

Serological screening of TORCH agents as an etiology of spontaneous abortion in Dhulikhel hospital, Nepal

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Abstract: The role of TORCH infections as a cause of spontaneous abortions is still debatable with conflicting results where geographical variation may play a significant role. This study was undertaken to discover the association of TORCH infections in women with spontaneous abortions by serological testing. The descriptive case control study was conducted from January to December, 2012. A serological evaluation was carried out to determine the presence of *Toxoplasma gondii*, Rubella, Cytomegalovirus (CMV) and Herpes Simplex Virus (HSV) specific immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies, using commercial diagnostic kits by enzyme-linked immunosorbent assay (ELISA) technique. Mean age of the study and control subjects were 24.8 \pm 6.4 and 23.8 \pm 3.8 years respectively where 72.8% of the study subjects were of the age between 20 to 35 years and 23% of the women with spontaneous abortion were below 20 years. Most of the cases were of incomplete abortions (43%) followed by complete abortions (26%). Only 1.3% of both IgG and IgM seropositivity against TORCH agents were noted among the study subjects whereas highest IgG seropositivity was detected with Rubella (86.8%) followed by HSV-I (72.8%). An infection susceptibility rate of 77.9% to *Toxoplasma gondii*, 11.7% to Rubella, 51.9% to CMV, 36.4% to HSV-I and 84.4% to HSV-II was noted. No significant difference in relation to age and type of abortion was found in seropositivity between the study and the control subjects. This study, probably the first of its kind from Nepal, suggests that current infection with TORCH agents might not be the possible etiology of spontaneous abortion. Serological TORCH screening may not be conclusive.

Keywords: Screening, TORCH Agents, Spontaneous Abortion, Immunoglobulin, Seropositivity

1. Introduction

The maternal infections that are transmissible *in utero* at various stages of the pregnancy, can be caused by many organisms, of which the members of the TORCH complex, namely *Toxoplasma gondii*, Rubella virus, Cytomegalovirus (CMV), Herpes Simplex Virus-I (HSV-I) and Herpes Simplex Virus-II (HSV-II) occupy prominent positions (1, 2). Previous studies have shown that these infections are associated with unfavorable foetal outcomes like multiple abortions, sterility, intrauterine foetal deaths, still births, congenital malformations

and other reproductive failures (3- 5). However, the causes of these unfavorable fetal outcomes may be genetic, hormonal, abnormal maternal immune response or maternal infection. The possible role of TORCH infections is not conclusive, particularly as an etiology of spontaneous abortion (6-8).

Spontaneous abortion, which is the loss of a pregnancy without outside intervention before 20 weeks of gestation, affects up to 20 % of recognized pregnancies and is subdivided into threatened abortion, inevitable abortion, incomplete abortion, missed abortion, septic abortion, complete abortion, and recurrent spontaneous abortion (9). Firm evidence on the

causes of recurrent miscarriage is scant (10). Moreover, though some high-risk factors were demonstrated to be etiologies of spontaneous abortion, it is likely that different races and different communities have different etiologies, and protocols for the diagnostic evaluation of spontaneous abortion (11). Due to the lack of a national screening programme in Nepal, there is no baseline serological data regarding the immune status of TORCH infection in pregnant women. However, several hospital based studies from South Asia have shown the incidence of acute TORCH infections as an etiology of spontaneous abortions and other negative obstetric outcomes (5, 12-14).

The demonstration of seroconversion in the patient sera *in vitro* is a highly established method chosen in developing countries to predict spontaneous abortion.⁵ It is suggested by previous studies from around the globe not to use TORCH serology for the detection of possible infection in women with bad obstetric history (BOH), as the measurement of specific antibodies is doubtful (11, 15, 16). It is our impression that the TORCH test has not been optimally used in hospital settings in Nepal. Hence, this study has been conducted in order to discover the immune status of TORCH agents in women who have experienced spontaneous abortion in Dhulikhel Hospital-Kathmandu University Hospital (DH-KUH), Nepal.

2. Materials and Methods

2.1. Study Site

This descriptive case control study was conducted from January to December, 2012 at the antenatal clinic in the Department of Obstetrics and Gynecology and the Department of Microbiology, DH-KUH, located 30 km from the capital city Kathmandu. This community hospital has later metamorphosized into a teaching hospital of Kathmandu University School of Medical Sciences (KUSMS), and now serves the focal population of four districts (Kavrepalanchowk, Sindhupalchowk, Ramechhap and Dolakha) all of rural origin. With more than 15 outreach centers around the country, DH-KUH is a model hospital in the South Asian region.

2.2. Study Subjects

The study group was comprised of 151 antenatal women of reproductive age who experienced spontaneous abortions during the study period. The control group was comprised of age-matched antenatal women with successful delivery who attended the same department clinic (mean age of study subjects vs. mean age of control subjects = 24.8 \pm 6.4 vs. 23.8 \pm 3.8 years). The investigator visited the antenatal clinic every day, selected the study subjects, and screened them using a predesigned pretested schedule using inclusion and exclusion criteria. Women with spontaneous abortions due to hypertension, diabetes mellitus, syphilis, Rh incompatibility, or physical causes of abortion were excluded, whereas women with abortions of unknown etiology were included.

2.3. Serological Analysis

For serological analysis, 3.0 mL of venous blood was collected in a red cap vacutainer from each study subject. The serum was separated and stored in numbered aliquots at -20°C until assayed. All the serum samples collected from the study and control groups were tested for *Toxoplasma gondii*, Rubella virus, CMV, HSV-1 and HSV-II IgM and IgG antibodies using commercially- available International Organization for Standardization (ISO) certified and Food and Drug Administration (FDA) registered ELISA kits (Diagnostic Automation/ Cortez Diagnostics, Inc. 23961 Craftsman Road, CA 91302, USA). The results were calculated by an ELISA Reader (Lab Life 2007, RFCL, India) according to manufacturer's instructions and compared in a parallel manner with controls. Rubella IgG and IgM were performed using a Sandwich ELISA technique whereas others were performed by an Indirect ELISA technique. The results were interpreted as positive, equivocal or negative by determining the immunoglobulin index. IgG or IgM indexes <0.9, 0.9-0.99 and >1.0 were suggestive for negative, equivocal and positive respectively.

2.4. Data Analysis

Data were entered in Microsoft Excel and analyzed using SPSS version 16 (SPSS Inc; Chicago, IL, USA). The results were interpreted according to frequency distribution and percentage. A Chi-square test was employed to determine any association between categorical data. Statistical significance was set at $p \leq 0.05$.

2.5. Ethical Approval

All study subjects were informed about the study and verbal informed consents were obtained from all subjects. Confidentiality was assured by using hospital registration numbers instead of names. Ethical clearance was obtained from the Institutional Review Committee of DH-KUH.

3. Results

In the one year period there were 151 cases of spontaneous abortion in DH-KUH who had TORCH screen performed. Of these only two cases of IgM seropositivity were detected. The first case was of 27 years old women who experienced a missed abortion and who was IgM positive for Rubella and second case was of a 20 years old woman who was IgM positive for Rubella, CMV and HSV-I. IgM seropositivity of any TORCH agents was not detected among the control subjects. However, a high number of the study, as well as, the control subjects showed IgG seropositivity as shown in Table 1. The IgG seropositivity of TORCH agents in control subjects was found as *Toxoplasma gondii*: 22.1%, Rubella: 88.3%, CMV: 48.1%, HSV-I: 63.6% and HSV-II: 15.6% i.e. this study showed that our study population has an infection susceptibility of 77.9% to *Toxoplasma gondii*, 11.7% to Rubella, 51.9% to CMV, 36.4% to HSV-I and 84.4% to HSV-II. No significant difference was noted among the

seropositivity rate of any TORCH agents.

Table 1. Seropositivity of TORCH agents among study and control groups

TORCH agents	Seropositivity in study subjects (N=151)			Seropositivity in control subjects (N=77)			p-value
	IgG only	Both IgG and IgM	Total % positivity	IgG only	Both IgG and IgM	Total % positivity	
<i>Toxoplasma gondii</i>	32	0	21.2	17	0	22.1	0.80
Rubella	131	2	86.8	68	0	88.3	0.80
Cytomegalovirus	97	1	64.2	37	0	48.1	0.20
Herpes Simplex Virus-I	110	NT	72.8	49	NT	63.6	0.17
Herpes Simplex Virus-II	10	1	6.6	12	0	15.6	0.30

NT=Not tested; *= a significant difference

Most of the study and control subjects were between the ages 20 to 35 years (72.8% study and 78% control subjects) which is the ideal age for pregnancy. Surprisingly, almost 23% of the study subjects were women below 20 years of age, as shown in Table 2. Due to the lack of sufficient sample size in each age category, we haven't performed the statistical tests according to age strata.

The cases of spontaneous abortion were divided into four

different clinical categories. Most of the cases were of incomplete abortions (43%) followed by complete abortions (26%), missed abortion (19.2%) and threatened abortion (11.9%). In all the cases of abortion, the most predominant seropositivity rate was of Rubella followed by HSV-I as shown in Table 3. The least rate of seropositivity was seen in infection by HSV-II.

Table 2. Seropositivity in relation to age of the subjects

TORCH agents	Age (Years)	Study subjects		Control subjects	
		Sera tested	Positive sera (%)	Sera tested	Positive sera (%)
<i>Toxoplasma gondii</i>	<20	35	5 (14.3)	16	6 (37.5)
	20-35	110	27 (24.5)	60	11 (18.3)
	>35	6	0 (0.0)	1	0 (0.0)
Rubella	<20	35	33 (94.3)	16	14 (87.5)
	20-35	110	94 (87.5)	60	54 (90.0)
	>35	6	4 (66.7)	1	0 (0.0)
Cytomegalovirus	<20	35	22 (62.9)	16	11 (68.8)
	20-35	110	71 (64.5)	60	25 (41.7)
	>35	6	4 (66.7)	1	0 (0.0)
Herpes Simplex Virus-I	<20	35	24 (68.6)	16	11 (68.8)
	20-35	110	82 (74.5)	60	38 (63.3)
	>35	6	4 (66.7)	1	0 (0.0)
Herpes Simplex Virus-II	<20	35	4 (11.4)	16	6 (37.5)
	20-35	110	4 (3.6)	60	6 (10.0)
	>35	6	2 (33.3)	1	0 (0.0)

Table 3. Seropositivity in relation to type of abortion outcomes

TORCH agents	Complete abortion (n=39)		Incomplete abortion (n=65)		Missed abortion (n=29)		Threatened abortion (n=18)	
	Positive sera	(%)	Positive sera	(%)	Positive sera	(%)	Positive sera	(%)
<i>Toxoplasma gondii</i>	8	20.5	16	24.6	6	20.6	2	11.1
Rubella	37	94.8	51	78.4	26	89.6	17	94.4
Cytomegalovirus	29	74.3	36	55.3	19	65.5	13	72.2
Herpes Simplex Virus-I	31	79.4	46	70.6	23	79.3	10	55.5
Herpes Simplex Virus-II	5	12.8	4	6.1	1	3.4	0	0.0

4. Discussion

The current study is one of the few studies in Nepal to explore the immune status of TORCH agents among the most important clinical categories in immunocompetent hosts- pregnant women. Infections have been an established etiology of negative obstetric outcomes as suggested by various studies around the world (5, 12-14, 17, 18). Most of the viral pathogens usually cause primary maternal viremia which may infect the placenta and, thereby, the fetus, except HSV-I and HSV-II, which causes an ascending infection via the genital tract to fetal

membranes and then to the fetus (19, 20). Demonstration of seroconversion in patient sera in vitro has been a highly established method of choice in developing countries (5) and, hence, this method was employed in this study.

The first and foremost striking but convincing outcome of our study is the extremely low yield of both IgG and IgM seropositivity of TORCH agents (1.3%) in DH-KUH to identify the possibility of TORCH infection in patients experiencing spontaneous abortion. The rate of seroconversion occurring in pregnancy has been estimated to be around 1% only (15). This low rate of detection was not

surprising as few studies have suggested no detection or extremely low detection of infection undergoing TORCH testing (16, 21). The low rate of seroconversion and detection might be the reason in our study to the low yield of IgM seropositivity in women with spontaneous abortion. In our study, serological screening of TORCH infections in women with spontaneous abortions revealed the IgG seropositivity of 21.2%, 86.8%, 64.2%, 72.8% and 6.6% for *Toxoplasma gondii*, Rubella, CMV, HSV-I and HSV-II respectively, whereas the seropositivity among control subjects revealed 22.1%, 88.3%, 48.1%, 63.6% and 15.6% for *Toxoplasma gondii*, Rubella, CMV, HSV-I and HSV-II respectively. Similar incident rates of TORCH seropositivity in study and control subjects suggest the presence of immunity to the women towards the specific agents.

Reportedly, nearly half of the Nepalese are *Toxoplasma gondii* seropositive according to a study conducted almost 16 years ago (22). Occasional hospital based studies have revealed different rates of seropositivity for TORCH agents (12, 13, 23). An 80% positive rate of anti-HSV antibodies was found in early childhood (1-4 years) that further increased with age (96.1% positive in greater than 15 years age). In addition, antibodies against CMV were positive in all the subjects studied, as shown by a study conducted on the Nepalese population (24). The results obtained from these studies did not exhibit the actual seroprevalence, as they were case oriented rather than population oriented and, in addition, were hospital based studies. However, these handfuls of studies are suggestive for the presence of immunity to TORCH agents in Nepalese population. The seroconversion depends upon the prevalence of organism followed by the maternal immunity on the specific geography. Our results with high number of IgG positivity might suggest that women in our study site are with high maternal immunity to TORCH agents. This could be justified by doing extensive community based research to find out the immune status of pregnant women along with the seroprevalence of TORCH agents.

The seroprevalence of TORCH infections as indicators of acute infection has been reported from various parts of India which were focused mainly on reproductive women, women with negative obstetric histories, or pregnant women (5, 14, 25). Studies from Europe and USA also revealed significant prevalence of TORCH agents in women with BOH (17, 18). But our study is not in accordance with these findings. In addition, there is more than one *T. gondii* strain with a difference in virulence mechanisms and outcomes among isolates in nature (26). The occurrence of strain differences of TORCH agents could be one explanation regarding the prevalence. Hence, the genetic study to identify the strain specific occurrence of TORCH agents in Nepal is necessary to understand no role of these infections to cause negative obstetric outcomes along with spontaneous abortion.

Seropositivity rates in relation to age and type of abortion outcomes do not differ significantly among study and control subjects in our study. A high rate of HSV seropositivity

among the adult population of Nepal was reported in a previous study, however this study was on general population not in women experiencing spontaneous abortion (24). The occurrence of past infection with rubella is noted in all abortion categories followed by HSV-I infection. The high rate of IgG seropositivity of rubella has been reported in Indian population (5, 14, 27). May be the amendment of rubella vaccination in Nepalese and Indian routine immunization schedule has yield the high rate of IgG seropositivity which is suggestive for infection resistance.

The high incidence of spontaneous abortions, despite not finding the association of TORCH agents as a cause of acute infection (IgM seropositivity) in our study, could reflect other etiological factors of abortion than TORCH infections. The similar seropositivity rate that was observed in control subjects suggests to us the possibility that TORCH agents have no role in causing spontaneous abortions. Almost nil seropositivity of TORCH agents in women with previous abortion episodes was reported from a study done in Arabic countries (28). Not all the cases of abortion were associated with TORCH agents and Parvovirus B19 in a study conducted by Kishor *et al.* (27). Summers *et al.* (7) suggested that infection is an occasional cause of sporadic spontaneous abortion and this is consistent with statistical probability. In the medical literature, the limited evidence linking infection and recurrent pregnancy loss in humans remains largely anecdotal and generally cannot be reproduced in prospective studies. Chromosome abnormalities, congenital and acquired anatomical defects of the uterine fundus and cervix, parental chromosomal rearrangements, gene mutations, antibodies to cardiolipin, and luteal phase defects each make a small contribution in spontaneous abortions where infections are probably not relevant according to a study done by Stirrat *et al.* (10). It is likely that different races and different communities have different etiologies (29) which may be in accordance with our study as we did not find any direct association of current TORCH infection in spontaneous abortion unlike studies from India, China and other Asian and European countries (4, 5, 14, 17, 25).

As stated earlier, various studies revealed that congenital infections caused by TORCH agents are a significant cause of neonatal mortality and childhood morbidity worldwide. Because of their nonspecific clinical manifestations and the importance of early recognition of in utero infection, serologic screening for these pathogens has been considered a routine practice in many parts of the world (25). However, it is also noted that the implementation of widespread TORCH screening programs have been questioned due to several factors, including potential overuse, lack of consistent and reliable serologic methods, high cost, and misinterpretation of results (15, 21, 30-32). The conventional single serum assays do not make a clear distinction between a recent and a chronic infection because of the presence of even IgM antibodies at high levels for long periods of time (33-34). Serological tests may be inaccurate or inaccurately interpreted, which may

influence a woman's decision to terminate the pregnancy. A study from the USA suggested that a confirmatory serologic testing in a reference laboratory, communication of those results, and their correct interpretation by an expert to the patient's physician decreased the rate of unnecessary abortion by 50% among women for whom IgM *Toxoplasma gondii* positive results had been reported by outside laboratories (35). In our study, the women with negative IgM still yield abortions. It is possible that there are other better techniques and technologies for the correct diagnosis of TORCH infections. There are more sensitive, specific, and reliable methods for serological screening. To determine the IgG avidity, to perform TORCH tests in paired samples, molecular methods of organism identification are few of them (34). Hence, our study also doubts the single serum assay for TORCH screening in our setting.

5. Conclusion

Two possible results might be concluded from our study. First, acute infection of TORCH agents might not be the only possible etiology of spontaneous abortion and secondly, serological TORCH screening may not be conclusive. Our finding is in accordance with various studies which have concluded that TORCH screening is no longer recommended due to various reasons, one of them being its unreliability. Because it has been suggested that the prevalence of TORCH agents differ in communities, races and geography, we might take our results on a positive note, which suggests better immunity conditions against TORCH agents at least in our study population. This study is the first of its kind from this country, to our knowledge. However, we would recommend conducting this sort of study in large populations, in various geographical regions and in various communities before concluding the association of TORCH agents with spontaneous abortion and reliability of serological screening.

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