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Prevalence of Serum Antibodies to TORCH Infections among the Women of Child Bearing Age Visiting National Public Health Laboratory, Teku

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Abstract

The primary infection of *Toxoplasma gondii*, Rubella virus, Cytomegalovirus (CMV) and *Herpes simplex* virus (HSV) abbreviated as TORCH, remain a major problem in the women of child bearing age in Nepal. The main aim of this study was to determine the seroprevalence of TORCH infections among the women of child bearing age visiting the National Public Health Laboratory (NPHL), Kathmandu. Serum samples collected from 302 patients were tested for TORCH infections by IgM Enzyme Linked Immunosorbent Assay (ELISA). The seropositivity rate was found to be 18.82% (54/287) for *T. gondii*, 10.07% (28/278) for Rubella, 16.49% (46/279) for CMV and 23.34% (67/287) for HSV. The seropositivity rates in pregnant women were 17.92% (19/106) for *T. gondii*, 11.54% (12/104) for Rubella, 19.23% (20/104) for CMV and 25% (26/104) for HSV, the statistical association of TORCH infections with pregnancy was insignificant (P>0.05). Similarly, the seropositivity rates in women with bad obstetric history (BOH) were 17.84% (43/241) for *T. gondii*, 11.06% (26/235) for Rubella, 18.57% (44/237) for CMV and 26.14% (63/241) for HSV. The statistical association of CMV and HSV with previous obstetric performance were significant (P>0.05) while that of *T. gondii* and Rubella were insignificant (P<0.05). The seropositivity rate was found to be highest for HSV infection (23.34%) followed by *T. gondii* (18.82%), Cytomegalovirus (16.49%) and Rubella (10.07%)

Key words: TORCH infection, IgM ELISA, pregnant women, women with BOH

Introduction

Toxoplasmosis is caused by a protozoan, *Toxoplasma gondii*. It is asymptomatic in healthy individual and if acquired during pregnancy, especially as a primary infection may damage to the fetus (Yasodhara *et al.* 20011). Although of little concern in normal immunologically competent hosts, *T. gondii* infections can be fatal when the patient's immune system is unable to control the infection. Such is the case of congenital toxoplasmosis when *T. gondii* crosses the placenta during a primary maternal infection and causes disseminated infection of the fetus (Luft *et al.* 1984). Rubella is caused by an RNA virus and transmitted through airway or direct contact. In most cases, the disease is asymptomatic or has minor flu-like symptoms and generally is without sequelae (Su & Guo 2002). In pregnant women, however, rubella might cause multiple organ defects in fetuses, which is called congenital rubella syndrome (CRS) (Webester 1998). Cytomegalovirus (CMV) infection is the most important congenital infection globally (Gandhoke *et al.* 2009). Human CMV poses an important public health problem as it may cause serious morbidity and mortality in congenitally infected newborns and immunocomprimised patients, most notably transplant recipients and HIV infected persons (Chakravarti *et al.* 2009). The infected infants may suffer sensorineural hearing loss, ocular damage, or impairment of

cognitive and motor function (Fowler *et al.* 1993). The spectrum of infections caused by *Herpes simplex* Virus Type 1 (HSV-1) and *Herpes simplex* Virus Type 2 (HSV-2) includes oropharyngeal, genital and ocular lesions, neonatal infections, central nervous system infections and multisystem infections in the immunocomprimised individuals (Fatahzadeh & Schwartz 2007). HSV-1 is usually associated with primary infections of the orofacial area and latent infection of the trigeminal ganglion, while HSV-2 is usually associated with genital infections and latent infection in sacral ganglia (Whitley 2001).

TORCH infections are associated with recurrent abortion, intrauterine growth retardation, intrauterine death, preterm labour, early neonatal death and congenital malformations (Surpam *et al*.2006). Serologic testing of both mothers and neonates could be useful for detecting malformations (Golalipour *et al.* 2009). All antenatal cases with bad obstetric history should be routinely screened for TORCH complex as early diagnosis and appropriate intervention of the infection will help in proper management of these cases (Turbadkar *et al.* 2003).

In developing country like Nepal, where most of women belong to the low socio-economic group, may be exposed to one or more of the TORCH agents that may lead to serious congenital malformations. Therefore, appropriate intervention measures, early diagnosis and treatment of TORCH infection have to be carried out to reduce these infections during pregnancy and their effect on outcome of pregnancy.

Methodology

The study was conducted at the Immunology section of National Public Health Laboratory (NPHL), Kathmandu from March to August, 2010. A total of 302 female patients of child bearing age suspected of TORCH infections were recruited in this study. Informed verbal consent was taken from all the patients orally. *Toxoplasma gondii*, Rubella virus, CMV and HSV-II IgM antibodies were tested as per the instruction of the manufacturer (Human, Germany). Data were statistically analyzed using Chi-square test.

Data collection

Data were collected from each female patient of child bearing age by interview through a questionnaire. Clinical history (name, age, sex, signs and symptoms, past history of TORCH test, marital status, pregnancy and number of miscarriage or stillbirth, if any) of the patients were collected.

Specimen collection and storage

Following aseptic precautions, blood specimens were collected by vein puncture from female patient of child bearing age and were kept in labelled, clean and dry test tubes. The blood samples in the tubes were allowed to clot for 30 minutes at room temperature. The samples were then centrifuged for serum separation. The separated serum was then kept in other labelled tubes. The sera were then refrigerated at 2-8º C until tested. Few serum specimens for TORCH tests referred from different hospitals situated at Kathmandu valley were also received directly at NPHL.

Enzyme linked immunosorbent assay (ELISA) procedure

ELISA techniques for the detection of IgM antibodies to TORCH agents (*Toxoplasma*, Rubella, Cytomegalovirus and Herpes simplex virus) were performed as per the instruction of the manufacturer (Human, Germany).

Data analysis

Data were analyzed using Chi-square test with 5% level of significance.

Results and Discussion

In this study, out of 302 suspected patients, 54 women (18.82%) among 287 suspected cases were positive to *Toxoplasma* (IgM); 28 women (10.07%) among 278 cases were positive to Rubella (IgM); 46 women (16.49%) among 279 suspected cases were positive to CMV (IgM) and 67 women (23.34%) among 287 suspected cases were positive to HSV (IgM).

The seroprevalence of HSV infection was found to be highest (25%) compared to other TORCH agents. The seroprevalence of HSV was followed by Cytomegalovirus (19.23%), Toxoplasma (17.92%) and Rubella (11.54%). HSV infection was found to be highest among the TORCH infections in patients with bad obstetric history (BOH) followed by Cytomegalovirus, Toxoplasma and Rubella whose seropositivity were 26.14%, 18.57%, 17.84% and 11.06% respectively.

TORCH infections were associated with inadvertent outcomes like multiple abortions, sterility, congenital

malformations, intrauterine foetal deaths, still births and other reproductive failures, especially when they were acquired during the first trimester of the pregnancy. The detection of IgM antibody against TORCH was the best approach for the identification of these infections (Li *et al.* 2009). In this study, highest IgM seropositivity was seen with HSV (23.34%) followed by *T. gondii* (18.82%), CMV (16.49%) and Rubella (10.07%). HSV (IgM) and *Toxoplasma* (IgM) was found to have higher seroprevalance because in addition to congenital transmission, HSV was transmitted sexually and *T. gondii* was transmitted orally by eating raw meat or exposure to infected cat faeces. Similarly, the lower seroprevalance of Rubella (IgM) could be due to naturally acquired immunity.

T. gondii is known as an etiological agent in recurrent pregnancy wastages and its IgM seropositivity rate in pregnant women was found to be 17.92% in our study. In a study done in India, it was found to be 13.1%. Lower seroprevalance were found in studies done in other countries (Ribeiro *et al*. 2008, Alvarado-Esquivel *et al*. 2009). Among the women with BOH, prevalence of IgM antibodies against *T. gondii* was found to be 17.84% which was in concordance with the finding of a comparable study (15.43%) conducted earlier (Lamichhane *et al*. 2007). In a recent study in India, 21.9% of women with BOH were positive to *T. gondii* IgM antibodies (Sarkar *et al*. 2012).

Table 1. Correlation of TORCH infections with pregnancy

Pregnancy		Seropositivity percentage of		
	Toxoplasma (IgM)	Rubella (IgM)	Cytomegaloviurs (IgM)	Herpes Simplex Virus (IgM)
Pregnant	17.92 (19/106)	11.54 (12/104)	19.23 (20/104)	25 (26/104)
Non-Pregnant	19.34 (35/181)	9.2 (16/174)	14.86 (26/175)	22.40 (41/183)
P value			P>0.05	

Rubella or German measles is an infectious disease caused by Rubella virus which can pass from the pregnant women's bloodstream to infect her unborn child causing congenital Rubella syndrome (Hirsch 2006). In the present study, Rubella IgM seropositivity rate of pregnant women was found to be 11.54%. In a study done in Nigeria, 3.9% of pregnant women were positive to Rubella IgM and was found to be 7.7% in another study (Grace *et al.* 2009, Linguissi *et al.* 2012). Some recent studies reported lower seroprevalance of Rubella (Vilibic-Cavlek *et al.* 2011 Qin *et al.* 2011). In pregnant women, rubella might cause multiple organ defects in fetuses, which is called congenital rubella syndrome (CRS) (Webester 1998). In our study, Rubella IgM seropositivity in women with BOH was 11.06% which was higher than found in an earlier study in Nepal (4%) (Lamichhane *et al.* 2007). In recent studies, Rubella in women with BOH were found to be 30.4% and 4.65% respectively (Sen *et al.* 2012, Sadik *et al.* 2012*)*.

CMV infections were mostly inapparant, the virus could cause wide range of diseases in neonates (Kim 2010). The present study showed CMV IgM seropositive rate of 19.23% in the pregnant women. However, lower seroprevalance was found in other studies (Guo 1992, Chakravarty *et al.* 2005, Munro *et al.* 2005). In this study, CMV IgM seroprevalance was found to be 18.57% in BOH patients. Similar study done in Nepal showed 9.33% (Lamichhane *et al.* 2007). In a study done in India recently, 34.7% BOH patients were positive to CMV IgM (Sen *et al.* 2012) while other studies showed lower seroprevalence (Turbadkar *et al.* 2003, Sadik *et al.* 2012).

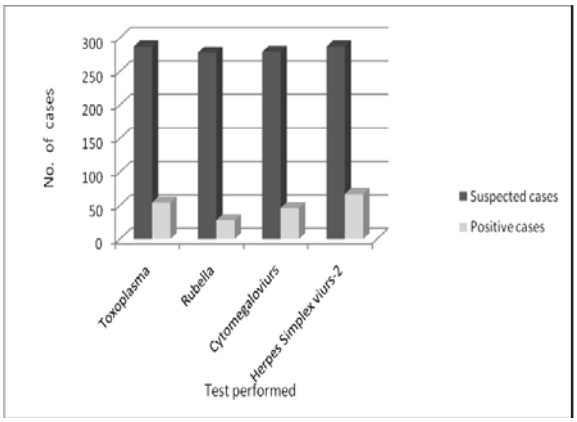

Fig 1. TORCH (IgM) test results

Likewise, our study showed that 25% of pregnant women were positive to HSV IgM antibodies. Studiesdone in Croatia and China have reported

seropositivity rate of 1.2% and 2.45% respectively (Vilibic-Cavlek *et al*. 2011, Qin *et al*. 2011). Seropositivity rate of HSV IgM among the BOH patients of our study was 26.14%. The seropositivity was found to be 11.33% in a previous study (Lamichhane *et al.* 2007). A study done in India showed higher seroprevalance of 33.5% (Sen *et al.* 2012). However, lower seroprevalance was reported in a study done in South India (Sadik *et al*. 2012).

This showed that the TORCH infections in the women of reproductive age group was mostly due to the infections which had very common route of transmissions in Nepal, for example, HSV was transmitted sexually and *T. gondii* was transmitted mainly from the food borne route. The low seroprevalance of Rubella might be because of the fact that it was self-limiting illness and provides lifelong natural immunity. The overall result emphasized the need for protection against these infections by safe sexual practice, maintenance of good hygiene and vaccination.

Table 2. Correlation of TORCH infection with previous obstetric performance

Previous obstetric performance	Seropositivity percentage of			
	Toxoplasma (IgM)	Rubella (IgM)	Cytomegalovirus (IgM)	Herpes Simplex Virus (IgM)
BOH	17.84 (43/241)	11.06 (26/235)	18.57 (44/237)	26.14 (63/241)
Normal obstetric performance	23.91 (11/46)	4.65 (2/43)	2.76 (2/42)	8.70 (4/46)
P value	P > 0.05		P < 0.05	

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