Himalayan Journal of Science and Technology (2018)

ISSN No: 2565-5132 (*Print*)
:2616-0234 *(Web)*

Original Research Article

Toxoplasma, Rubella, Cytomegalo and Herpes Viral Antibody Level in Patients at National Public Health Laboratory (NPHL), Kathmandu

Binod Dhungel[1], Nabaraj Adhikari[1], Upendra Thapa Shrestha[1], Bishnu Prasad Updhayaya[2], Komal Raj Rijal[1] and Prakash Ghimire[1]*

[1]Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu
[2]National Public Health Laboratory (NPHL), Teku, Kathmandu

***Corresponding Author:** Komal Raj Rijal, Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu. *E-mail: rijalkomal@yahoo.com*

Abstract:

The primary infection of *Toxoplasma gondii*, Rubella virus, Cytomegalovirus (CMV) and Herpes simplex virus (HSV) abbreviated as TORCH has remained a major problem in the women of childbearing age in Nepal. The main objective of this study was to determine the seroprevalence of TORCH infections among the women of childbearing age visiting the National Public Health laboratory (NPHL), Teku, Kathmandu. A total of 314 blood samples were collected and tested for TORCH infections by Chemiluminescent Immunoassay (CLIA). Of the total 314 patients, different patterns were observed in the requested test series. Highest number of patients (243) requested for CMV IgM test while only 195 requested for the same for Rubella. The IgM seroprevalence rates were 4.20% (9/214) for *Toxoplasma gondii*, 5.12% (10/195) for Rubella, 4.91% (12/244) for Cytomegalovirus and 5.56% (13/234) for Herpes Simplex virus. Significant portion of the test exhibited the equivocal result making this latest technology further complicated. Equivocal results were 2.33 % (5/214) for *Toxoplasma gondii*, 3.58% (7/195) for Rubella, 3.27% (8/244) for Cytomegalovirus and 4.27% (10/234) for Herpes Simplex Virus. The seropositivity rate in pregnant women was 11.97 % (17/142), in which the seropositivity in pregnant women with Bad Obstetric History (BOH) was found to be 12.5% (13/104) and women without BOH was found to be 10.52% (4/38). The statistical association of any one of the TORCH infections with previous obstetric history was significant ($p<0.05$). The seropositivity rate was the highest for HSV infection followed by Rubella, CMV and *T. gondii*.

Keywords: TORCH, Bad Obstetric History, CLIA, Seroprevalence, Seropositivity

Introduction

The infection of *Toxoplasma gondii*, Rubella virus, Cytomegalovirus (CMV) and Herpes simplex virus (HSV) abbreviated as TORCH has remained a major problem in the women of childbearing age in Nepal. Nowadays, TORCH screening has been routine practice to monitor the plight of mother and growing fetus. Infections caused by TORCH and others agents like *Chlamydia trachomatis*, *Treponema pallidum*, *Neisseria gonorrhoeae*, and HIV are the major causes of Bad Obstetric History (BOH) (McCabe and Remington, 1988; Turbadkar et al, 2003). BOH implies previous unfavorable fetal outcomes in terms of two or more consecutive spontaneous abortions, early neonatal deaths, stillbirths, intrauterine fetal deaths, intrauterine growth retardations and congenital anomalies. Maternal infections transmissible in utero at various stages of gestation lead to recurrent pregnancy wastage. Infections caused by TORCH are the major cause of BOH (McCabe and Remington, 1988).

It has been estimated that of all congenital anomalies, prenatal infections alone account for 2 to 3%. TORCH infections are some of the most common infections associated with congenital anomalies. Most of the infections caused due to TORCH result mild maternal morbidity but may have serious fetal consequences and treatment on maternal infection frequently has no advantage on fetal outcome. Therefore, it is to be noted that once the maternal infection is recognized, clinicians should carry out the treatment of maternal disease

ISSN No: 2565-5132 (*Print*)
:2616-0234 *(Web)*

coupled with fetal monitoring. The knowledge on these diseases will enable the clinicians to provide appropriate counseling service to the mothers thus providing preventive measures to avoid these infections, and will aid in counseling parents on the potential for adverse fetal outcomes when these infections are present (Stegmann and Carey, 2002). The TORCH agents are responsible for very frequent infection in HIV positive patients with progressively lowered immunity. Such individuals required to be diagnosed at an early stage. Hence, treating clinicians are requested to investigate the patients accordingly to modify their treatment regimens and necessary prophylaxis as required. Many sensitive and specific tests are available for serological diagnosis of TORCH complex (Newton, 1999). ELISA for IgM antibodies against these infections is highly sensitive and specific (Malhotra and Bhardwaj, 1991).

In developing countries like Nepal, where most of the 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. Studies on epidemiology of TORCH in Nepalese people are increasing yet the results obtained from these studies do not provide actual seroprevalence, as they are case oriented rather than population oriented and, in addition, are hospital-based studies. However, these handfuls of studies are suggestive for the presence of immunity to TORCH agents in Nepalese population. This study aims to determine current status of TORCH and assess risk factors among immunocompromised patients visiting National Public Health Laboratory (NPHL), Teku during the study period. At NPHL, the scheme for diagnosing the infection is done through TORCH panel which is solely dependent upon the detection of IgM antibodies against those organisms, and only few of them are subjected further for the analysis of IgG antibodies in serum sample. The detection of only IgM antibodies limits the establishment of prevalence among people. This study aims to determine the prevalence of anti-TORCH antibodies in suspected serum samples and establish the relation of risk factors with the infection. The study compares and contrasts the status of infection in suspected individuals visiting NPHL.

Materials and Methods

The hospital/laboratory based prospective cross-sectional study was conducted in NPHL, Teku, Kathmandu, during November, 2014 to August, 2015. Three hundred fourteen blood samples received for the testing of TORCH agents from suspected TORCH patients were processed during study period. Suspicions of the TORCH cases classification were defined by following guidelines (CDC, 2013).

Rubella / German measles:

Suspected Case: Any generalized rash illness of acute onset that does not meet the criteria for probable or confirmed rubella or any other illness. Probable: In the absence of a more likely diagnosis, an illness characterized by all of the following i.e. Acute onset of generalized maculopapular rash, temperature greater than 99.0°F or 37.2°C, arthralgia, arthritis, lymphadenopathy, or conjunctivitis, lack of epidemiologic linkage to a laboratory confirmed case of rubella and non-contributory or no serologic or virologic testing. Confirmed: A case with or without symptoms who has laboratory evidence of rubella infection confirmed by one or more of the following laboratory tests i.e. isolation of rubella virus; or detection of rubella-virus specific nucleic acid by polymerase chain reaction; or IgG seroconversion or a significant rise between acute- and convalescent-phase titers in serum rubella IgG antibody level by any standard serologic assay; or Positive serologic test for rubella IgM antibody.

Genital Herpes (Herpes Simplex Virus):

it is a condition characterized by visible, painful genital or anal lesions and diagnosed by isolation of herpes simplex virus from cervix, urethra, or anogenital lesion, or demonstration of virus by antigen detection technique in clinical specimens from cervix, urethra, or anogenital lesion, or demonstration of multinucleated giant cells on a Tzanck smear of scrapings from an anogenital lesion .Probable: A clinically compatible case (in which primary and secondary syphilis have been excluded by appropriate serologic tests and darkfield microscopy, when available) with either a diagnosis of genital herpes based on clinical presentation (without laboratory confirmation) or a history of one or more previous episodes of similar genital lesions Confirmed: A clinically compatible case that is laboratory confirmed Comment: Genital herpes should be reported only once per patient. The first diagnosis for a patient with no previous diagnosis should be reported (CDC, 2013).

Genital Warts: An infection characterized by the presence of visible, exophytic (raised) growths on the internal or

ISSN No: 2565-5132 (*Print*)
:2616-0234 *(Web)*

external genitalia, perineum, or perianal region and diagnosed by histopathologic changes characteristic of human papillomavirus infection in specimens obtained by biopsy or exfoliative cytology or demonstration of virus by antigen or nucleic acid detection in a lesion biopsy. Probable: A clinically compatible case without histopathologic diagnosis and without microscopic or serologic evidence that the growth is the result of secondary syphilis. Confirmed: A clinically compatible case that is laboratory confirmed (CDC, 2013).

Toxoplasmosis

Confirmed Case: Clinical illness with laboratory confirmation of infection by detection of IgM and IgG antibodies to *T. gondii* from a single blood specimen; or detection of rise in IgG serology units taken at least 2-3 weeks apart or demonstration by microscopy or nucleic acid amplification of organism (CDC, 2013).

Cytomegalovirus, Congenital infection

Confirmed case: Laboratory confirmation of cytomegalovirus (CMV) infection with or without clinical illness: Direct detection of CMV, using PCR or viral culture, from urine, saliva, blood or CSF collected from an infant at less than 3 weeks of age. If CMV is detected in saliva, repeated testing should be performed using urine; or prenatal diagnosis through direct detection of CMV, using PCR or viral culture, in amniotic fluid; or histopathological evidence of CMV inclusion disease from an appropriate clinical specimen, including a post-mortem specimen. Probable Case: Unexplained clinical illness consistent with congenital CMV with evidence of CMV infection not meeting the criteria above; for example direct detection of virus in a child older than 3 weeks of age, or positive CMV IgM antibody in fetus or newborn (CDC, 2013).

Inclusion Criteria: Patients visiting NPHL were enrolled by fulfilling following inclusion criteria i.e. individuals visiting lab for the diagnosis of infection with one or more or entire TORCH agents, individuals with disease history (HIV/AIDS, Fungal meningitis, Tuberculosis), pregnant women and/or women having Bad obstetric History (BOH), individuals who have undergone organ transplantation, individuals with prior history of prolonged antibiotic therapy, patients with malignancy and elderly people and children. Patients fulfilling the afore-mentioned criteria were enrolled under study with written consent and obtained demographic information and information related to infection by filling predesigned structured questionnaire form.

Collection, transport and interpretation of blood Specimens from suspected cases of TORCH: Five milliliters of venous blood was aseptically collected from suspected individuals at National Public Health Laboratory, Kathmandu. Collected blood samples were transported immediately to Immunology Department of NPHL. In the laboratory, serum was separated, labeled and stored at -20 °C until use. The separated serum samples were run under chemiluminescent immunoassay equipment as per manufacturer's guidelines. Serum specimens were analyzed following the automated system following the guidelines (CDC, 2013). The results obtained were interpreted according to the guidelines provided i.e. reactive, nonreactive and equivocal. Data collection, entry and validation, organization and monitoring approaches were used to generate quality of data. Data were analyzed using SPSS software version 16.

Results and Discussion

A total of 314 blood samples were collected from patients visiting NPHL for TORCH analysis at NPHL and analyzed. Among 314 patients, 65.6% (n=206) were female and 34.4% (n=108) were male. Majority of the patients were between the age group 21-30 years in female and 31-40 years in male **(Figure 1)**.

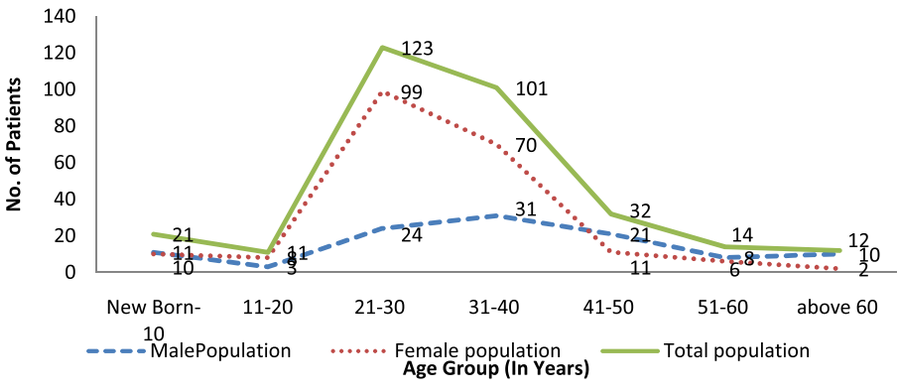

Figure 1: Age wise distribution of TORCH suspects cases at NPHL

ISSN No: 2565-5132 (*Print*)
:2616-0234 *(Web)*

Almost all of the population representing the women was from childbearing age whereas male population followed diagnosis only after the suspicion of the disease.

Seroprevalence of TORCH IgG antibodies:
Of the 314 patients, only 86 patients were tested for the TORCH IgG test and found high seroprevalence of IgG antibodies as compared to IgM. The seroprevalence of serum IgG antibodies against TORCH agents were 24.41% (21/86) for *T. gondii*, 93.24% (69/74) for Rubella, 89.18% (66/74) for CMV and 85.13% (63/74) for HSV. Out of 86 patients, 74 patients were tested for complete TORCH panel test whereas 12 patients requested only for detection of *Toxoplasma* IgG. None of test result was noted as equivocal **(Figure 2)**.

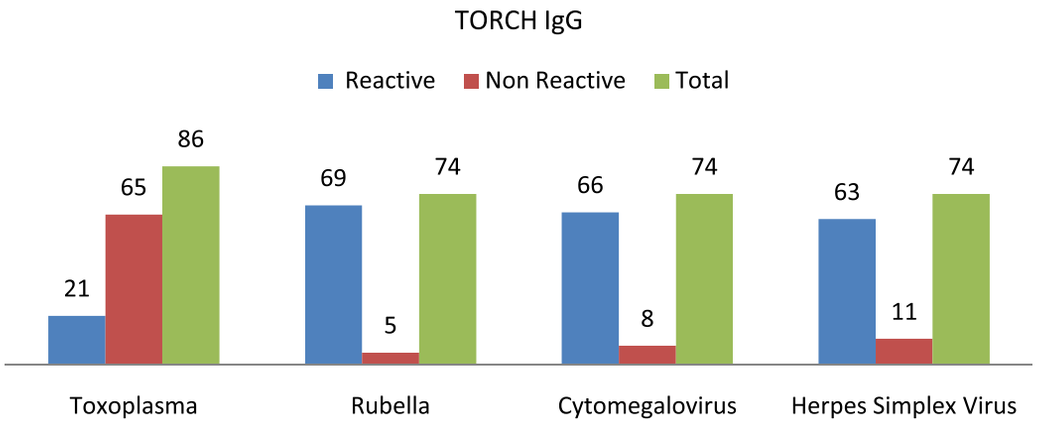

Figure 2: Seroprevalence of TORCH IgG antibodies

Seroprevalence of TORCH Agents by Using IgM Chemiluminescent Immunoassay (CLIA): The IgM seroprevalence rates were 4.20% (9/214) for *T. gondii*, 5.12% (10/195) for Rubella, 4.91% (12/244) for CMV and 5.56% (13/234) for HSV. Significant portion of the test exhibited the equivocal result making this latest technology further complicated. Equivocal results were 2.33 % (5/214) for *T. gondii*, 3.58% (7/195) for Rubella, 3.27% (8/244) for CMV and 4.27% (10/234) for HSV **(Figure 3).**

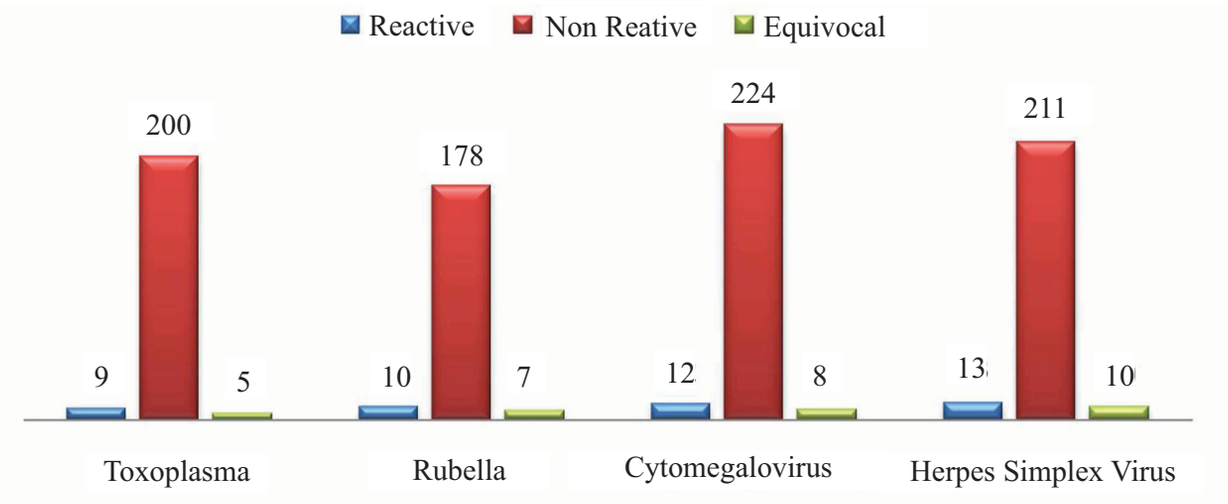

Figure 3:Seroprevalence of TORCH IgM antibodies

TORCH Infection among HIV Positive Patients: Among 314 patients, 11 patients (two female; nine male) were living with HIV/AIDS.The seropositivity rates among the suspects were 9.09% (1/11) for *T.gondii*, 20% (2/10) for Rubella, 20% (2/10) for CMV and 10% (1/10) for HSV. The overall seropositivity was 18.18% **(Figure 4)**.

ISSN No: 2565-5132 (*Print*)
:2616-0234 *(Web)*

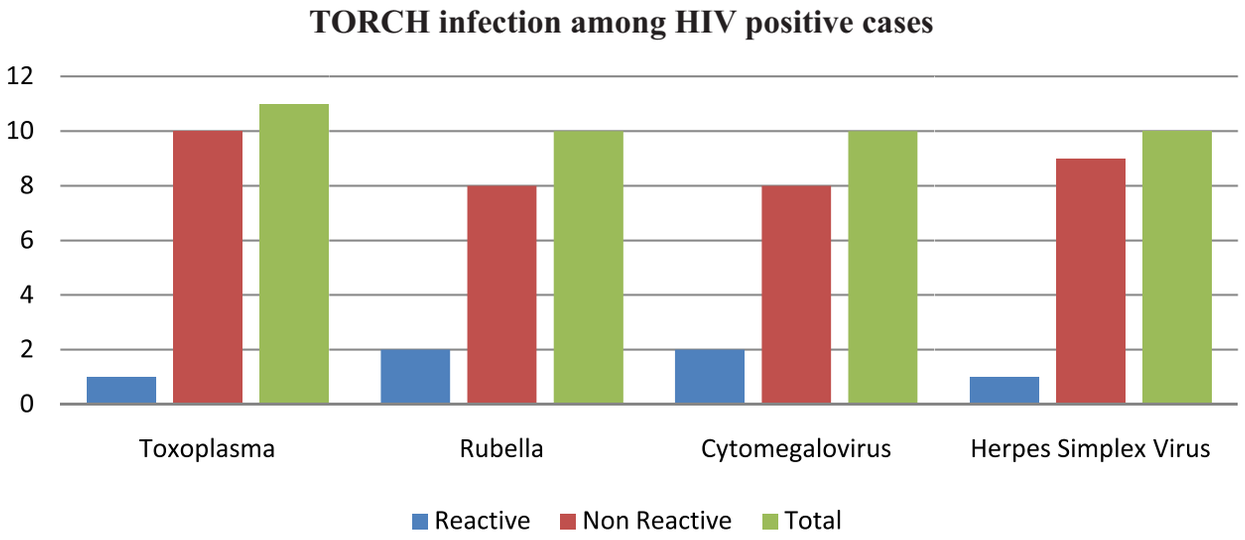

Figure 4: TORCH infection among HIV positive cases

TORCH infection among pregnant women with or without Bad Obstetric History (BOH): Among 206 female patients, 142 were pregnant. Among total pregnant women, 104 women had Bad Obstetric History (BOH).Seropositivity rates were 12.5% (13/104) for women with BOH and 10.52% (4/38) for pregnant women without BOH. The overall seropositivity rate among pregnant women was 11.97% (17/142). Thus obtained result was statistically significant ($p < 0.05$) (**Table 1**).

Table 1: Correlation of TORCH infections with pregnancy

Test result	Reactive	Equivocal	Non- reactive	Total	P- value
Pregnant with BOH	13	10	81	104	<0.05
Pregnant without BOH	4	10	24	38	
Total	17	20	105	142	

In this study, highest IgM seropositivity was observed with Herpes simplex virus (5.56%) followed by Rubella virus (5.12%). The seropositivity of Rubella was observed higher as compared to other TORCH agents despite profound Immunization programme with MMR vaccine during childhood. To the best of our knowledge, baseline data on seropositivity on the local populations are not available from any part of Nepal (Lamichhane et al, 2015). The highest number of female patients was found in the age group 21-30 followed by age group 31-40 years. This is probably because of the fact that females of these age groups (21-40 years) are of child bearing and screened for antibodies against TORCH agents at their prenatal visits. Nowadays, in addition to the higher prevalence rate, the trend of recommendation for pregnant women to undergo the diagnosis for TORCH is increasing.

Women with child bearing age group are recommended by doctor for the diagnosis even when symptoms in relation to TORCH infections are not observed. The increasing trend, off course, is a good signal for maternal health and is reflected in the study also. The number of male patients was higher in the age group (31-40 year). The difference in trend is probably because of the fact that most of the male patients have trend to visit lab only after the onset of the symptoms either related to TORCH or other else. Most of the patients have habit to visit only after undergoing illnesses (Rai et al, 2011).

The highest seroprevalence (93.34%) of the Rubella specific IgG antibodies was detected which may be due to the previous immunization of the individuals with MMR vaccine. The prevalence rate was followed by Cytomegalovirus (89.18%); Herpes simplex Virus (85.13%) and *T. gondii* (24.41%) in our study. This prevalence rates for other TORCH agents were higher than previous findings. Pradhan (2015) estimated a prevalence rates of 9.2, 78.9 , 58.7 and 35.8% for *Toxoplama*, Rubella, Cytomegalovirus and Herpes Simplex virus respectively in the study done at eastern Nepal in 2012 to 2014 by using ELISA. In another study carried out on 2010-2011 in TU Teaching hospital, prevalence rates of 35.9, 54.2, 81 and 66% for

ISSN No: 2565-5132 (*Print*)
:2616-0234 *(Web)*

Toxoplasma, Rubella, CMV and HSV respectively were reported on women with spontaneous abortions (Guddy et al, 2015).

The present study result was found highly deviated than other similar studies due to the fact that NPHL is a tertiary care center and most of the patients had habit of visiting the center only after the onset of the problems. Most of the patients were found with previous disease history. Only a small fraction of our study was focused to detect IgG antibodies against the agents. Therefore, IgG persists for long time after an infection is established. This could be the principal reason for the pattern. None of the study has done yet to compare the sensitivity of the ELISA and CLIA. The sensitivity issue could be another reason for this large deviation.In another study done in 2012 at Dhulikhel, Kathmandu University, prevalence rates were estimated as 77.9% for *T. gondii*, 86.8% for Rubella, 51.9% for CMV and 84.4% for HSV(Acharya et al, 2014). The finding of this study was comparable to our study. Although the result is highly deviated for *Toxoplasma* and CMV, rest of the infections had higher prevalence rate. The highest seropositivity for Rubella can again be acknowledged for the previous immunization practice in Nepal.Although, nearly half of the Nepalese are *T. gondii* seropositive (Rai et al, 1998), this study is not likely to support the established fact. This could be because of increasing awareness and sanitation practice targeted against the infection.

The main aim of this study was to determine the overall seroprevalence rate of IgM antibodies against TORCH infection. The seropositivity rates were found to be 4.20% (9/214), for *T. gondii*, 6.67% (13/195) for Rubella, 4.52% (11/243) for CMV and 5.15% (12/233) for HSV. In another study conducted in 2015 found prevalence of serum antibodies against TORCH infections as 18.82% for *Toxoplasma*, 10.07% for Rubella, 16.49% for CMV & 23.34% for HSV (Lamichhane et al, 2015). The seropositivity rates were also far higher than our estimates. Similarly, in another study; the rates were estimated as 26.6% for *Toxoplasma*, 9.2% for Rubella, 17.4% for CMV & 4.6% for HSV (Pradhan, 2015). This finding was also highly deviated from our study. In another study, the seropositivity rate was estimated as 23.2% for one or more TORCH agents. Similarly, prevalence rates of IgM antibodies were 13.7% for *Toxoplasma*, 4.7% for Rubella, 11.7% for CMV & 11.9% for HSV (Lamichhane et al, 2015). Leaving behind other TORCH agents, the prevalence rate of Rubella was comparable to our study. This low rate can again be accredited to the aggressive immunization practice carrying out in national immunization program by government itself. In another study, the seropositivity rates among women with spontaneous abortions were reported as 16.3% for *Toxoplasma*, 3.3 % for Rubella, 2% for CMV and 3.3% for HSV (Guddy et al, 2015). The study is comparable to our findings. The analogy could be due to the fact that the study was also carried out in tertiary care centre Tribhuvan University Teaching Hospital. These studies do not correlate with our present studies. This is because of the main reason that most of the studies are focused to pregnant women and with Bad Obstetric performance rather than overall population group. Our study found 11.97% overall seroprevalence rate of one or more TORCH agents among pregnant women. The stratified results based on individual agent, if provided, still become lower than the previous findings. Our focus was to estimate the overall prevalence rate in pregnant women rather than stratified results on individual agents from TORCH groups. However, the seroprevalence rates among HIV positive individuals are comparable to all other studies. This is because of lowered immunity among the individuals. The infection rate among such individual is well documented. The first and foremost striking but convincing outcome of our study is the extremely low yield of IgM seropositivity as compared to that of IgG antibodies. The rate of sero-conversion occurring in pregnancy has been estimated to be around 1% only (Jauniaux et al, 2006). This low rate of detection was not surprising as few studies have suggested no detection or low detection of infection undergoing TORCH testing (Cullen et al, 1998; Leland et al, 1983). The low rate of sero-conversion and detection might be the reason in our study behind the high sensitivity of the CLIA method over traditional ELISA method existing in our laboratories. The high yield of equivocal result might have possibilities to be reported otherwise as false positive in traditional ELISA method.

Seropositivity rates in relation to status of pregnancy and BOH outcomes differed significantly in our study. Almost all studies revealed higher prevalence among women in childbearing age. Various studies revealed that congenital infections caused by TORCH agents were a significant cause of neonatal mortality and childhood morbidity worldwide. In our study, the patients with negative IgM still had symptoms related to the disease and those women with negative IgM still had unfavorable pregnancy outcomes. There are more sensitive, specific and reliable methods for screening. To determine the IgG avidity, to perform TORCH tests in paired samples, molecular methods of organism identification is necessary (Owen et al, 2006). Hence, our study also doubted the single serum assays for TORCH screening.

ISSN No: 2565-5132 (*Print*)
:2616-0234 *(Web)*

Further, the cost of the whole TORCH panel test being very high, most general population from developing countries like Nepal, cannot afford the cost. Moreover, during pregnancy, periodic monitoring is required, which aids further difficulty to the people. By including vaccination against rubella virus (MMR vaccine) in the national immunization schedule, the incidence of congenital rubella can be reduced greatly. When elimination is achieved by prevention and vaccination, this component can be deleted from the TORCH-panel investigations, thereby reducing their cost.

Conclusions

The IgM seroprevalence rates were 4.20% (9/214) for *T. gondii*, 5.12% (10/195) for Rubella, 4.91% (12/244) for CMV and 5.56% (13/234) for HSV. So it is recommended that previous history of pregnancy wastage and the serological reactions for TORCH infections during current pregnancy must be considered while managing BOH cases to reduce the adverse fetal outcome.

Acknowledgements

We would like to extend our thanks to the staffs of NPHL for their support during study and all the TORCH suspects' patients for providing blood sample to us. We are grateful to University Grant Commission for providing thesis support grant to principle investigator under the scheme of person with disabilities. We would also like to thank all the staffs of Central Department of Microbiology for their help during study period.

References

Acharya D, Shrestha A, Bogati B, Khanal K, Shrestha S, Gyawali P. Serological screening of TORCH agents as an etiology of spontaneous abortion in Dhulikhel Hospital, Nepal. Am J Biomed Life Sci, 2014, 2, 34-9.

Case Definition, 2013. https://wwwn.cdc.gov/nndss/conditions/rubella/case-definition/ 2013. Accessed November 2016.

Center for Disease Control and Prevention. Rubella /German measles, 2013.

Center for Disease Control and Prevention. STD Surveillance Case Definitions Part 2. 2013. https://www.cdc.gov/std/stats13/app- casedef-2.htm. Accessed November 2016.

Cullen A, Brown S, Cafferkey M, O'Brien N, Griffin E. Current use of the TORCH screen in the diagnosis of congenital infection. J Infection,1998, 36, 185-8.

Guddy K, Pant P, Shama J, Pokharel B, Singh M, Pradhan N. Association of TORCH antibodies in women with spontaneous abortions. Heal Renaissance, 2015,12, 124-9.

Jauniaux E, Farquharson RG, Christiansen OB, Exalto N. Evidence-based guidelines for the investigation and medical treatment of recurrent miscarriage. Hum Reprod, 2006, 21, 2216-22.

Lamichhane B, Pudasaini B, Upadhyay B, Sharma M and Khanal SP. Prevalence of Serum Antibodies to TORCH Infections among the Women of Child Bearing Age Visiting National Public Health Laboratory, Teku. Nepal J Sci Technol, 2015, 15**,** 85-90.

Leland D, French ML, Kleiman MB, Schreiner RL. The use of TORCH titers. Pediatrics, 1983,72, 41-3.

Malhotra V and Bhardwaj Y. Comparison of enzyme linked immunosorbant assay and indirect haemagglutination test in serological diagnosis of toxoplasmosis. J Communicable Dis, 1991, 23, 154-6.

McCabe R and Remington JS. Toxoplasmosis: the time has come. New Eng J Med,1988, 318, 313-5.

Newton ER. Diagnosis of perinatal TORCH infections. Clin Obstet Gynecol, 1999, 42, 59-70.

Owen WE, Martins TB, Litwin CM Roberts WL. Performance Characteristics of Six Immulite 2000 TORCH Assays. Am J Clin Pathol, 2006,126, 900-5.

Pradhan S. Epidemiological and serological profiles of TORCH infection in pregnancy. J Pathol Nepal, 2015,5, 705-8.

Rai S, Sharma A, Shrestha R, Pradhan P. First case of congenital toxoplasmosis from Nepal. Nep Med. Coll J, 2011, 13, 64-6.

Rai SK, Shibata H, Sumi K, Rai G, Rai N, Manandhar R, Gurung G, Ono K, Uga S, Matsuoka A. Toxoplasma antibody prevalence in Nepalese pregnant women and women with bad obstetric history. Southeast Asian J Trop Med Public Health,1998, 29**,** 739-43.

Stegmann BJ and Carey JC. TORCH Infections. Toxoplasmosis, Other (syphilis, varicella-zoster, parvovirus B19), Rubella, Cytomegalovirus (CMV), and Herpes infections. Curr Womens Health Rep, 2002, 2, 253-8.

Turbadkar D, Mathur M and Rele M. Seroprevalence of torch infection in bad obstetric history. Indian J Med Microbiol, 2003, 21, 108.