CHAPTER - I

1. INTRODUCTION

The acronym TORCH was introduced to highlight a group of agents which causes congenital and prenatal infection, namely *Toxoplasma gondii*, Rubella virus, Cytomegalovirus and Herpes simplex virus (Lim *et al.,* 1994). Klein and Remington in 2001 have suggested that this classification is too limiting and that several additional infectious agents should be considered in the category, such as enterovirus, *Borrelia burgdorferi* and of course HIV. This research, however only covers the traditional TORCH infection. This acronym has become one of the most recognized in the field of neonatal and prenatal medicine (Jonson, 2006). TORCH infection also poses threat to immunosuppressed patients sun such as HIV / AIDS patients, individual under chemotherapy, transplant recipient, pregnant women, neonates. It can also pass through pregnant woman's blood stream to infect her unborn child (Hirch, 2006). Infection caused by TORCH is the major cause of BOH, asymptomatic and chronic, the social and reproductive maladjustment because of repeated pregnancy wastages, cost of treatment and morbidity caused to infants make the TORCH of infection major cause of concern. The prevalence of this infection varies from one geographical area to another (Surpam *et al.,* 2006). Primary infection with TORCH in pregnant women can lead to serious complication that is initially unapparent or asymptomatic. Between 2% and 5% of all congenital anomalies are attributed to prenatal infection. Typically, these organism cause only asymptomatic or mild infection in the mother but can cause more serious consequences in the fetus (Tamer, 2009). The first trimester of pregnancy is an important period often fraught with complication like bleeding, pain, leading to severe apprehension in the mother (Fluorence *et al.,* 1999). Maternal infection, especially during the early gestation, can result in fetal loss or malformations because the ability of fetus to resist infectious organism is limited and fetal immune system is unable to prevent the dissemination of infectious organism to various tissue (Maladina *et al.,* 2002).

The nature of TORCH infections has changed dramatically as a result of new vaccines, more sophisticated diagnostic testing, and greater public awareness of the need for early prenatal care. The impact of the "new genetics" may enable the identification of those at risk for these infections even before conception.(http://www.medscape.com/viewarticle/472409). The TORCH test, which is sometimes called the TORCH panel, belongs to a category of blood tests called infectious-disease antibody titer tests. This type of blood test measures the presence of antibodies and their level of concentration in the blood. The name of the test comes from the initial letters of the five disease categories.

Since the TORCH test is a screening or first-level test, body fluids or tissues may be recommended to confirm the diagnosis of a specific infection. In the case of toxoplasmosis and Rubella, cerebrospinal fluid may be obtained from the infant through a spinal tap in order to confirm the diagnosis. In the case of CMV, the diagnosis is confirmed by culturing the virus in a sample of the infant's urine. In HSV infections, tissue culture is the best method to confirm the diagnosis.

The five categories of organisms whose antibodies are measured by the TORCH test are grouped together because they can cause a cluster of symptomatic birth defects in newborns. This group of defects is sometimes called the TORCH syndrome. A newborn baby with these symptoms will be given a TORCH test to see if any of the five types of infection are involved. The increased use of polymerase chain reaction (PCR) amplification of a sample of amniotic fluid and ultrasound during pregnancy are more recent advancements that support earlier case finding which is important because the mother can be treated to prevent fetal infection (Boyer and Boyer, 2004). Nepal, being a developing country, has people who are less awareness about health conscious. Most of the people in our country do not have access to safe drinking water, hygienic food and proper sanitation practices because of poor socialistic, educational and economic status. In such context, diseases such as TORCH infections are not given much importance and this inattention may ultimately lead to serious complications in pregnant women, infants, children and immunosupressed patients. Therefore, early detection and treatment of TORCH infections and preventive measures have to be carried out in order

to reduce the incidence of later occurring life threatening consequences. As very few Research have been carried out in the past to find out the prevalence rate of TORCH infections in our country, the results obtained from the present research may help to bring awareness about TORCH infections and their preventive measures in the general population. The present study was conducted among various types of patients visiting OM HOSPITAL AND RESEARCH CENTRE suspected of TORCH infections. The main objective of this study was to determine the seroprevalence of TORCH infections among the suspected patients of different age groups and gender. In this study, IgM and IgG antibodies present in the serum of suspected patients to be tested were detected by Enzyme Linked Immunosorbent Assay (ELISA) which reveals recent infection where as IgG antibodies indicates past infections. Normal result of a TORCH panel shows the normal level of IgM antibody in specimen. When the concentration of IgM exceeds the cutt off value, it indicates the recent infection. Presence of IgG and absence of IgM in pregnant women indicate past infection and in infant, may reflect the passive transfer of Maternal antibody (http://pregnancy.ehealthforum.com/health/topic48127.html).

CHAPTER-II

2. OBJECTIVES

2.1 GENERAL OBJECTIVE

To determine the seroprevalence of TORCH infections among the patients visiting OM Hospital and Research Centre and to identify risk factor for the disease.

2.2 SPECIFIC OBJECTIVES

1. To determine the seroprevalence of TORCH infections among different types of patients of different age group and gender visiting OM Hospital & Research centre.
2. To identify the risk factor of disease

CHAPTER III

3.1 LITERATURE REVIEW

3.1.1 *Toxoplasma gondii*

The name *Toxoplasma* is derived from the Greek word Toxon meaning arc or bow, reflecting to the curved shape of trophozoite (Paniker, 2007). *Toxoplasma gondii* is an obligate intracellular coccidian parasite first described by Nicolle and Manceaux in 1908 in smear made from spleen and liver of a North African rodent gundi, *Ctenodactylus gundi* and hence the name, *Toxoplasma gondii*, is a parasite of the intestinal tract of the cat family but is transmissible to many other mammals. Only one species exits and there appears to be little strain to strain variation (Chakraborty, 2005; Paniker, 2007).

It causes toxoplasmosis in humans. The parasite probably is the only protozoan, whose all the stages (tachyzoite, tissue cyst and oocyst) are infectious for human. It is found inside the reticuloendothelial cells and other nucleated cells of the host. The life cycle of the parasite was fully described only in 1970, when it was known that cats are the definite hosts and man and other warm blooded animals are the intermediate hosts (Parija, 2004). *Toxoplasma gondii* is an obligate intracellular protozoan responsible for infection through the world in wide range of hosts including humans. Primary infection is usually subclinical but in some patients cervical lymphadenopathy or ocular disease can be present. Infection accquired during pregnancy may cause severe damage to the fetus. In immunocompromised patients, reactivation of latent infection can cause life threatening encephalitis. *Toxoplasma gondii* infection can be diagnosed indirectly with serological methods and directly by polymerase chain reaction (PCR), hybridization, isolation and histology. Where as indirect serological methods are widely used in immunocompitent patients, definite diagnosis in immunocompromised people mostly undertaken by direct detection of parasite (Cheesebrough, 2005).

In laboratory, the diagnosis of infection by *Toxoplasma gondii* is carried out by the detection of specific anti-*Toxoplasma* immunogobin (IgM & IgG) and to discriminate chronic from reactivated infection, IgG avidity is also determined with VIDAS instruments (biomericux, France); moreover the diagnosis of toxoplasmosis using bioptic tissue sample, blood, and urine is done detecting *T. gondii DNA* by a Real Time PCR Fluorescence Resonance Energy Transfer (FRET), targeting *T. gondii* 529 bp repeated region. (http://www.medsci.org/v06p0135.htm)

Morphology

There are three important morphological form of parasite as Tachyzoites, Tissue cysts and Oocysts. Among them, Tachyzoites and Tissue cysts are important diagnostic form seen in humans. Tachyzoites are the actively proliferating trophozoites, which are found in any organ but most commonly in the brain, skeletal muscle, and heart muscle during the acute stage of infection. Intracellular infection can occur in all mammalian cells except erythrocytes. Once inside a cell, they multiply within a vacuole by a process known as endodyogeny. Intracellular multiplication continues until host cells lyse or a tissue cyst is formed. In an immunocompetent host, Tachyzoites are eliminated and Tissue cysts form. Tissue cysts are the extremely resistant resting forms of the parasite to the host's defences. Most commonly found in the brain, skeletal and cardiac muscle during chronic stage of the infection. These cysts contain slowly growing trophozoites known as bradyzoites. Each cyst contains hundreds of bradyzoites. Oocysts are shed only by the members of the cat family (Parija, 2004). The trophozoite and Tissue cysts represent stages in asexual reproduction (schizogony), while Oocyst is formed by sexual reproduction (gametogony or sporogony) (Chakraborty, 2005; Ichhpujani, 2002).

Life cycle

Digenetic i.e The Life cycle of *Toxoplasma gondii* has two phases, namely, the definite host **(**Primary host): The sexual part of the life cycle takes place only in members of the Felidae family (enteric cycle) and the indefinite host (Secondary host): the asexual life

cycle can take place in any warm-blooded animal, like other mammals, including felines and birds (exoenteric cycle) (Parija, 2004; Chakraborty, 2005).

Asexual cycle

Human and other intermediate hosts acquire infection by ingestion of water and food contaminated with infectious sporulating oocysts or tissue cysts present in the raw or undercooked meat (mutton, pork, etc.) from another intermediate host, or transplacentally (Parija, 2004).Sporozoites from the oocysts and bradyzoites from the tissue cysts invade the intestinal mucosa and in epithelial cells multiply as tachyzoites by endodyogeny. In the cells, tachyzoites continue to multiply and may spread locally to mesenteric lymph nodes by invading new host cells. They also spread to distant extra-intestinal organs (e.g. brain, eye, liver, spleen, heart, skeletal muscle and placenta of pregnant mother) by invading lymphatics and blood. With the development of immunity, many of the tachyzoites are destroyed in visceral organs and acute infection is resolved. Some tachyzoites may still persist and continue to grow and develop into large tissue cysts in the brain, heart muscles and also in the skeletal muscles. These tissue cysts, which contain hundreds of bradyzoites, remain viable for years, thus retaining the potential for their re-activation. The immunosupression of the host causes reactivation of the cyst and renewed infection in the host. The infection in human is dead end (Parija, 2004).

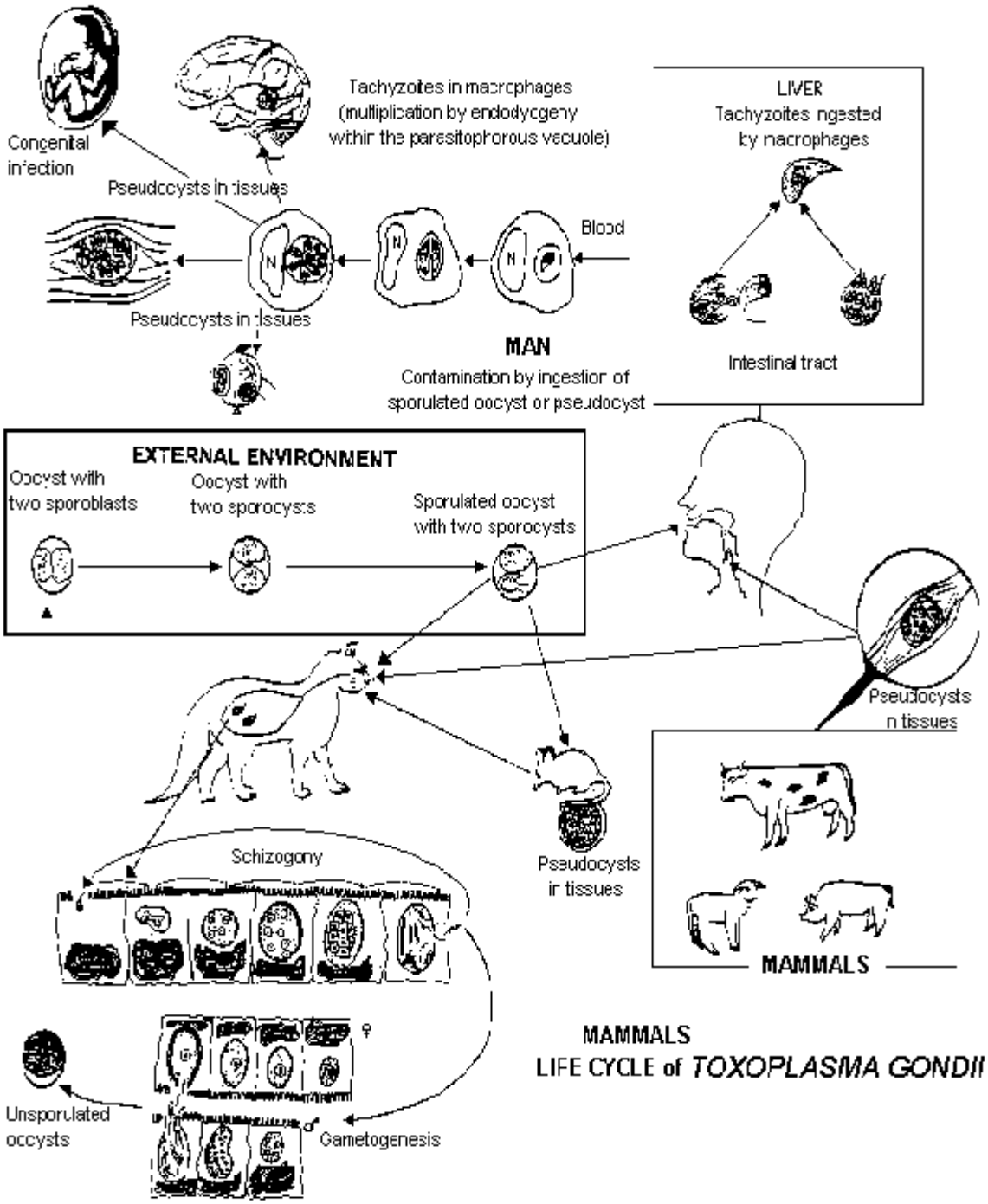

Fig. Adopted from Int J Med Sci (2009); 6:135-136@ivyspring international publisher.

Sexual cycle

Cats acquire infection by their predatory habit of feeding on infected mice, which harbor the tissue cysts or by being fed raw meat of domesticated animals containing these cysts (Parija, 2004). Cats acquire *Toxoplasma* by ingesting any of three infectious stages of the organism. Fewer than 50 percent of cats shed oocysts after ingesting tachyzoites or oocysts, whereas nearly all cats shed oocysts after ingesting tissue cysts. The bradyzoites released in the small intestine penetrate the mucosal epithelial cells of the small intestine in which, they undergo several cycles of asexual generation before the sexual cycle begins. The sexual cycle ends with the production of zygote which is surrounded by a thin but remarkably resistant and rigid wall to form an oocyst. The oocysts are then released into the lumen of intestine by rupture of the host cells. Millions of these oocysts, which are non-infectious and non-sporulated, are excreted in faeces of cats daily, for a period of 1-3 weeks. In the environment, the sporogony occurs in oocysts for up to 21 days. During sporogony two sporocysts, each containing two sporozoites is formed in each oocyst in the next 3-4 days at room temperature. With sporulation, these oocysts become infective and remain infectious for more than one year in warm, humid environments. Man acquires infection by ingesting these sporulating oocysts and the cycle is repeated (Parija, 2004).

Epidemiology

Transmission

Infection usually acquired by ingestion of contaminated food, such as improperly cooked meat, food and drink contaminated by infective Oocyst from cat faeces, transplacentaly usually during first trimester of pregnancy (Chakraborty, 2005), rarely by blood transfusion or transplantation from infected donors (Paniker, 2007) and occupational transmission to laboratory personnel and slaughter house workers and organ transplantation (Ichhpujani and Bhatia, 2002)

Pathogenesis

Intermediate host, usually man gets infection by conjumption of contaminated food, drinks with oocyst. The Oocyst remains infectious for more than 1 year (Nematollahi, 2008). Oocyst thus conjumed undergoes for asexual cycle resulting in the formation of tissue cyst or cyst in all nucleated cells, those of brain (Nematollahi, 2008). Human being also gets infection by conjumption of contaminated material with tissue cyst. Sporozoites from Oocyst and bradyzoites from the tissue cyst invade the intestinal mucosa and in epithelial cells, multiply as tachyzoites by endodyogeny in acute infection. Tachyzoites proliferate in the gastrointestinal as well as in extraintestinal sites. At these sites, they cause disruption and death of cells, producing necrotic foci, which are surrounded by mononuclear cells. In immunodificient host and even some apparently healthy normal hosts, the acute infection progress and may cause potentially lethal conditions such as encephalitis, pneumonitis and myocarditis (Parija, 2004). With the development of immunity due to exposure of tissue cyst or cyst, Parasites are cleave out slowly but in chronic case, infected cells form pseudocyst that contain large no. of bradyzoites surrounded by thick cell wall. The pseudocyst persists for many years particularly in certain immunologically privilege sites of body such as CNS and eye. In immunocompromised older patients e.g. HIV, reactivation of latent toxoplasmosis pose a special problem (Chakraborty, 2005).

Immune responses

Development of both the antibody and cell-mediated immunities (CMI) significantly alter the course of *Toxoplasma* infection and its clinical manifestations and resolve the acute infection in the immunocompetent hosts. *Toxoplasma* specific IgM antibodies are first to appear, hence their detection is suggestive of acute infection. The IgG antibodies appear late but are present in the circulation for a longer period as in chronic infection. The CMI, through activated macrophages and monocytes, is suggested to play an important role in conferring resistance to re-infection as well as in the development of

initial resistance in toxoplasmosis, possibly in co-operation with humoral antibodies (Parija, 2004).

Clinical manifestations

Toxoplasmosis in human may occur as congenital, acquired, or ocular infections in the immunocompetent hosts or in the immunocompromised hosts (Parija, 2004).

Congenital Toxoplasmosis

It occurs when a non-immune susceptible woman becomes infected during pregnancy, leading to transplacental transmission of *T. gondii* to the fetus (Parija, 2004). The risk of fetal infection rises with the progress of gestation from 25% when mother acquire primary infection in the first trimester, to 65% in third trimester but the clinical severity of infection decline as gestation proceeds (Paniker, 2007). If the mother had been infected by *Toxoplasma*; prior to the current pregnancy, the offspring donot suffer from congenital toxoplasmosis because of failure of transplacental transmission of trophozoites from chronic infection. The classic clinical triad of congenital toxoplasmosis is retinochoroiditis, cerebral calcification and convulsions, Microcephaly, hydrocephalus, Organomegali other less frequent manifestation (Parija, 2004).

Acquired toxoplasmosis: It may be of the following 3 types:

Toxoplasmosis in immunocompetent host

The vast majority of *Toxoplasma* infection i.e. 80%-90% in immune competent host remain asymptomatic but only 10%-20% of infection remains as symptomatic .Lymphadenophaty is classic clinical sign but supra clavicular,sub occipital,axillary and inguinal lymphnode are also less frequently involved.The usual clinical sign associated with this infection are sore throat,fever,malaise,night sweat and myalgias.The condition is also associated with a maculopapular skin rash that does not affect the palms and soles(Parija,2004).

Toxoplasmosis in the non-AIDS immunocompromised host

This condition is observed in immunosupressed patients receiving immunosuppressive therapy for malignancies and persons receiving bone marrow and solid organ transplantations. The condition may be a newly acquired disease or reactivation of cysts in a chronic infection. Central nervous system is mainly affected in 50% of patients. The condition manifests as encephalitis, meningoencephalitis, myocarditis and pneumonitis. Hemiparesis, seizures, mental status changes and visual changes are the common clinical symptoms (Parija, 2004).

Toxoplasmosis in HIV/AIDS patients

Toxoplasmosis is one of the opportunistic infections that AIDS patients develop. Approximately 3% to 10% of all AIDS patients die of toxoplasmosis (Singh, 2003).Brain is must commonly involved. Toxoplasmic encephalitis (TE) with or without focal CNS lesion is most characteristics and lung is next common organ. Pulmonary toxoplasmosis is most frequently encounter in patients with AIDS without proper therapeutic and clinical management. This condition is mostly noticed in patient, having CD4 cells count $<50/mm^3$ (Parija, 2004). Most ocular infections are congenital in origin and remain latent. Reactivation of latent infection may occur young and adolescents leading to retinochoroiditis and impairment of vision (Chakraborty, 2005).

Ocular toxoplasmosis

Most ocular infections are congenital in origin and remain latent. Reactivation of latent infection may occur in young and adolescents leading to retinochoroiditis and impairment of vision. The retinochoroiditis usually is unilateral in acquired toxoplasmosis while it is bilateral in congenital toxoplasmosis. Blurred vision, photophobia, pain, and scotoma are the symptoms (Parija, 2004).

Global Scenario:

The seroprevelence rate was observed to the 56.3% IgG positive in female and 41.1% IgG positive in male whereas 7.6% and 4.5% positive for IgM value (suay *et al.,* 2004), 32.3% IgG and 50.7% IgM in female (Sebastian. *et al.,* 2008),

21.05% IgG and 0% IgM in normal newborn infants, 8.57%IgG and 0% IgM in suspected congenital neonates and 13.6% IgG and 1.7% IgM positive in pregnant women (Tantivanich *et al.,* 2009), 48.3% IgG, 0.4% IgM and 1.6% both IgM+IgG positive in pregnant women in western region of Turkey (Sonmez *et al.,* 2009), 14.66% IgM in women with BOH (Surpam *et al.,* 2006), 17.2% IgG in consecutive antenatal women seen in KK woman's and children's hospital (Wong *et al.,* 2007), 42.5% IgM in pregnant woman with BOH and 71.8% IgM in aborted patients (Chopra *et al.,* 2004], 3.45% IgG in women of childbearing age (Lim *et al.,* 1994), 20% IgM and 55% IgG positive in women wih BOH of Nainital,India (Thapaliyal *et al.,* 2005), 10.52% IgM and 42.10% IgG positive in pregnant women with BOH (Turbadkar *et al.,* 2003), 20.3% IgG and 3.6% IgM positive in healthy voluntary blood donors in Karnatka, India (Sunder *et al*., 2007), 35.8% IgG and 3.4% IgM positive out of 232 women sampled at the two hospital clinics and 34.9% IgG and 11.9% IgM positive out of 218 woman at the health centres. (Samnel *et al.,* 2008), 30.9% IgG [34% (34/100) men and 26.2% (17/65) woman] in the immunoconpitent adults, 67.8% IgG [56/82 men, 04/07 woman] in HIV infected host , 20.5% (8/39), 34.8% (16/46) and 38.4% (5/13) IgG in the 2^{nd} ,3^{rd} ,4^{th} and 5^{th} decades in healthy adults , 69% (29/42) IgG in the 3^{rd} and antitoxoplasma IgM was negative in all (Meisher *et al.,* 1997), 71% IgG positive in out of 612 pregnant women(Saffer *et al.,* 1999), 53.14% IgM positive in 2371 women with recurrent abortions and 69.35% IgM positive in 310 women with neonatal deaths (Zagar *et al.,* 1999), 6.1% IgG positive and 0% IgM positive in out of 343 women (Alvarado *et al.,* 2006).

Nepalese Scenario

Overall 57.9% antitoxoplama antibody, 64.1% positive in Chitwan (71.2% positive in female > 56.9% positive in males) > 51.0% positive in Mustang (57.9% in males > 43.3% in females). Almost equal positive rate was observed among males in both study area (Rai *et al.,* 1994), Overall seropositive rate; 48% IgG in central and 49% in Western regions (district wise; Nuwakot, Kathmandu valley, Chitwan : 38%, 46% and 64% respectively) and 49% IgG in western region (district wise; Mustang, Surkhet and Banke; 51%, 67% and 44% respectively) and 1% IgM positive (Rai *et al.,* 1996), Overall 55.4% (191/ 345), prevalence rate 59.0% in older age group(27-36 year) > 52.2% in younger age group (16-26). (Rai *et al.,* 1998), Overall seroprevalence rate observed in western Nepal – 65.3% out of 404 (Achham: 66.9% and Dang: 63.5%) (Rai *et al.,* 1999). Overall, 50.7% antitoxoplasma antibody positive, out of which 5.7 % IgM positive, patients with malignancy had highest positive rate; 68.7%, women with BOH; 25 % (Rai *et al.,* 2003), 0% IgG and 5% IgM positive in Nepalese women (Kafle, 2004), 4.88% IgM of males and 15.73% IgM positive in females with overall prevalence 15.43% (Lamichhane *et al.,* 2007).

3.1.2 Rubella

Rubella virus was first described by 2 German physicians, Bergen and Orlow, in the mid eighteenth century. At that time, it was frequently known by the German name 'Roteln', and it was due to early interest of the German physicians and the general acceptance of a German name that the disease subsequently became known as 'german measles'(Bantavala and Best,1998).

It was initially considered to be a variant of measles or scarlet fever and was called "third disease." It was not until 1814 that it was first described as a separate disease in the German medical literature. In 1914, Hess postulated a viral etiology based on his work with monkeys. Hiro and Tosaka in 1938 confirmed the viral etiology by passing the disease to children using filtered nasal washings from acute cases. Following widespread Australian epidemic of Rubella infection in 1940, Gregg, an Australian

ophthalmologist, reported in 1941 the occurrence of congenital cataracts among 78 infants born following maternal rubella infection in early pregnancy. This was the first reported recognition of Congenital Rubella Syndrome (CRS) (Bantavala and Best, 1998).

Rubella virus was first isolated in 1962 by Parkman and Weller. It is classified as a togavirus, genus *Rubivirus*. It is most closely related to group A arboviruses, such as Eastern and Western Equine Encephalitis viruses. It is an enveloped RNA virus, with a single antigenic type that does not cross-react with other members of the togavirus group. Rubella virus is relatively unstable and is inactivated by lipid solvents, trypsin, formalin, and ultraviolet light, extremes of pH and heat, and amantadine. Although it's morphologic feature & physiochemical feature place it in the toga virus group, Rubella is not transmitted by arthropods and unlike togavirus group it has no invertebrate hosts (Brooks *et al.,* 2007; Chakraborty, 2003). It is an enveloped RNA virus and has mean diameter of 58nm with 30nm core. The core is surrounded by a lipoprotein envelope with surface haemaglutinin spikes of 5-8 nm in length. The virion is pleomorphic. The symmetry of nucleocapsid has been difficult to establish because of its instability, but rotational analysis of thin section of Rubella virion suggested that the core had a T= 3 icosahedral symmetry and 32 capsomeres (Bantavala and Best, 1998; Greenwood *et al.*, 2002).

Rubella - commonly known as German measles or 3-day measles — is an infection that primarily affects the skin and lymph nodes. It is usually transmitted by droplets from the nose or throat that others breathe in. It can also pass through a pregnant woman's bloodstream to infect her unborn child. It's a generally mild disease in children; the primary medical danger of rubella is the infection of pregnant women because it can cause congenital rubella syndrome in developing babies. Rubella (German measles) Virus antigen is medically significant because of its teratogenic effect when contracted by childbearing women. Maternal infection, especially during the first trimester of pregnancy, can result in a range of congenital birth defects including deafness, cataracts, diabetes and cardiac and bone abnormalities. The characteristic multisystem

defects resulting from a Rubella prenatal infection are termed the Congenital Rubella Syndrome (CRS) (Chakraborty, 2003). Before a vaccine against Rubella became available in1969, Rubella epidemics occurred every 6-9 years, most often among kids 5 to 9 years old. Many cases of congenital Rubella occurred as well. After being conducted immunization program, there are far fewer cases of Rubella and congenital Rubella. Most Rubella infections today appear in young, non-immunized adults rather than in kids. In fact, experts estimate that 10% of young adults are currently susceptible to Rubella, which could pose. (http://kidshealth.org/parent/ infectionsbacterial /viral/ german_measles-html)

Epidemiology

Pathogenesis

Humans are the only known host. Rubella virus is transmitted by the respiratory route and the virus replicates in the nasopharyngeal mucosa, followed by multiplication in the cervical lymphnodes. Following incubation period of multiplication, viramia develops after 7-9 days that spread out the virus to different parts of the body like skin, joint, placenta and last until the appearance of antibody on about days 13-15.The development of the body coincides with the appearance of rash, suggesting the immunological basis for the rash. After the rash appears, the virus remain detectable only in the nasopharynx (Brooks *et al.,* 2007; Chakraborty, 2003).Virus can be detected not only in individual lesion of macular rash but also in unaffected skin (Greenwood *et al.,* 2002). During the period of maternal viremia, the placenta may become infected, followed by fetus because of lack of maternal immunity IgG during the midgestation of early pregnancy and may result in multisystem disease (Webster, 2006). Tondury and Smith studied that Rubella virus enter the fetus via the chorion, in which it induces necrotic changes in epithelial cells as well as in endothelial lining of the blood vessels. The damaged endothelial cells are desquamated into the lumen of the vessel and the transported as virus-infected emboli in to the fetal circulation to settle in an infect various fetal organs and may result in congenital Rubella syndrome as cataract, artharitis, heart failure and deafness, etc (Banatvala and Best, 1998).

Immune responses

Cell mediated immune responses develop within a few days of onset of rash in mother. Prior to the development of the maternal immune response, the Rubella virus spread through the blood and may infect multiple maternal tissues, including the placenta. Maternal antibody production subsequently results in the disappearance of the virus from the blood but it may persist for months in the placenta. Probably as a result of placental damage the virus frequently crosses the placenta and infects the fetus for at least the first trimester, the fetus is incapable of making a normal immune response as occurs in the mother. Instead, it must rely on transfer across the placenta of maternal antibodies. This process starts in the first half of gestation with Rubella specific IgG detected in coelonic fluid at 6 weeks gestation but placental transfer appears to be insufficient at this stage. Transfer increases progressively throughout the gestation and cord maternal IgG levels at term may exceed those of the mother. Hence, in the first trimester, the fetus appears to be relatively defenseless against the virus and most damage occurs after infection during this period.

In second trimester, the risk of the fetal infection decreases significantly, this is thought to be due to the changes in placenta rather than an enhanced fetal immune response. Since fetal infection still takes place later in gestation. Fetal damage is rare if fetal infection occur after about 16 week's gestation. This implies that at these stages the combine fetal response and maternal antibodies is sufficient to limit the viral activity. From about mid gestation, the fetal progressively has a capacity to launch both a humeral and cytotoxic response to the virus. Together with the maternal IgG the fetus, can largely protect itself from viral damage for the remainder of the gestation (Webster, 1998).

Clinical manifestation

Congenital manifestation

If the fetus is infected during primary maternal infection, a wide spectrum of abnormalities may occur. The classical Congenital Rubella Syndrome (CRS) triad consists of abnormalities of the eyes (bilateral or unilateral, cataracts, opthalmia, and glucoma), ear (deafness) and heart defects (Greenwood *et al.,* 2002).

Postnatal acquired manifestation

Monitoring of CRS survivors for up to 50 years has revealed that they show a number of late onset manifestation including insulin dependent diabetes (50 times the rate in the general population), thyroid dysfunction and rare a neurodegenerative disorder-panencephalitis. This condition may result from prenatal damage, damage from ongoing infection, immune mediated cell destruction and autoimmune response triggered by molecular mimicry (Webster, 1998). The Commonest complication of postnatal acquired Rubella is joint involvement, although this is rare among children and adult males, it may occur in up to 60% of post pubertal females. Symptoms generally develops as rash subsides and vary in severity from mild stiffness of small joints of the hand to a frank arthritis with severe pain, joint swelling and limitation of movement. The duration of these symptoms is usually about 3 days but occasionally they may persist for up to month (Bantavala and Best, 1998).

Global Scenario

As for rubella, it was detected as 90.8% positive female and 90.3% positive male donors for IgG values. While it was 9.7% and 9.2% IgM positive respectively (Suay et *al.,* 2004), 9.6% IgG and 11.3% IgM positive in female (Sebastian et *al.,* 2008). The seropositives for anti-rubella IgG, IgM and IgG + IgM together in pregnant woman in Turkey were found as 96.1%, 0.2%, 1.8% respectively (Tamer *et al.,* 2009), 4.66% IgM positive in pregnant woman and early neonatal death (8%) were maximally associated with rubella virus (Surpam *et al.,* 2006), 17.5% IgM positive in pregnant women with

BOH and 59.9% IgM positive in aborted care. (Chopra *et al.,* 2004), 28.6% IgM and 66.6% IgG positive in pregnant women of Nainital with BOH (Thapliyal *et al.,* 2005), 26.8% IgM and 61.3% IgG positive in pregnant women with BOH (Turbadkar *et al.,* 2003), 2.2% IgM positive in children (Zakzouk and Muhaimeed, 1996), the seroprevalance study of Rubella among primary and pre-primary school pupils of Kenya by using ELISA test kit showed that overall, rubella seropositivity rate was 80% and it increased with age from 59% (among ages 4-6 years) to 94% (ages 14-20 years) (Janeth J.K *et al.,* 2009), 69.9% IgG positive in women before marriage and pregnancy in Isfan (Allameh *et al.,* 2004).

Nepalese Scenario

In Nepalese women of childbearing age, 0% IgG and 3.63% IgM positive was observed (Kafle *et al.,* 2004), 9.37% IgM in male and 3.37% IgM positive in female (Lamichhane *et al.,* 2007), 49.3% IgM positive in Nepalese population was found. The suspected rubella cases were higher in male (55.14% of total) but the positive cases were found higher in female (52.38% of total positive). Out of total positive cases, the highest positive cases (46.67%) were from the age group 5-10 years followed by 28.57% from the age group below 5 years. Rubella positive cases were from Bhaktapur (13.33%), followed by Rauthat (9.52%), Bara (7.62%) and Dadeldhura (7.62%). The highest suspected cases were reported from CDR (131 cases) with 58 positive cases (52.24% of total positive). The rubella cases were highest in spring season (58.1%) followed by winter season (29.52%). The rubella cases were highest in March (30/61 cases) followed by February (18/28 cases), April (18/24 cases), January (13/26 cases) and May (13/27 cases) (K.C, 2007).

3.1.3 Cytomegalovirus

Cytomegalovirus (from the Greek Cyto-, “Cell” and –megalo-, “large”) is a herpes viral genus of the herpes virus group: in human it is commonly known as HCMV or Human Herpes virus 5 (HHV-5). CMV belong to the *B herpesvirinae* subfamily of herpesviridae (http://en.wikiperdia.org/wiki/cytomegalovirus#cite_note-sherris-0).

The name ‘cytomegalovirus’ was chosen on account of the swollen state of infected cell as seen in culture and tissues. Nuclei of productively infected cells contain a large inclusion body, giving a typical ‘owl’s eye’ appearance (Greenwood *et al*., 2006). They are very species specific and cell type – specific. All attempts to infect animals with human Cytomegalovirus have failed. A number of animal Cytomegalovirus exit, all of them species – specific (Brooks *et al*., 2007). It possesses both intranuclear & cytoplasmic inclusion bodies. There is single serotype of human CMV, although different strain can be identified using restriction endonuclease mapping techniques (Volk *et al.,* 1996).

CMV has double strandd DNA, a protein capsid and a lipoprotein envelope. Like other herpes viruses, CMV demonstrates icosahedral symmetry, replicates in the cell nucleus and can cause either a lytic and productive or a latent infection (Brooks et al., 2007). CMV has the largest genetic content of the human herpes viruses. One, a cell surface glycoprotein, acts as an Fc receptor that can non specifically bind the Fc portion of immunoglobins. This may help infected cell evade immune elimination by providing a protective coating of irrelevant host immunoglobins (Britt, 1998).

HCMV infections are frequently associated with salivary gland, though they may be found through out the body. HCMV infection can also be life threatening for patients who are immunocompromised *e.g.* patients with HIV, organ transplant recipients, or neonates (Madigan *et al.*, 2006). Other CMV viruses are found in several mammal species, but species isolated from animals differ from HCMV in terms of genomic structure, and have not been reported to cause human disease. HCMV is found through out all geographic locations and socioeconomic groups, and infects between 50% and 80% of adults in the United States (40% worldwide) as indicated by the presence of antibodies in much of the general population. Seroprevalence is age-dependent: 58.9% of individuals aged 6 and older are infected with CMV while 90.8% of individuals aged 80 and older are positive for HCMV. HCMV is also the virus most frequently transmitted to a developing fetus. HCMV infection is more widespread in developing countries and in communities with lower socioeconomic status and represents the most significant viral cause of birth defects in industrialized countries. CMV "seems to have a large impact on immune parameters in later life and may contribute to increased

morbidity and eventual mortality" (http://en.wikipedia.org/ wiki/cytomegalovirus #cite_note-sherris-0).

It is estimated that fetal infection following primary maternal infection during pregnancy results in damage 5-10 times more often than infection that follows reactivation or reinfection in women with preconceptional immunity. Naturally acquired intrapartum or postnatal CMV infection in normal new born infants has not been associated with infection (Britt, 1998). Most people in the United States have the virus by the time they turn 40. Almost all gay and bisexual men are more 3 out of 4 people living with HIV carry the virus (Project INFORM, 2007).

Types of CMV

The three main types of CMV infections are, Acquired (or primary) CMV: a CMV infection that is contracted for the first time, Reoccurring CMV: a previously dormant CMV infection that reoccurs due to a weakened immune system and Congenital CMV: a CMV infection that develops in pregnancy and can affect the unborn baby. (http://en.wikipedia.org/wiki/cytomegalovirus#cite_note-sherris-0).

Epidemiology

Transmission

The main possible route of CMV infection occur through transplacental, intrapartum, breast milk, nosocomial / transfusion, sexual contact, bodyfluid contact of infected individual and infected organ transplant (The International Herpes Management Forum, 1999; Britt 1998; www.OTISpregnancy.org).

Pathogenesis

The pathogenesis of CMV infection must be viewed as a lytic viral infection, resulting from a failure of host immunity to control effectively viral replication and spread. However, it is almost a certainty that CMV exerts its pathogenic potential through non - lytic mechanism as well. These may include modulation of normal host cell function by

limited expression of its genome and perhaps by affecting neighboring cellular function through induction of cytokine production. Other pathway of host cell damage may include the generation of host derived immunopathological response that result in significant organ damage (Grundy *et al.,* 1887). CMV genes block apoptosis, interfere with the expression of immune recognition molecules and HLA on the surface of infected cells to avoid lysis by natural Killer or cytotoxic T cells, and inhibit the antiviral effects of IFNs. CMV gene products that attract phagocytic cells may promote transfer to cells of the monocyte / macrophage lineage, and other genes facilitate establishment of latency and persistence in these cells. The virus infects placental cytotrophoblasts, and through placental infection, it may be transferred to the fetus. With this genetic repertoire, CMV achieves high levels of infectious virus in peripheral blood and in epithelial cells during primary infection. Over time, the immune response suppresses replication, although CMV is not eliminated. During chronic CMV infection, the quantity of virus in peripheral blood may increase intermittently and the virus can replicate actively at epithelial sites throughout the lifetime of the host. Recent work suggests that the virus infects maternal endothelial cells, from which there is spread to placental cytotrophoblasts and ultimately, from infected placental cells to the fetal circulation (Arvin *et al.,* 2004).

A recent study links infection with CMV to high blood pressure in mice, and suggests that the result of CMV infection of blood vessel endothelial cells (EC) in humans is a major cause of artherosclerosis. Researchers also found that when the cells were infected with CMV, they created a protein called rennin that is known to contribute to high blood pressure (http://en.wikipedia.org/wiki/cytomegalovirus#cite_note-cheng_et _al _2009-7).

Immune Response

Both innate and adaptive arms of the immune response are important for the control of CMV infection, and within the adaptive response, both T cells and antibodies have been shown to protect from acquisition of CMV or from serious disease in different settings.

Some studies suggest that transplacentally acquired or passively administered antibody protects against CMV disease in neonates and transplant recipients. Antibodies, presumably capable of neutralizing infectivity, can have a role in protection against infection or disease, but they are not typically sufficient to control CMV infection. Virus-specific T cell immunity is the most important adaptive immune component responsible for suppressing CMV dissemination to lungs or other organs in which life-threatening damage may occur.

The innate immune response may shape or augment the adaptive immune response, and the magnitude of the initial adaptive immune response is important in determining the numbers of antigen-specific memory T cells. During the transition from an innate immune response to adaptive anti-CMV immunity, natural killer cells may be an important source of IFN-g, facilitating the expansion of antigen- specific helper T cells that are critical for CMV control. During initial infection, the number of CMV-specific CD8+ T cells increases to an extraordinarily high level. As suppression of viral replication is established, many CMV-specific T cells die, but by comparison with other common viral pathogens, the numbers of circulating T cells that recognize CMV peptides remain quite high. The high frequencies of CD4+ and CD8+ T cells associated with natural CMV infection may reflect boosts" that result from CMV reactivation and replication at epithelial sites, or they may reflect antigen presentation by virus that persists in monocytes (Arvin *et al.*, 2004).

Clinical Manifestation

CMV infections most commonly cause Congenital and perinatal infection that occur when the mother suffers a primary infection (or reactivation) during pregnancy. Congenital infections are actually more common in industrialized countries and higher socioeconomic groups than in poorer communities (**Error! Hyperlink reference not valid.**.) Cytomegalovirus infection can also acquired by the infants from exposure to virus in the mother's genital tract during delivery and maternal breast milk. In this case, the infant usually have received some maternal antibody and the perinately acquired

infection (Brooks *et al.,* 2007) and others are mononucleosis, Pnemonitis, GI diseases, retinitis.

Global Scenario

It was observed in respect as 83.8% IgG in female and 81.2% IgG in males whereas 12.9% IgM in female and 14.9% IgM in male donors (Suay *et al.,* 2004), Infection susceptibility in terms of IgG 3.2% and 28.2% IgM in aborted cases (Sebastian *et al.,* 2008). The seroprevelance of CMV among pregnant women in Turkey showed that 96.4% IgG, 0.7% IgM and 1.9% for IgG+ IgM (Tamer *et al.,* 2009), 5.33% IgM positive in pregnant woman with BOH (Surpam *et al.,* 2006), 87% IgG positive antenatal woman in KK Womans and Children Hospital (Wong *et al.,* 2000), 23.5% IgM positive in pregnant woman with BOH in and around Amritsar (Chopra *et al.,* 2004), 26.7% IgM and 93% IgG positive in pregnant woman of Nainital with BOH (Thapliyal *et al.,* 2005), 8.42% IgM and 91.05% IgG positive in pregnant woman with BOH (Turbadkar *et al.,* 2003), 93% IgG and 5.4% IgM in woman of childbearing age in Iran. Seroprevolence rate was higher in woman from rural as compared to those of urban areas (Arabpour *et al.,* 2007), 0% IgM and 95% IgG positive among voluntary blood donor in Delhi, India (Kothari *et al.,* 2002).

Nepalese Scenario

In Nepalese of childbearing age, 0% IgG and 5% IgM positive was obsered (Kafle *et al.,* 2003). In a research done by Lamichane et al., in 2007, 14.70% IgM positive of male and 11.04% IgM positive of female were observed.

3.1.4. Herpes simplex virus

Herpes simplex virus 1 and 2 (HSV-1 and HSV-2) is two species of the Herpes virus family, herpesviridae, which cause infections in human. As with other herpesviridae, Herpes Simplex Virus may produce life-long infections. It is estimated that 4 billion people worldwide carry the Herpes Virus. WHO figures show that 550,000,000 people are estimated to have HSV2 of the genitals. (http://en.wikipedia.org/ wiki/

Herpes_simplex_ virus).They are also called *Human Herpes Virus 1* and *2* (*HHV-1* and *HHV-2*) and are neurotropic and neuroinvasive virus. They enter and hide in the human nervous system, accounting for their durability in the human body. HSV-1 is commonly associated with herpes outbreaks of the face known as cold sore or fever blisters, whereas HSV-2 is more often associated with genital herpes. An infection by a herpes simplex virus is marked by watery blister in the skin or mucus membrane of the mouth, lips or genitals (Madigan, 2006). Lesions heal with a scab characteristic of herpetic disease. However, the infection is persistent and symptoms may recur periodically as outbreaks of sores near the site of original infection. After the initial or primary infection, HSV becomes latent in the cell bodies of nerves in the area. Some infected people experience sporadic episodes of viral reactivation, followed by transportation of the virus via the nerve's axon to the skin, where virus replication and shedding occurs.(http://en.wikipedia.org/wiki/ Herpes_simplex_virus). The usual site of latency for HSV-2 are cranial sacral ganglia and lumber or sacral ganglia respectively. The lesion caused by HSV-1 are, in general, above the waist where as those caused by HSV-2 are below the waist (Levinson and Jawetz, 2000).

Viral structure

Herpes viruses have a characteristic morphology, which is the main factor in assigning viruses to this family. Virus are comprised of 4 distinct structural elements; envelope tegument, capsid & core (Greenwood, 2006). The Herpes simplex viruses are approximately 150nm in diameter, contains icosahedral capsid made of 162 capsomere, double stranded DNA genome and are surrounded by a lipid envelope derived from the nuclear membrane of host cell (Chakraborty, 2003). HSV-1 enters cells by a fusion event that occurs at neutral PH at the plasmamembrane and which is mediated by irus encoded glycoproteins (spear, 1993). The diameter of the enveloped virion is 150 to 200nm whereas that of naked capsid is about 100nm (Volk *et al.,* 1996). Herpes virus encodes several glycoprotein for viral attachment, fusion and immune escape (Chakraborty, 2003). The DNA of HSV possesses unique structural features not found in the DNA of other viruses. If the 3’ ends of the double stranded DNA are partially

digested with DNA exonuclease III, Subsquently annealing will result in the formation of double stranded circles, indicating the presence of terminally redundant sequences (Volk *et al.,* 1996).

Epidemiology

Transmission

HSV transmission occurs horizontally with close contact with an infected person who is shedding virus from the skin, in saliva or in secretion from the genitals. In addition, vertical transmission of HSV may occur between mother and child during childbirth, which can be fatal to the infant (Madigan, 2006).

Herpes Pathogenesis

The portal of entry in primary infection is the damaged skin or mucosa and the classic lesion is vesicle beneath the keratinized squamous epithelial cells. The infection of epithelial cells is cytolytic; the cell loses adhesion occasionally become multinucleate as a result of virus induced cell fusion and contains cowdry type A nuclear inclusion (Minson, 1998). After primary infection, the virus travels by retrograde intra- axonal flow to sensory root ganglia which innervate the area of infection. They settle within the neurone in sensory ganglia either the trigeminal ganglia (HSV-1) or sacral ganglia (HSV-2) remain latent. These get reactivated when provoked by various stimuli or by transiently depressing CMI (Chakraborty, 2003).

Clinical manifestation

Major complication of HSV infections are Gingivostomatitis - clinical manifestation with redening and swelling of gum and multiple ulcerated lesions occur on the membrane of the mouth, Herpetic Keratoconjuntivitis - Infection of the eye by HSV1 (cold sore viruses) causes an extremely painful ulceration of the cornea. In most cases, primary lesions heal within 2 to 3 weeks. However recurrent keratitis can result in severe corneal ulcers and in frequent causes of corneal blindness in the United States

(Volk *et al*. 1996), Meningitis - HSV meningitis, most often caused by HSV-2, is usually mild and symptoms resolve their own, Neonatal HSV infection - acquired by passage through infected birth canal of mother, by HSV-2 (Chakraborty, 2003), Cervical carcinoma - caused by HSV-2, Alzheimer's disease – by HSV-1 (http://en.wikipedia.org/wiki/Herpes_simplex_virus, Volk *et al.*, 1996).

Global Scenario

HSV-1 was found out as 89.7 % positive in females and 87.2 % positive in males for IgG values where as IgM values were detected as 6.9 % positive for females and 8.5 % positive for male donors. HSV-2 IgG positivity was discovered as 90.6 % in females and 87.8 % in males while rates for IgM positivity were 8.6 % and 10.5 % respectively (Suay *et al*., 2004). Anti HSV IgG was found as 61.3 % and IgM as 59.2 % positive in abortion cases (Sebastian *et al*., 2008). HSV IgM 8.66 % positive in women (Surpam *et al*., 2006). Neonatal Herpex Simplex virus (HSV) is very rare in Hong Kong (Lim *et al*., 1994). 26.7 % IgM and 73 % IgG positive for HSV was observed (Thapaliyal *et al*., 2005). 3.6 % IgM and 33.58 % IgG positive for HSV-2 (Turbadkar *et al*., 2003). HSV-2 IgG and IgM antibodies were found in 63.1 % and 11.3 % in pregnant women (Nizami *et al*., 2004).

Nepalese Scenario

58.53 % IgG for HSV-1 and 65.85 % IgG for HSV-2 positive and 0 % IgM for HSV in Nepalese women of child bearing age (Kafle et al.,2004). 11.16 % IgM positive to HSV in patients of different age groups and gender in NPHL (Lamichhane et al., 2007).

3.2 Laboratory Diagonosis

Diagnosis of *Toxoplasma*

Diagnosis of *Toxoplasma gondii* can be done directly by microscopy- Tachyzoites or Cysts can be detected in tissue specimens such as bone marrow aspiration, brain biopsy, Molecular method in which PCR is being increasingly used for detection of *Toxoplasma*

gondii DNA in CSF, amniotic fluid, bronchoalveolar lavage (BAL) and blood and imagin method by Ultrasound, CT Scan etc, Biologically by animal inoculation and tested for presence of tachyzoites and the most sensitive indirect method is Serodiagnosis. A wide number of serological tests are available to detect either *Toxoplasma* antigen in serum or other body fluids eg. ELISA, Sabin-Feldman dye tests (Parija, 2004).

ELISA is most commonly and widely used technique for the detection of both IgG and IgM antibodies. In acute infection, IgG and IgM antibodies level generally raise within one to two weeks of infections. The presence of elevated levels of *Toxoplasma gondii* specific IgG antibodies indicates that infection has occurred but does not distinguish between recent infection and infection acquired in past. Detection of *Toxoplasma gondii* specific IgM antibodies has been used as an aid in determining the time of infection. A negative IgM test result with positive IgG results usually indicates infection at least 6 months previously. However, the interpretation of *Toxoplasma gondii* specific IgM positive results is complicated by the persistence of IgM antibodies up to 18 months after infection and by false positive reaction in commercial test (Jones et al., 2003).

Diagnosis of Rublla

Diagnosis of Rubella virus can be done directly by Molecular method in which PCR used for genomic analysis, Biologically by virus isolation from specimen is usually obtained by culture method and the most sensitive indirect method is Serodiagnosis; e,g ELISA, IFA- demonstration of Rubella specific IgM antibodies in the infant's blood or sera. In infants with CRS, IgM antibody persists for at least 6-12 months, Documentation of persistence of serum Rubella IgG titre beyond the time expected from passive transfer of maternal antibody (Brooks *et al.*, 2007; Bantvana & Best, 1998).

Diagnosis of CMV

Diagnosis of CMV virus can be done directly by Molecular method in which PCR used for detection of viral genetics, Biologically by virus culture from specimen and the most sensitive indirect method is Serodiagnosis;. e,g ELISA, IFA- demonstration of CMV specific IgM and IgG antibodies in the infant's blood or sera (Greenwood *et al.*, 2006; Chakraborty, 2003).

Diagnosis of HSV

Diagnosis of HSV is directly done by Microscopy in which smear made from scraping from base of vesicle is stained by toludene blue. Multinucleate giant cells (Tzank cells) are found. It can be also detected by culture and isolation technique in which specimen is directly inoculated into cell cultures, Chorioallantoic membrane, and human embryonic tissue. Among them, most advanced technique used indirectly is serological tests such as CFT, Neutralization test, ELISA test are used for detection patients serum antibody (Chakraborty, 2003; Greenwood *et al.*, 2007).

3.3 Treatment and Prevention of TORCH infections

Treatment of Toxoplasmosis

Treatment of toxoplasmsis depends upon the host immune status. Three form of therapy used at present includes; (a) pyrimethamineand sulphadiazine, (b) Spiramicin, and (c) Spiramycin and Sulfadiazine (Ichhpujani and Bhatia, 1994). Congenital toxoplasmosis, whether symptomatic or asymptomatic, should be treated with combination of Sufadiazine and pyrimethamine. If the presence of acute toxoplasmosis infection in pregnant woman is confirmed, treatment with spiramicin can be initiated as an effort to prevent transmission to fetus. If fetus infection is confirmed through amniocentesis, the woman may be switched to pyrimethamine and sulfadiazine. Combination of pyrimethamine and sulfadiazine is also effective against toxoplasmosis in immunocompromized patients (Levinson and Jawetz, 2000).

Prevention of Toxoplasmosis

Morbidity and Mortality rate from congenital infection can be reduced effectively mainly through three interventions namely, educating all the risk individuals about the prevention measure against the mode of transmission, Screening of pregnant women and new born child to indentify causal factors and treat congenital infections and use of well sophisticated techniques for rearing of animal and strictly avoid of contact of diseased animals with immune compromised individuals (Ross *et al*, 2006).

The main preventive measures for toxoplasmosis is blocking of route of transmission as food born route by cooking meat up to high temperature ,usually 145 – 180 ^{o}F temperature before consumption. Fruits and vegetables should be peeled and washed with clean water before intake. Contaminated material is always sterilized with suitable chemical and only use after ward. Pregnant women and immune deficient must be away from animals as its wastes may contain oocyst of *Toxoplasma*. Pregnant women should wear gloves when gardening and during any contact with soil or sand because cat waste might be in soil or sand. After gardening or contact with soil or sand, hands should be washed thoroughly. As most of complication of *Toxoplasma* seen in women of child bearing age, Health-care providers should educate pregnant women at their first prenatal visit about food hygiene and prevention of exposure to cat feces. The government and the meat industry should focus to reduce *Toxoplasma* in meat (Hughes *et al*, 2000).

Treatment of Rubella

There is no any treatment measure recognized yet. Since there is only one strain of Rubella exit, treatment is applied only as preventive measure by vaccination at their child stage. Most commonly used vaccine is MMR.

Prevention of Rubella

Since there is only one serotypes of Rubella, Rubella can be prevented by a rubella vaccine. Once immunize, it persists up to life time. The rubella vaccine is usually given as a combined measles-mumps-rubella (MMR) inoculation. Children should receive the

MMR vaccine between 12 and 15 months of age, and again between 3 and 6 years of age (Doctor NDTV Team, 2004). The prevention of congenital rubella obviously is dependent upon adequate early immunization, resulting in a high prevalence of immunity in women of childbearing age. If there is any doubt that they are immune, women should be screened for rubella immunity at the beginning of pregnancy. Contact isolation is required for neonates suspected to have congenital rubella (Boyer and Boyer, 2004). Pregnant women who are not immune should avoid anyone who has the illness and should be vaccinated after delivery so that they will be immune during any future pregnancies (Hirsch, 2006).

Treatment of Cytomegalovirus infection

Some antiviral agents for CMV infections are available but serious side effects limit their use to life or sight threatening complication. Ganciclovir is the agent most often used for serious CMV diseases given intravenously twice a day and is moderately effective against CMV retinitis and pneumonia in AIDS patients. Clinically, treatment has been successful in CMV colitis and encephalitis and progression of CMV retinitis in AIDs has been controlled with prolonged maintainance therapy. Gancioclovir resistant viruses are also reported. Foscarnet is used as an alternative agent for such cases. Other medicine prescribed against to CMV infection is Formiversin (Levinson and Jawetz, 2000)

Prevention of Cytomegalovirus (CMV) infection

Throughout the pregnancy, practice good personal hygiene, especially hand washing with soap and water, after contact with diapers or oral secretions (particularly with a child who is in day care). Women who develop a mononucleosis-like illness during pregnancy should be evaluated for CMV infection and counseled about the possible risks to the unborn child. Laboratory testing for antibody to CMV can be performed to determine if a woman has already had CMV infection. Recovery of CMV from the cervix or urine of women at or before the time of delivery does not warrant a cesarean section. The demonstrated benefits of breast-feeding outweigh the minimal risk of

acquiring CMV from the breast-feeding mother. There is no need to either screen for CMV or exclude CMV-excreting children from schools or institutions because the virus is frequently found in many healthy children and adult.

Treatment of HSV infections

Antiviral agents used to treat HSV infections are nucleoside Analogues. Acyclovir is the treatment of choice for encephalitis and systematic diseases caused by HSV-1. It is also useful for treatment of primary and recurrent genital herpes. Acyclovir is also used to treat neonatal infection caused by HSV-2. Mutants of HSV-1 resistant to Acyclovir have been isolated from patients: forscarnet can be used in these cases. For HSV-1 eye infections, other nucleosides analogues, e.g. Trifluridine are used topically. Panciclovir, a derivative of acyclovir can be used to treat reccurences of orolabial HSV-1 infection in immunocompitent adults. Valacyclovir and Famciclovir are used in the treatment of genital herpes and in the suppression of recurrences (Levinson and Jawetz, 2000).

Prevention of Herpes simplex virus (HSV) infection

Herpes infections can be prevented by avoiding direct contact with sores or ulcers of someone who has an active herpes infection - either on the mouth or on the genitals. Teens that are sexually active should properly use a latex condom during sexual activity, but even condoms will not completely eliminate the risk of spreading genital herpes while there are active lesions. The only surefire way to prevent genital herpes is abstinence (Homeier, 2005). Prevention of neonatal HSV requires the prevention of acquisition of HSV in the third trimester of pregnancy. Identification of women or couples susceptible to acquisition of HSV in pregnancy through serologic screening is receiving increasing attention, and such screening is being used with increasing frequency (Corey, 2003). Exposure of the infant at birth can be avoided if delivery by caesarean section is performed in the early stage of labour, but this is only to be recommended when lesions are present in the mother or virus has been demonstrated at that time (Ogilvie, 1997)

CHAPTER IV

4. MATERIALS AND METHODS

The present study was performed in the immunological section of OM Hospital and Research Center, Chabahil. The study was carried out from 15th July 2009 to 30th March 2010. During this period, a total of 161 blood samples from patients suspected of TORCH infections were collected and processed according to the standard laboratory protocols.

4.1 MATERIALS

All materials required for present work are listed in the appendix-II

4.2 METHODS

4.2.1 Study Site

Study was performed at the OM Hospital and Research Centre, Chabahil, Kathmandu. As this is one of the research centre and renounced hospital, the patients flow very high in comparison to others. So, this is the best suitable site for this study.

4.2.2 Study Population

Study population covers the patients visiting OM Hospital and Research Centre of age variation 0 to 50 or over during 15th July 2009 to 30th March 2010. It includes both male and females gender for observing statistical significance. Here all together 161 patients (151 females and 10 males) were studied.

4.2.3 Data collection

Data collection was done from each patient by interview through questionnaire given in appendix I. Clinical history (name, age, sex, signs and symptoms, past history of TORCH test) of patients were collected. Female patients were also asked about marital status, pregnancy and number of miscarriage or stillbirth, if any.

4.2.4 Specimen collection and storage

1. Following aseptic precaution, blood specimens were collected by vein puncture from each patient.

2. Specimen was kept in a labeled, clean and dry test tube.
3. Blood specimens were then centrifuged at 300 rpm for 5 minutes for serum separation.
4. Separated serum was transferred into other labeled test tubes with the help of micropipette.
5. The sera were then refrigerated at 2-8^{oc} until tested.

4.2.3 Reagent preparation

1. All reagents were allowed to reach room temperature before use and were mixed gently.
2. Wash buffer was diluted 20 times with distilled water.

4.2.4 ELISA Technique (Source: GB-1091 supplied by General Biologics Corporation)

1. The desired number of coated strips was placed into the holder.
2. 1:40 dilution of test sample, Negative control, Positive control and Calibrator were prepared by adding 5 µl of the sample to 200 µl of sample diluents and they were mixed well for homogenization.
3. 100 µl of diluted sera, calibrator and controls were dispensed into the appropriate wells. For reagent blank, 100 µl sample diluents were dispensed in 1A well position. The holders were tapped to remove air bubbles from the liquid and were mixed well.
4. Then the loaded wells were incubated at 37^{oc} for 30 minutes.
5. At the end of incubation period, liquid was removed from all wells. The micro titer well was ringed and flicked 4 times with diluted wash buffer (Phosphate buffer and tween 20) and one time with distilled water.
6. 100 µl of enzyme conjugate was dispensed to each well and incubated at 37^{oC} for 30 min.
7. Enzyme conjugate from all wells were removed and the micro titer wells were again ringed and flicked 4 times with diluted wash buffer and then one time with distilled water.

8. 100 µl of TMB reagent were dispensed into each well and were mixed gently for 10 seconds.
9. They were incubated at 37^{oC} for 15 minutes**.**
10. 100 µl of stop solution (1N HCl) was added into each well to stop reaction.
11. They were gently mixed for 30 seconds.
12. The O.D. (Optical Density) was read at 450 nm within 15 minutes with a micro well reader.
13. Data analysis was done by using SPSS software.
14. Safe disposal: Used micro titer strips and pipette tips were first treated with sodium hypochlorite solution and then discarded in the plastic containers in sealed condition. Test tubes and other glassware's used were washed and then sterilized by autoclaving.

CHAPTER V

5. RESULTS

Among the TORCH suspected patients, anti toxoplasmosis IgM was found 0.6 %, IgG in 26.4 % with both IgM + IgG in 0.6 %. Consequently, anti Rubella IgM was observed 0.6 %, IgG in 59.3 % along with both IgM + IgG in 0.6 %. Similarly, anti CMV IgM was discovered in 6 %, IgG in 32.7 % with both IgM + IgG in 2 %. While anti HSV-1 IgM was prevalenced in 8.7 %, IgG in 5.4 % and both IgM + IgG in 0 % and anti HSV-2 IgM was found in 16.8 %, IgG in 25.5 % with both IgM + IgG in 4.0 %.

Among the result observed, highest prevalence was observed for anti HSV-2 IgM in16.8 %, for anti Rubella IgG in 59.3 % and 4.0 % both IgM + IgG for HSV-2. The highest prevalence of IgM in HSV-2 observed may be due to the ease of transmission as it also transmitted prenatally and sexually along with close contact with infected person. Where as highest prevalence of IgG for Rubella observed, may be due to pre-immunization programs in childhood (table -1)

Table 1. **Overall distribution of TORCH profile in whole population**

TORCH profile	IgM positive %	IgG positive %	IgM+IgG positive %
Toxoplasma gondii	0.6 (1/155)	26.4 (41/155)	0.6 (1/155)
Rubella Virus	0.6 (1/150)	59.3 (89/150)	0.7 (1/ 150)
CMV	6 (9/150)	32.7 (49/150)	2 (3/150)
HSV-1	8.7 (13/149)	5.4 (8/149)	0 (0/149)
HSv-2	16.8 % (25/149)	25.5 (38/149)	4.0 (6/149)

Overall age wise distribution study of TORCH profile in whole population, positivity rate of IgM for *Toxoplasma* was observed highest in 0.9 % (1/112) to age group, 21-30

years, followed by 0 % in remaining all age group with no suspected patient was observed in age group 3-10 and > 51. Where as Highest prevalence for IgG was in 31 % (9/29) to age group 31-40, followed by 27.7 % (31/112) to 21-30 years, 20 % (1/5) to 11-20 years, 0 % (0/5) to 11-20 years and 0 % (0/4) to 0-2 years along with highest for both IgM + IgG in 0.9 % (1/112) to age group 21-30 years , followed by 0 % to all other age group. In our observation, out of 4 infants, none of them was positive to *Toxoplasma*.

As for Rubella, highest prevalence of IgM was observed in 3.6 % to age group 31-40 years, IgG in 62.9 % to age group 21-30 years, followed by 57.1 % to 31-40 and so on with highest rate for both IgM + IgG was in 0.9 % to 21-30 years, followed by 0 % in remaining all age group. Out of 4 Rubella suspected infants, 25 % (1/4) IgG positive was observed. It may be due to passive transfer maternal antibody up to 6 months and vaccination in childhood.

While highest prevalence rate was observed for anti CMV IgM in 40 % to age group 41-50 years, followed by 25 % to age groups 0-2 (infants) and so on with both IgM + IgG in 20 % (1/5) to age group 41-50 year, followed by 3.6 % to age group 31-40 year and so on.

Whereas highest prevalence rate for anti HSV-I IgM was discovered in 11.1 % to age group 31-40 years, followed by 9.25 % to 21-30 years and so on with highest prevalence for both IgM + IgG in 0 % in all age groups and highest prevalence rate was found out for anti HSV-2 IgM in 20.37 % to age group 21-30 years, followed by 11.1 % to 31-40 years, IgG 28.7 % to age group 21-30 years followed by 22.2 % to 31-40 years and so on with highest for both IgM + IgG; 4.6 % to age group 21-30 years followed by 3.7 % to 31-40 years and so on. Out of four infants suspected of HSV, no one had contracted by HSV infection (table-2)

Table-2, **Overall age wise distribution of TORCH profile in whole**

Age group	*Toxoplasma gondii*			Rubella virus			CMV			H
	IgM %	IgG %	IgM+ IgG %	IgM%	IgG %	IgM+ IgG%	IgM%	IgG %	IgM+ IgG%	Ig
0-2	0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)	25 (1/4)	0 (0/4)	25 (1/4)	0 (0/4)	0 (0/4)	0 (0
3-10	-	-	-	-	-	-	-	-	-	-
11-20	0 (0/5)	20 (1/5)	0 (0/5)	0 (0/5)	40 (2/5)	0 (0/5)	0 (0/5)	40 (2/5)	0 (0/5)	0 (0
21-30	0.9 (1/112)	27.7 (31/112)	0.9 (1/112)	0 (0/108)	62.9 (68/108)	0.9 (1/108)	2.8 (3/108)	32.4 (35/108)	0.9 (1/108)	9. (10
31-40	0 (0/29)	31.0 (9/29)	0 (0/29)	3.6 (1/28)	57.1 (16/28)	0 (0/28)	10.7 (3/28)	39.3 (11/28)	3.6 (1/28)	11 (3
41-50	0 (0/5)	0 (0/5)	0 (0/5)	0 (0/5)	40 (2/5)	0 (0/5)	40 (2/5)	20 (1/5)	20 (1/5)	0 (0
>51	-	-	-	-	-	-	-	-	-	-

populations

A total of 155 patients, 145 females and 10 males were tested for anti toxoplasmosis antibodies. Among the toxoplasmosis suspected patients included in TORCH group, anti toxoplasmosis IgG was found in 27.6 % and IgM in 0.7 % along with both IgM + IgG in 0.7 % among female patients, while those of male were 10 %, 0 % and 0 % for IgG, IgM and both IgM + IgG respectively.

A total of 150 patients, 141 females and 9 males were tested for anti - Rubella IgM and IgG antibodies. As for Rubella, it was detected in females IgG in 61 %, IgM in 0.7 % and both IgM + IgG in 7 % where as those of male were 33.3 %, 0 % and 0 % respectively.

A total of 150 patients, 141 females and 9 males were tested for anti CMV IgM and IgG antibodies. Anti CMV data was found for female as IgG in 33.3 % and IgM in 4.9 % along with both IgM + IgG in 1.4 % and these rates for male were as 25 %, 25 % and 12.5 % respectively.

A total of 149 patients, 140 females and 9 males were tested for anti HSV-1 IgM and IgG antibodies. Anti HSV-1 was discovered in female as IgG in 5 %, IgM in 9.3 % and both IgM + IgG in 0 % while for male patients, it was in 11.1 %, 0 % and 0 % respectively.

A total of 149 suspected patients, 140 females and 9 males were tested for anti HSV-2 IgM and IgG antibodies. Anti HSV-2 was found in females as IgG in 26.4 %, IgM in 15.7 % and both IgM + IgG in 4.3 % while for male, it was as IgG in 11.1 %, IgM in 0 % and both IgM + IgG in 0 % (table-3).

Table 3: Overall gender wise distribution of TORCH infected patients

TORCH profile	Male positive			Female positive		
	IgM %	IgG %	IgM+IgG %	IgM %	IgG %	IgM+IgG %
Toxoplasma	0 (0/10)	10 (1/10)	0 (0/10)	0.7 (1/145)	27.6 (40/ 145)	o.7 (1/ 145)
Rubella	0 (0/9)	33.3 (3/9)	0 (0/9)	0.7 (1/ 141)	61 (86/141)	0.7 (1 / 141)
CMV	25 (2/8)	25 (2/8)	12.5 (1/8)	4.9 (7/142)	33.3 (47 /143)	1.4 (2/142)
HSV-1	0 (0/9)	11.1 (1/9)	0 (0/9)	9.3 (13/140)	5 (7/ 140)	0 (0/140)
HSV-2	0 (0/9)	11.1 (1/9)	0 (0/9)	15.7 (22/140)	26.4 (37/140)	4.3 (6/140)

A total of 155 patients, 145 females were tested for anti toxoplasmosis antibodies. The highest no. of suspected cases was 109 from the age group 21-30. Positivity rate was highest for IgM in 0.9 % age group to 21-30 and for IgG in 34.6 % in age group 31-40, followed by 27.9 % to 21-30 years, 20 % in age group 11-20 and for both IgM + IgG in 0.9 % to 21-30 years in females suspected patients.

A total of 150 patients, 141 females were tested for anti Rubella IgM and IgG antibodies. The highest number of suspected cases was 105 from age group 21-30, followed by 26 in age group 31-40 years. Positivity rate was highest for IgM in 3.8 % in age group 31-40 and for IgG in 63.8 % in age group 21-30 years, followed by 57.7 % to 31-40, 50 % to 41-50 and 40 % in age group 11-20 along with for both IgM + IgG in 0.9 % positive to 21-30 years in females suspected patients.

A total of 150 patients, 141 females were tested for anti CMV IgM and IgG antibodies. The highest no. of suspected cases was 105 from age group 21-30, followed by 27 in age group 31-40 years. Positivity was highest for IgM in 25 % out of 4 in age group 41-50 years, followed by 11.1 % to 31-40 and 2.8 % to 21-30 and for IgG in 40 % in age group 11-20 year, followed by 37 % in 31-40 and then 33.3 % to 21-30 along with 0.9 % and 3.7 % both IgM + IgG in age group 21-30 and 31-40 year respectively.

A total of 149 patients, 140 females were tested for anti HSV-1 IgM and IgG antibodies. The highest no. of suspected patients was 105 from age group 21-30 year, followed by 25 in age group 31-40 year. Positivity rate was highest for IgM in 12 % in age group 31-40 year, followed by 9.5 % to 21-30 year and for IgG 20 % to 11-20, followed by 4.7 % to 21-30 year and 4.0 % to 31-40 year with both IgM + IgG 0 in % in all age groups.

A total of 149 suspected patients, 140 females were tested for anti HSV-2 IgM and IgG antibodies. The highest number of suspected cases was 105 from age group 21-30 year, followed by 25 to 31-40 years in case of female. The positivity rate was highest forIgM in 18.1 % in age group 21-30 years, followed by 12 % to 31-40 year and for IgG in 29.5 % to 21-30 year, followed by 20 % to 11-20 and 31-40 years along with both IgM+ IgG

in 4.7 % and 4% positive in age group 21-30 and 31-40 years respectively. This observation concluded that highest prevalence rate was observed at stage of childbearing age (table-4)

Table 4: Age wise distribution of TORCH in female

Age group	*Toxoplasma gondii*			Rubella			CMV			H
	IgM %	IgG %	IgM+ IgG %	IgM%	IgG %	IgM+ IgG%	IgM%	IgG %	IgM+ IgG%	Ig
0-2	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)	0
3-10	-	-	-	-	-	-	-	-	-	-
11-20	0 (0/5)	20 (1/5)	0 (0/5)	0 (0/5)	40 (2/5)	0 (0/5)	0 (0/5)	40 (2/5)	0 (0/5)	0
21-30	0.9 (1/109)	27.5 (30/109)	0.9 (1/109)	0 (0/105)	63 (67/105)	0.9 (1/105)	2.8 (3/105)	33.3 (35/105)	0.9 (1/105)	9. (1
31-40	0 (0/26)	34.6 (9/26)	0 (0/26)	3.8 (1/26)	57.7 (15/26)	0 (0/26)	11.1 (3/27)	37 (10/27)	3.7 (1/27)	12 (3
41-50	0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)	50 (2/4)	0 (0/4)	25 (1/4)	0 (0/4)	0 (0/4)	0 (0
>51	-	-	-	-	-	-	-	-	-	-

A total of 155 patients, 10 males were tested for anti - toxoplasmosis antibodies. The highest no. of suspected cases was 3 from the age group 21-30, 31-40, and 0-2. Positivity rate was highest for IgG in 33.3 % in out of 3 male patients, age group to 21-30 years while positivity rate for IgM were 0 % in all age group with both IgM + IgG 0 % in all age group.

A total of 150 patients, 9 males were tested for anti Rubella IgM and IgG antibodies. The highest no. of suspected cases was 3 from age group 21-30 and 0-2, followed by 2 in age group 31-40 years. Positivity rate was highest for IgG in 50 % in age group 31-40, followed by 33.3 % to 21-30 and 0-2 (infants) years while positivity for IgM among male patients were 0 % and both IgM + IgG in 0 % in all age group.

A total of 150 patients, 9 males were tested for anti CMV IgM and IgG antibodies. The highest no of suspected case was 3 from age group 0-2 and 21-30 years, followed by 1 in age group 31-40 and 41-50 years. Positivity rate was highest for IgM in 100 % out of 1 suspected patient in age group 41-50, followed by 33.3 % in age group 0-2 year (Infants) and for IgG in 100 % to 31-40 and 41-50 year with both IgM + IgG in 100 % in age group 41-50 years. 33.3 % infants were infected with CMV and this represents the congenital infecton.

A total of 149 patients, 9 males were tested for anti HSV-1 IgM and IgG antibodies. The highest no. of suspected patients were 3 from age group 0-2 and 21-30 year, followed by 2 in age group 31-40 in case of male. Positivity rate was highest for IgG in 50 % out of 2 suspected patients in age group 31-40 and for IgM in 0 % to all age group with both IgM + IgG in 0 % also in all age group. A total of 149 suspected patients, 9 males were tested for anti HSV-2 IgM and IgG antibodies. The highest no. of suspected cases was 3 from age group 0-2 and 21-30 year, followed by 2 to 31-40 years in case of male. Positivity rate was highest for IgG in 50 %, out of 2 suspected patients and for IgM in 0 % to all age group with both IgM + IgG in 0 % in all age group (table-5).

Table – 5. Age wise distribution TORCH profile in male.

Age group	*Toxoplasma gondii*			Rubella			CMV			HSV-1			HSV-2	
	IgM%	IgG%	IgM+ IgG%	IgM%	IgG %	IgM+ IgG%	IgM %	IgG %	IgM+ IgG %	IgM %	IgG %	IgM+ IgG %	IgM %	I %
0-2	0 (0/3)	0 (0/3)	0 (0/3)	0 (0/3)	33.3 (1/3)	0 (0/3)	33.3 (1/3)	0 (0/3)	0 (0/3)	0 (0/3)	0 (0/3)	0 (0/3)	0 (0/3)	0 (
3-10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11-20	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21-30	0 (0/3)	33.3 (1/3)	0 (0/3)	0 (0/3)	33.3 (1/3)	0 (0/3)	0 (0/3)	0 (0/3)	0 (0/3)	0 (0/3)	0 (0/3)	0 (0/3)	0 (0/3)	0 (
31-40	0 (0/3)	0 (0/3)	0 (0/3)	0 (0/2)	50 (1/2)	0 (0/2)	0 (0/1)	100 (1/1)	0 (0/1)	0 (0/2)	50 (1/2)	0 (0/2)	0 (0/2)	5 (
41-50	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)	100 (1/1)	100 (1/1)	100 (1/1)	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)	0 (
>51	-	-	-	-	-	-	-	-	-	-	-	-	-	-

CHAPTER-VI

6. DISCUSSION AND CONCLUSION

6.1 DISCUSSION

The acronym TORCH was introduced to highlight a group of agents which affects the fetus and newborn namely *Toxoplasma gondii*, Rubella Virus, Cytomegalovirus (CMV) and Herpes Simplex Virus (HSV). These agents often provide similar clinical picture which include one or more of the following clinical signs: low birth weight, prematurity, purpura, jaundice, anemia, microcephaly, hydrocephaly, cerebral calcification, chorioretinitis, cataracts, microphthalmia and pneumonitis (Lim *et al.*, 1994). TORCH infection is the major cause of Bad Obstetric History (BOH). Patients with BOH implies unfavorable fetal outcome in terms of two or more consecutives spontaneous abortions, early neonatal death, still child birth. Infection usually asymptomatic and chronic (Surpam, 2006). Maternal infection, especially during the early gestation can result in fetal loss or malformations because the ability of the fetus to resist infectious organism is limited and the fetal immune system is unable to prevent the dissemination of infectious organism to various tissues (Sebastian, 2008).

The present study was conducted among patients visiting OM Hospital & Research Centre, to determine the prevalence of antibodies, IgM and IgG against the agent causing TORCH infections. One hundred and sixty one blood samples were collected from the suspected patients and subjected to Enzyme linked Immunosorbant Assay (ELISA).

Out of 161 patients requesting for different panels of TORCH tests, 151 patients with one infant were female and 10 patients with 3 infants were males, with age variation 0-50 and above.

The highest no. of female patients, 114 (75.5 %) were found in age group 21-30, followed by 27 in the age group 31-40. This is probably because of the fact that females

of these age groups are of child bearing age and are screened for antibodies against TORCH infectious agents at their prenatal visits.

In the present study on the seroprevalence of antibodies IgM against the agents causing TORCH infections, HSV-2 was found to have the highest seroprevalence among the 4 TORCH agents. In addition to individual study of IgM and IgG, combined study of both IgM + IgG was done and result showed that among the five TORCH agents, Combined Seroprevalence of both IgM + IgG was found to have highest for HSV-2. It may be due to ease of transmission and because of in addition to congenital transmission; HSV-2 is also transmitted by close contact with an infected person who is shedding virus from skin, in saliva or in secretion from genitals. This research finding is comparable to global scenario as 8.6 % females and 10.5 % males positive to IgG in Dicle University Hospital (Suay *et al*., 2004), HSV IgM 8.66 % positive in women (Surpum *et al*., 2006), Neonatal HSV is very rare in Hong Kong (Lim *et al*., 1998), 26.7 % IgM positive (Thapaliyal *et al.*, 2005), anti HSV-2 IgM 3.6 % positive (Turbadkar *et al*., 2003), 11.3 % IgM positive in pregnant women (Nizami *et al.*, 2004), 0 % IgM for HSV in Nepales women of child bearing age (Kafle *et al*., 2004), 11.16 % IgM positive to HSV in patients of different age group and gender in NPHL (Lamichhane *et al*., 2007). During the same study period, among the TORCH profile, seroprevalence of Rubella IgG was observed to have highest. It may probably because of the fact that the entire individual at their child stage were immunized by MMR vaccine. Age wise study of TORCH agents on whole population showed that highest prevalence was observed 20 to 40 such as IgG for Rubella; 62.9 % (Table -2) in age group 21 – 30 years. This may be because of all the individual at their child stage are immunized with MMR vaccine and people of this age are nearly close to menopause and females are suffered from many kinds of reproductive problem and advised for screening TORCH and some TORCH agents are most commonly transmitted by sexual means, it usually encounter in sexually active group. Result of Rubella is also comparable to global scenario and Nepalese scenario as; 90.8% positive female and 90.3% positive male donors for IgG values (Suay et *al.*, 2004), 9.6% IgG positive in female (Sebastian et *al.*, 2008). The seropositives for anti-

rubella IgG, in pregnant woman in Turkey were found as 96.1% (Tamer *et al.,* 2009), 66.6% IgG positive in pregnant women of Nainital with BOH (Thapliyal *et al.,* 2005), 61.3% IgG positive in pregnant women with BOH (Turbadkar *et al.,* 2003), the seroprevalance study of Rubella among primary and pre-primary school pupils of Kenya by using ELISA test kit showed that overall, rubella seropositivity rate was 80% and it increased with age from 59% (among ages 4-6 years) to 94% (ages 14-20 years) (Janeth J.K *et al.,* 2009), 69.9% IgG positive in women before marriage and pregnancy in Isfan (Allameh *et al.,* 2004). In Nepalese women of childbearing age, 0 % IgG positive was observed (Kafle *et al.,* 2004). The suspected rubella cases were higher in male (55.14% of total) but the positive cases were found higher in female (52.38% of total positive). Out of total positive cases, the highest positive cases (46.67%) were from the age group 5-10 years followed by 28.57% from the age group below 5 years. Rubella positive cases were from Bhaktapur (13.33%), followed by Rauthat (9.52%), Bara (7.62%) and Dadeldhura (7.62%). The highest suspected cases were reported from CDR (131 cases) with 58 positive cases (52.24% of total positive). The rubella cases were highest in spring season (58.1%) followed by winter season (29.52%). The rubella cases were highest in March (30/61 cases) followed by February (18/28 cases), April (18/24 cases), January (13/26 cases) and May (13/27 cases) (K.C, 2007). Out of 4 infants, 25 % was observed IgM positive to CMV. This may be due to congenital infection from infected mother and 25 % was found out IgG positive to Rubella. This may be due to passively transfer of maternal antibody and Rubella vaccination at their child stage. Among the data observed in response to sex wise distribution, Prevalence (Rubella IgG) was observed highest in female. This may be because of females of child bearing age are suffered from many kinds of disease related to gynecology and prescribed for screening of TORCH profile and national level Rubella vaccine program at child stage.

6.2 CONCLUSION

This study was carried out to determine the prevelance of TORCH (IgM and IgG) among suspected patients visiting OM Hospital and Research centre, Chabahil. In the study period, 161 samples were collected. Among them, overall prevalence of IgG against TORCH profile as: 26.4 %, 59.3 %, 32.7 %, 5.4 %, and 25.5 % for *Toxoplasma*, Rubella, CMV, HSV-1 and HSV-2 respectively. This study concludes that wide range of population still suspectible to TORCH profile with high value of immune response to Rubella. Whereas overall prevalence of IgM was observed as: 0.65 %, 0.67 %, 6 %, 8.72 %, and 16.8 % for *Toxoplasma*, Rebulla CMV, HSV-1 and HSV-2 respectively. It concludes that HSV have high potential rate to cause infection, most commonly in women of childbearing age. Among the patient, female of childbearing age showed high prevalence rate in compared to male. It is therefore recommended that all antenatal cases should be routinely screened for these agents. Health education concerning the transmission of TORCH agent is strongly needed in order to prevent the spread of disease.

CHAPTER-VII

7. SUMMARY AND RECOMMENDATIONS

7.1 SUMMARY

1. The study was conducted among the patients suspected of TORCH infections visiting OM Hospital and Research centre, Chabahil from 17th July 2009 to 30th March 2010.
2. During the study period, total of 161 patients were studied. Out of which 10 (3 infants) were male and 151(1 infants) were female.
3. This study determine the overall anti Toxoplasma IgM, IgG and Both IgM + IgG positive in 0.6 %, 26.4 % and 0.6 % respectively where in female as 0.7 %, 27.6 % and 0.7 % respectively and in males 0 %, 10 % and 0 % respectively.
4. This study determine the overall anti Rubella IgM, IgG and Both IgM + IgG positive as 0.7 %, 59.3 % and 0.7 % respectively where in female as 0.7 %, 61 % and 0.7 % respectively and in males 0 %, 33.3 % and 0 % respectively.
5. This study determine the overall anti CMV IgM, IgG and both IgM + IgG positive as 6 %, 32.7 % and 2 % respectively where in female as 4.9 %, 33.3 % and 1.4 % respectively and in males 25 %, 25 % and 12.5 % respectively.
6. This study determine l anti HSV-1 IgM, IgG and both IgM + IgG positive as 8.7 %, 5.4 % and 0 % respectively where in female as 9.3 %, 5% and 0% respectively and in males 0 %, 11.1 % and 0 % respectively.
7. This study determine the overall anti HSV-2 IgM, IgG and both IgM + IgG positive as 16.8 %, 25.5 % and 4.0 % respectively where in female as 15.7 %, 26.4 % and 4.3 % respectively and in males 0 %, 11.1 % and 0 % respectively.
8. Highest prevalence was observed in female.
9. In out of 4 infants, 1(25%) IgG and 1 (25%) IgM positive to Rubella and CMV infection was observed. 25 % IgM shows intrauterine infection.

7.2 RECOMMENDATIONS

1) All woman of childbearing age should be examined for antibodies against TORCH agents before going to conceive pregnancy as it perinately transfer to new born child and responsible to cause congenital infection.
2) In this study, out of 4 infants, 1 (25%) IgG positive to Rubella and 1 (25%) positive IgM for CMV. So, this study recommends that Seroprevalece study of TORCH profile among infants should also be carried out.
3) Health education concerning the transmission of TORCH agents should be needed in order to prevent the spread of disease.

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