can result in fetal loss or malformations because the ability of the fetus to resist infections is limited and the fetal immune system is unable to prevent the dissemination of infectious organisms to various tissues. Infections with the *Toxoplasma gondii, Rubella,* Cytomegalovirus and Herpes simplex virus (TORCH) can bring serious consequences to the fetus. Detection of these infections in both mother and fetus is an important part of prenatal care. The main objective of this study was to assess the influence of TORCH infections in pregnancy related complications. The role of other parameters like pregnancy induced hypertension (PIH) and amniotic fluid index (AFI), etc. were also examined.

Materials and Methods

Pregnancy cases, attended at the Institute of Maternity and Child Health (IMCH), Calicut Medical College, Kerala from January 2001 to December 2003 were analysed. They included abortion cases (n=71) of various categories, full term pregnant women having babies with IUGR (n=63), congenital anomalies (n=66), and IUD (n=24). The control group included first trimester (n=30) and full term (n=83) normal pregnant women. The patient's history and other details like age, socioeconomic status, gravida, previous abortions, PIH, AFI, diabetes, infections with HIV, Hepatitis, etc. were collected.

Serum samples were collected at about 8-12 gestational weeks and stored in small screw capped vials at –20 °C until analysis. The maternal and chord blood samples were analyzed for the presence of IgM immunoglobulins against TORCH infections using ELISA kits from Equipar diagnostics. The immune status of the control population was studied using IgG ELISA method. The results were read at 450 nm in the ELISA reader (Sunrise model, Tecan, Austria). Tests to detect HIV and HbsAg infections were done in the medical college laboratory and the results were recorded. Various medical and clinical parameters including PIH, AFI, diabetes, and hepatitis

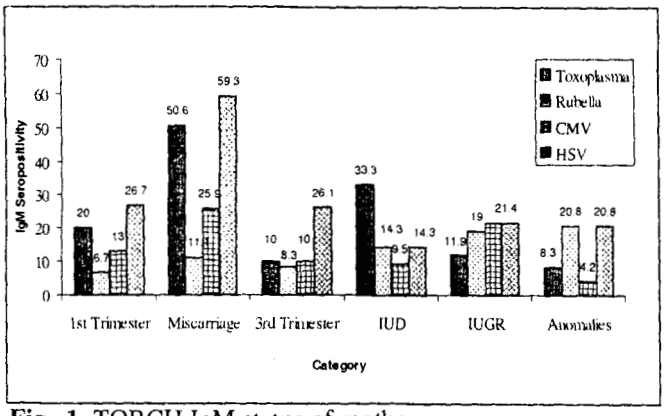

Fig. 1. TORCH IgM status of mothers

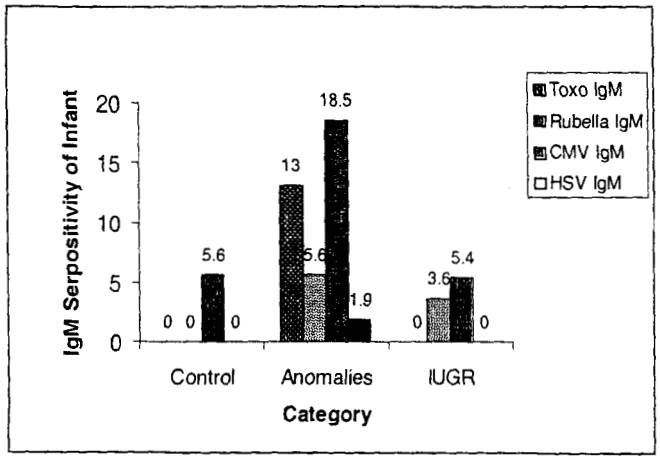

Fig. 2. TORCH IgM Serpositivity of infants

were also considered. Statistical analysis was done using the SPSS software and chi-square test was used to assess statistical significance.

Results

In this study, 10.2% of the mothers were teenagers. Also 14.3% of the miscarriage cases and 15.09% of IUGR cases were teenagers. The immunity of the general population was assessed using IgG ELISA and a susceptibility level of 32.3%, 9.6%, 3.2% and 61.3% was found against *Toxoplasma gondii, Rubella,* Cytomegalovirus and Herpes simplex virus respectively. The IgM ELISA results of the mothers and infants against TORCH infections are shown in Figures 1 and 2. PIH was noticed in 9.5% of congenital anomalies, 14.3% of IUD cases and 17.2 % ($p < 0.006$) of the IUGR cases, other categories being free of it. Socioeconomic status was low in more than 85% of the third trimester complications while 90% miscarriage cases were from middle class. About 20% of the first trimester and 44.73% of the 3rd trimester controls were primigravida, whereas 61.9%, 30%, 52.58% and 42.6% were primigravida in congenital anomalies, IUD, IUGR and miscarriage cases respectively. During the study, we have also noticed that IUGR is significantly related to AFI; 35.3 % of the test group had Oligohydramnios while only 7.4 % of the control group had it ($p<0.031$).

Discussion

A comparatively higher rate of teenage pregnancy was observed in our study group. When the previously observed Indian rate was only 6.3% (Harlap *et al.*, 1981), it was about 10.2% in this study. About 14.3% of the miscarriage cases and 15.09% IUGR cases of this study were teenagers. Though the parameters like gravida, parity, and prevalence of infections like HIV and hepatitis, etc. were considered, the incidence was not significant to correlate these factors with complications.

As per this study, 67.7% of the general population of Malabar area has IgG to *Toxoplasma gondii*, 90.4% to *Rubella,* 96.8% to CMV and 38.7% to HSV infections. Compared to similar studies in other places, the immune status of this population is higher except in case of HSV (Ghazi *et al.*, 2002; Ustacelebi *et al.*, 1986; Rodier *et al.*, 1995). This study reveals the high resistance of our populations towards *Rubella* and CMV infections. That is, majority of people in this area are pre-exposed and have acquired resistance to the viruses leaving a small percent susceptible to the infection. In abortion cases IgM seroprositivity against TORCH agents were as *Toxoplasma gondii* -50.7%, *Rubella* -11.3%, CMV -28.2% and HSV -59.2%. Compared to controls the infection with *Toxoplasma* ($p<0.03$)and HSV ($p<0.022$) were statistically significant. The various miscarriage categories like complete, threatened, missed, spontaneous and incomplete abortions were studied separately and it was found that *Toxoplasma* and HSV infections have almost the same rate of influence on missed, spontaneous and threatened abortions. It was also noticed that cross infection with one or more of the TORCH agents is present in a statistically significant ($p<0.03$) level.

The IgM assay in mothers of IUD cases showed a significant ($p<0.008$) correlation between *Toxoplasma* infection and IUD. When 33.3% of these mothers were infected, only 10% of the third trimester control mothers were affected. Infections with *Rubella*, CMV and HSV in these mothers were as 14.3%, 9.5% and 14.3 % respectively. These infections are not statistically significant. The role of TORCH infections in third trimester pregnancy is discussed by Barbara *et al.* (2002). It seems that PIH has some role in the intra uterine death since 14.3% of the IUD category suffered from PIH whereas only 2.9% of the control group had it.

The TORCH IgM in mothers of babies with congenital anomalies were not so significant. But the infants had a significant ($p<0.043$)

level of transplacental infection by *Toxoplasma gondii* (13%). The prevalence of other infections were as *Rubella* -5.6%, CMV -18.5% and HSV -1.9%. In controls, except to CMV (5.6%) no transplacental infection was observed. The incidence of the *Toxoplasma* infection in 13% of infants is to be considered as significant because it has been reported that 90% of the infected infants are asymptomatic at birth and may not demonstrate symptoms, such as mental retardation, blindness, learning disabilities and epilepsy until later in life (Lopez *et al.*, 2000). About 18.5% of CMV infection is also relevant in the light of the findings of Roizen (1999) that 60% of the infected infants will have subclinical infections in neonatal period and 71% will manifest infections within five years. Hearing loss is the most common of the permanent sequelae, affecting 68% - 93% of children, according to him.

No statistically significant serum levels of IgM against TORCH infections were observed in IUGR mothers in this study, though Khan and Kazzi (2000) have reported a 41% prevalence of TORCH infections in their IUGR cases. But none of the infants had positive IgM titers for TORCH infections in their study. The overall seropositivity of the IGUR cases was found to be lesser than the control group. Only 34.1% of mothers of the test group had seropositivity against one or other of the TORCH agents while 40% of the control group had it. *Rubella* and CMV had shown to have more influence in causing IUGR with an infection prevalence of 19% and 21.4% respectively compared to control groups where it is in the order of 8.3% and 10%. The IUGR neonates had transplacental infections with *Rubella* (3.6%) and CMV (5.6%) though not to a significant level. Even though maternal infections have little influence, the intrauterine infections may be one of the factors affecting IUGR. The influence of neonatal infections in causing IUGR is pointed out by Chaturvedi and Desai (1989).

Some previous workers (Prada and Tsang,1998) had already reported PIH as one of the factors influencing IUGR. A prevalence of 19% PIH was reported by Khan and Kazzi (2000). A statistically significant ($p<0.058$) high prevalence (22.2%) of pregnancy induced hypertension is observed in patients of middle class socio economic status compared to the patients with low socioeconomic status (7.9 %). Bhatia *et al.* (1984) have pointed out the influence of low socioeconomic status and consequent undernourishment as a factor leading to low birth weight babies. In this study 85% of the IUGR cases were from this category. About 35.3 % of the IUGR cases had Oligohydramnios, while only 7.4 % of the control group had it ($p<0.031$). This suggests a role of Oligohydramnios in causing IUGR.

Conclusion

As per this study, the general population of Malabar area has an IgG level of 67.7% to *Toxoplasma gondii*, 90.4% to *Rubella*, 96.8% to CMV and 38.7% to HSV infections. The findings support the high susceptibility of the general population to *Toxoplasma* and HSV compared to *Rubella* and CMV and their significant role in causing first trimester miscarriage. No considerably significant correlation could be established between IUGR and TORCH infections. But IUGR was observed as more prevalent in teenage pregnancies. PIH, socioeconomic status and Oligohydramnios were significantly influencing IUGR. IUD and congenital anomalies were significantly related to transplacental infections.

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References

Barbara, J. Stegmann, M. D., Christopher, J. and Carey, M.D. (2002). TORCH infections. *Current Women's Health Reports* 2: 253-258.

Bhalerao, A.R., Desai, S.V., Dastur, N.A. and Daftray, S.N. (1990). Outcome of teenage pregnancy. *J. Postgrad. Med.* 36(3):136-39.

Bhatia, B.D.1984). Growth pattern of intrauterine growth retarded (IUGR) babies in first nine months of life. *Acta Paediatr. Scand.* 73(2): 189-196.

Chaturvedi, P. and Desai, P.C. (1989). Cord and maternal serum IgM in IUGR babies. *Indian Pediatr.* 26(7): 660-664.

Ghazi, H.O., Telmesani, A.M. and Mahomed, M.F. (2002). TORCH agents in pregnant Saudi women. *Med. Princ. Pract.* 11(4):180-182.

Harlap, S., Shino, P.H. and Romchoran, S. (1989). Life table of spontaneous abortion and the effects of age, parity and other variables. *In:* (Eds. I.H. Porter, E.B. Hook), Human Embryonic and Fetal Death. New York: Academic Press, pp.104-106.

Khan, N.A. and Kazzi, S.N. (2000). Yield and costs of screening growth-retarded infants for torch infections. *Am. J. Perinatol.* 17(3): 131-135.

Lopez, A., Wilson, M., Navin, T.R. and Jones, J.L. (2000). Preventing congenital toxoplasmosis. *MMWR.* 49.

Prada, J.A. and Tsang, R.C.(1998). Biological mechanisms of environmentally induced causes of IUGR. *Eur. J. Clin. Nutr.* 52 (Suppl .1): 21-27.

Rodier, M. H., Berthonneau, J., Bourgoin, A., Giraudeau, G., Agius, G., Burucoa, C., Hekpazo, A. and Jacquemin, J.L. (1995). Seroprevalence of Toxoplasma, Malaria, Rubella, Cytomegalovirus, HIV and treponemal infections among pregnant women in Cotonou Republic of Benin. *Acta Tropica.* 59:271-277.

Roizen, N.J. (1999). Etiology of hearing loss in children. Non-genetic causes. *Pediatr. Clin. North Am.* 46:49-64.

Ustacelebi, S., Koksal, I., Canturk, H., Saify, S.J., Ersoz, D and Sellioglu, B. (1986). Detection of antibodies against TORCH agents during pregnancy. *Mikrobiyol. Bull.* 20(1):1-8.