Association of TORCH infections and foetal outcome in women with bad obstetric history in a tertiary care hospital of Eastern India

Soumya Nayak[1],Kumudini Panigrahi[2],BasantiKumari Pathi[3],Nirmala Poddar[4],Dipti Pattnaik[5],IpsaMohapatra[6],NarahariAgasti[7],Nirmal Mohakud[8],

[1]Assistant Professor, Department of Microbiology, Kalinga Institute of Medical Sciences, KIIT Deemed to be University, Bhubaneswar, Odisha, India

[2]Professor, Department of Microbiology, Kalinga Institute of Medical Sciences, KIIT Deemed to be University, Bhubaneswar, Odisha, India

[3]Professor, Department of Microbiology, Kalinga Institute of Medical Sciences, KIIT Deemed to be University, Bhubaneswar, Odisha, India

[4]Professor, Department of Microbiology, Kalinga Institute of Medical Sciences, KIIT Deemed to be University,Bhubaneswar, Odisha, India

[5]Professor, Department of Microbiology, Kalinga Institute of Medical Sciences, KIIT Deemed to be University, Bhubaneswar, Odisha, India

[6]Associate Professor, Department of Preventive and Social Medicine, Kalinga Institute of Medical Sciences, KIIT Deemed to be University, Bhubaneswar, Odisha, India

[7]Professor, Department of Obstetric and Gynaecology, Kalinga Institute of Medical Sciences, KIIT Deemed to be University, Bhubaneswar, Odisha, India

[8]Professor, Department of Paediatric, Kalinga Institute of Medical Sciences, KIIT Deemed to be University, Bhubaneswar, Odisha, India

Corresponding Author- NirmalaPoddar, Professor, Department of Microbiology, Kalinga Institute of Medical Sciences, KIIT Deemed to be University, Bhubaneswar, Odisha, India

Abstract:

TORCH complex infections- Toxoplasma gondii, Rubella virus, Cytomegalovirus and Herpes simplex virus during pregnancy are linked with congenital disorder, unfavorable foetalconsequences and successive reproductive failures. TORCH complex contaminations are normally mild in mother yet can be harmful to the foetus. The objective of thepresentstudy is to evaluate the seroprevalence of TORCH among women with Bad obstetric history and to find its correlation with socio-demographic characteristics and foetal outcomes. For this, we executed a cross-sectional study for a period of two years in 334 women. Blood sampleanalysis was conducted for the presence of specific Immunoglobulin M (IgM) and Immunoglobulin G (IgG) antibodies against each agent of TORCH complex by Enzyme Linked Immunosorbent Assay (ELISA). Statistical analysis was carried outusing independent sample t-test / Chi=square tests. Our results for BOH patients showed IgM seropositivity for HSV-II was 22.64%, Toxoplasma 8.97%, Rubella 7.26%, HSV-I 6.4% and CMV 3.41% whereas, IgG seropositivity for CMV was 70.51%, Rubella 55.99%, HSV-I 42.3%, HSV-II 29.9% and Toxoplasma 19.2%. Cross infection with multiple TORCH agent was observed. TORCH positive pregnant women indicates that most of the newbornsexperience congenital cardiac malformation (35%), followed by Cataract (30%), Hydrocephalus (20%), and congenital rubella syndrome (15%). These Congenital anomalies are linked with multiple TORCH infections. Timely detection and early treatment of infected pregnant women can prevent transfer of infection to foetus.

Key words: TORCH, Bad obstetric history, ELISA, Immunoglobulin M, Immunoglobulin G.

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Introduction:

Bad obstetric history (BOH) statespreviouscritical fetal outcome with regard totwo or more successiveunplanned abortions, early neonatal demise, stillbirths, intrauterine foetal deaths, intrauterine growth retardation and congenital anomalies [1]. BOH etiology can be genetic, hormonal, unusual maternal immune response, and maternal infection.

Toxoplasma gondii (TG), Rubella virus, Cytomegalovirus (CMV) and Herpes simplex virus (HSV) are the members of TORCH complex causing maternal infections transmitted in utero at different stages of pregnancy[1,2]. These infections in pregnancy are commonly found in association with poor pregnancy outcomes like habitual abortions, neonatal deaths, intrauterine fetal death (IUFD), still births, intrauterine growth retardation, congenital malformations and other reproductive failures.Overall TORCH infections bring out mild maternal morbidity; still can havecritical fetal outcome. The capability of the foetus to withstand infectious organisms is finite;also the foetal immune system is not able to stop the dissemination of infectious organisms to numerous tissues [3].

Toxoplasma is a parasitic infectious disease caused by a protozoan TG, which is transmitted to humans through the infection of contaminated food or water [4]. This infection canbring out congenital toxoplasmosis with major foetal outcomes like hydrocephalus, retinitis pigmentosa, or even death in utero. An airborne viral infection of RNA virus of paramyxovirus group brings out Rubella. Approximately 30%–50% foetusesif exposed with Rubella during initial three months of pregnancy will adversely affected. Congenital rubella syndrome (CRS) may include spontaneous abortion, stillbirth, hepatosplenomegaly, thrombocytopenia, and purple rash [5]. The infected newborn may have one or both vision and hearing disability, heart defects, and accumulation of calcium in the brain. In India, 10 to 20 percent of female in childbearing age are susceptible to Rubella infection [6]. CMV infection causes due to DNA virus of herpes group. The common modes of infection are through saliva, urine, stool, breast milk, and unscreened blood transmission [4].Cytomegalovirus is a primesource of inherited infections and long-term neurodevelopmental disabilities among children [4].HSV have similar group of DNA virus as CMV and such infection in newborn is oftenget by exposure with the mother's contaminatedvagina [4].

The magnitude of TORCH contaminationchanges among different geographical areas. As mostly asymptomatic or mild clinical course of these four diseases, detectionin pregnancy is often missed. So, the most effective way to control birth defects due to prenatal infection by these four microorganisms is preventive antenatal screening and counseling, leading to early diagnosis [7].

The current study planned to evaluate the seroprevalence of TORCHin women with BOH visiting for antenatal check up and to obtain its association with socio-demographic characteristics and foetal outcomes.

Methods

A cross-sectional study was executedin the Department of Microbiology in collaboration with the Department of Obstetrics and Gynaecology at Kalinga Institute of Medical Sciences, Bhubaneswar, Odishafor a period of two years.Womenincluded incurrentstudy were categorized aswomen with BOH (n=234) and withoutany indication of BOH (n=100).

Every women with priorBOH (previous critical foetal outcome with regard to two or more successiveunplanned abortions, clinical

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history of foetal death/s, IUGR, early neonatal demise, and/or congenital anomalies) and pregnant women without any previous BOH were taken as comparative group were included. Whereas, pregnant femalehaving previous history of other known causes of critical foetal consequenceslike high blood pressure, diabetes, eclampsia of pregnancy, Rh incompatibility and physical causes of miscarriage were excluded through the research study.

Five milliliter of venous blood specimenwas drawn from each group of women. Extraction of sera was done using centrifugation method and it was hold at -20˚C until use. IgG and IgM levels against TG, Rubella virus, CMV and HSV were measured using Bio-radImmunoglobulinG(IgG)andImmunoglobulin M(IgM) ELISA kits (Bio-Rad 96 well ELISA, France) individually for each organism. ELISA was executed on automated Analyzer Reader (iMark^{TM} Microplate Absorbance Bio-Rad Laboratories, Japan), and the optical density (OD) was read at 450nm absorbance. All the ELISA kits had 99% Kappa agreement with reference ELISA method according to manufacturer's kit insert. The interpretation of test results was based on the antibody index (AI) which is calculated by dividing the optical densityof each sample by cutoff value (calibrator optical density multiplied by calibrator factor).Questionnaire with details of age, age of marriage, education, occupation, socio-economic status, family size, place of residence, past obstetric history, current obstetric history, blood parameters for TORCH were used as study tool and convenience sampling was used as sampling technique.

All data collected was statistically analyzed using Epi Info software (version 7.3.2). Quantitive data demonstrated as mean, standard errors (SE) and standard deviation (SD) to indicate the magnitudeand precision of data. The independent sample t-test / Chi=square tests were used as tests ofsignificance.

Results:

In current study, out of234 women havingBOH were categories clinically as abortion in 82.4% (193/234), as pre-term labour in 16.6% (39/234), as IUFD in 15.8%(37/234),as still birth in 13.6%(32/234), early neonatal death within two weeks seen in 12.3%(29/234) and foetus with congenital disorder were 8.5%(20/234) as reflected in Table 1. Among them, Seropositivity against TORCH agent was found maximum in women with early neonatal death and congenital anomalies(100%) each, followed by patients with still birth (93.75%), preterm labour patient(92.3%) and patients with consecutive abortion(90.6%).

Adverse Foetal outcome	Total Number =234 n (percentage)	TORCH positive n (%)	TORCH negative n (%)
Consecutive abortions	193 (82.47%)	175 (90.67%)	18 (9.32%)
IUFD	37 (15.81%)	31 (83.78%)	06 (16.21%)
Stillbirth	32 (13.67%)	30 (93.75%)	02 (6.25%)
Early Neonatal Death	29(12.39%)	29 (100%)	0
Preterm Labour	39 (16.66%)	36 (92.3%)	3 (7.69%)
Congenital Anomalies	20 (8.54%)	20 (100%)	0

Table 1: Magnitude of TORCH infection and foetal outcome in women with BOH.

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As shown in table 2, IgMseropositivity was most prevalent for HSV-II infection (22.64%) followed by Toxoplasmosis (8.97%), Rubella (7.26%), HSV-I (6.4%) and CMV (3.41%).But based on IgG,seropositivity was mostprevalent for CMV (67.09%), followed by Rubella(48.71%), HSV-I(35.89%), Toxoplasma (10.25%) and HSV-II (7.26%), in women with history of BOH.

TORCH agent	IgM n (%)	IgG n (%)	Total n (%)
Toxoplasma gondii	21(8.97%)	24 (10.25%)	45(19.2%)
Rubella virus	17 (7.26%)	114 (48.71%)	131 (55.9%)
Cytomegalovirus	8 (3.41%)	157 (67.09%)	165 (70.51%)
Herpes I virus	15 (6.4%)	84 (35.89%)	99 (42.3%)
Herpes II virus	53(22.64%)	17(7.26%)	70 (29.9%)
TOTAL	114 (48.71%)	396	510

Table 2: Serological data of IgM and IgG antibodies against TG, rubella, CMV, and HSV type I and type II infections in patient with BOH.

With respect to age group, majority of BOH occurrence were found in females aged between 25-30years followed by 19-24years and 31-36years respectively. This is described in table 3.

Adverse Foetal outcome	19-24y	25-30y	31-36y	37-42y	> 43y	N (%)
Consecutive abortions	49	97	34	11	2	193(82%)
IUFD	13	13	9	2	0	37(16%0
Stillbirth	6	17	7	1	1	32(14%)
Early Neonatal Death	5	19	2	3	0	29(12%)
Preterm birth	12	14	9	3	1	39(17%)
Mothers with H/o baby born with Congenital disabilities	4	7	7	1	1	20 (9%)

Table 3: Clinical presentation of BOH with respect to age groups (n=234).

Comparative study of women with BOH and without BOH were carried out as illustrated in table 4, and it was observed that IgM and IgG antibody of TORCH agent were linked with BOH as the p value isless than 0.005.

TORCH profile	Antenatal women with BOH [n=234]	Antenatal women without BOH [n=100]	p-value
T.gondii			
IgM positive	21	0	0.004
IgG positive	24	0	0.002
Both IgM & IgG positive	1	0	0.66

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Neither IgM & IgG positive	188	100	<0.0001
Rubella			
IgM positive	17	0	0.01
IgG positive	114	03	<0.0001
Both IgM & IgG positive	07	0	0.18
Neither IgM & IgG positive	96	97	<0.0001
CMV			
IgM positive	08	0	0.14
IgG positive	157	06	<0.0001
Both IgM & IgG positive	5	0	0.33
Neither IgM & IgG positive	64	94	<0.0001
HSV-1			
IgM positive	15	0	0.02
IgG positive	84	1	<0.0001
Both IgM & IgG positive	04	0	0.44
Neither IgM & IgG positive	131	99	<0.0001
HSV-2			
IgM positive	53	0	<0.0001
IgG positive	17	02	0.10
Both IgM & IgG positive	0	0	-
Neither IgM & IgG positive	164	98	<0.0001

Table 4: Comparative study of magnitude of TORCH complex infection in women with BOH and without BOH

The characteristics of studied participants is as indicated in table 5, the age group between 19-24 and 31-36 and working women were the risk factors for the development of BOH as p value is less than 0.001 and 0.01 respectively.

Characteristics	Antenatal women with BOH [n=234]	Antenatal women without BOH [n=100]	p-value
Mean age			
Age group(years)			
19-24	63(26.92%)	10(10.0%)	**0.001**
25-30	116(49.58%)	43(43.0%)	0.33
31-36	41(17.52%)	35(35.0%)	**0.0008**
37-42	11(4.70%)	11(11.0%)	0.06
>43	03(1.28%)	01(1.0%)	0.74
Education			
Educated	208(88.89%)	92(92.0%)	0.51

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Uneducated	26(11.11%)	08(80.0%)	
Employment Status			
Working	36(15.38%)	05(5.0%)	**0.01**
House-wife	198(84.62%)	95(95.0%)	
Socio-economic status			
High	15(6.41%)	5(5.0%)	0.63
Middle	175(74.79%)	72(72.0%)	
Low	44(18.80%)	23(23.0%)	

Table 5: Characteristics of study participants (N=334).

The association between various foetal outcomes with types of antibody for TORCH complex infection is shown in table6.

BOH	**T.gondii**		**Rubella**		**CMV**		**HSV-1**		**HSV-2**	
	IgM n(%)	IgG n(%)	IgM n(%)	IgG n(%)	IgM n(%)	IgG n(%)	IgM n(%)	IgG n(%)	IgM n(%)	IgG n(%)
Abortions N=193	22 (11.3)	23 (11.9)	16 (8.2)	100 (51.8)	08 (4.14)	130 (67.35)	16 (8.2)	78 (40.4)	53 (27.4)	14 (7.25)
IUFD N=37	0	02 (5.4)	05 (13.5)	26 (70.2)	03 (8.1)	27 (72.9)	02 (5.4)	02 (5.4)	14 (37.8)	03 (8.1%)
Still birth N=32	0	05 (15.6)	2 (6.25)	19 (59.3)	02 (6.25)	22 (68.75)	07 (21.8)	16 (50)	07 (21.8)	02 (6.25)
Early neonatal deathN=29	05 (17.2)	01 (3.4)	10 (34.4)	15 (51.7)	01 (3.4)	23 (79.3)	02 (6.8)	12 (41.3)	05 (17.2)	01 (3.4)
Preterm Labour N=39	01 (2.5)	04 (10.2)	03 (7.6)	24 (61.5)	02 (5.1)	35 (89.7)	03 (7.6)	12 (30.7)	09 (23.07)	02 (5.1)
Congenital Anomalies N=20	02 (10)	02 (10)	09 (45)	15 (75)	03 (15)	18 (90)	0	10 (50)	02 (10)	03 (15)

Table 6: association between various foetal outcomes with types of antibody for TORCH infection

On analyzing the association between various foetal outcomes with types of antibody for TORCH infection, it was observed that, out of 193 patients with history of consecutive abortions, maximum IgM seropositivity for HSV-II i.e. 27.4 (53/193), but IgG seropositivity was maximum in CMV, 67.35%(130/193) following Rubella 51.8% (100/193) and HSV-I40.4% (78/193). Out of 37IUFD cases, maximum IgM seropositivity was for HSV-II (37.8%),but IgG seropositivity for CMV was72.9%(27/37) following Rubella 70.2% (26/37). Out of 32 women with clinical history of still birth, IgM antibodies were positive for HSV in 21.8 % (7/32) patients however IgG seropositivity was maximal in CMV (68.75%) following Rubella (59.7%) and HSV-I 50% (16/32).Out of 29 female with history of early neonatal death maximumIgM seropositivity for Rubella 34.4%(10/29) and IgG seropositivity was maximum

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79.3%(23/29) in CMVfollowed by Rubella 51.7%(15/29).Out of 39 female with clinical history of preterm labour, maximum IgM seropositivity was for HSV-II which is 23.07% (9/39) and IgG seropositivity was maximum of 89.7% in CMV following 61.5% (24/39) in Rubella. Out of 20 females with clinical history of congenitaldisabilityin newborns, maximum IgM seropositivity was in Rubella 45% (9/20) and IgG seropositivity was 90% in CMV following 75% in Rubella and 50% in HSV-I .

Relationship of TORCH infections in pregnant women along with congenital disorder of foetus is illustratedin table 7.

Parameters in infant	IgM-positive mother	TORCH pathogens involved in congenital disorders in infants
Cardiac anomaly	7 (35%)	T+R+C+H , R+C+H-3, T+R+C-2, R+C
Hydrocephalus	4 (20%)	R+C+H-3, R+C,
CRS (Congenital Rubella Syndrome)	3 (15%)	R+C, R+C+H-2
Cataract	06 (30%)	T+R+C+H-1, R+C+H-3, R+C-2

Table 7: Association of congenital anomalies withTG, Rubella, CMV, and HSVinfections in pregnant women

Follow-up of TORCH IgM positive pregnant women divulge that, most of the women had multiple TORCH complex infection also the newbornshave congenital cardiac disorder in 35%(7/20), followed by congenital cataract 30% (6/20) hydrocephalus20%(4/20) and congenital rubella syndrome 15%(3/20).

Discussion:

Women with BOH (n=234), comprise of consecutive abortion in 193 (82.4%), preterm labour in 39 (16.6%), IUFD in 37 (15.8 %), still birth in 32 (13.6 %), early neonatal demise in 29 (12.3%) and congenital disorder 20 (8.5%). It is clear from the table 1, that magnitudeof TORCH contamination was found to be higher in early neonatal death and congenital disorder with 100% rate followed by 93.75% of still birth, 92.3% of preterm labour,90.67% of consecutive abortion and83.78% of IUFD.

In current study, IgM positivity of TORCH contaminationin pregnant female was observed to be 48.7% which is in contrast with Padmavathy et al. [8]were the reported seropositivity rate was 13.8%.The difference in rate of seropositivity represents the geographic dissimilarity of rate of infection and risk elements. Additionally, IgM positivity in pregnant women of New Delhireported 45.56% byTiwari etal. [9].In India, seroprevalence of toxoplasmosis is reported up to 80% [10,11] In current study, 8.97% and 10.25% pregnant women showed anti-toxoplasma IgM and IgG antibodies, respectively whereas Shrivastava et al. [11] reported 29.68% and 9.37% toxoplasma IgM and IgG antibodies in pregnant women from Indore, Madhya Pradesh. The seroprevalence of toxoplasmosis in different countries ranges between 7.7% and 76.7%[12,13]. Toxoplasmosis isabsolutelycurablein pregnancy using antibiotics; thus, timelydiagnosis and quick therapycan prevent the foetal complications. Further, various researchshowed that encysted forms of TGget activelater in pregnancy which cancause infection in the initial three months of pregnancy and frequently causes abortions [11, 13].Thus, the patientwith seroconversion must

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beexaminedthroughout pregnancy. Further, if not attended vaccination for toxoplasmosis, the inhibitioncould be achieved by hygiene, sanitization and right guidance by health-care authorities.

In current study, the seroprevalence against Rubella virus was 55.9%; however, Rubella IgM was present only in 7.26% of cases. This is possibly due to the extensive rubella vaccination program since 2014 [14]. World Health Organization, South-East Asia Region had set a 2023 (from earlier 2020) target to stop rubella and reduce the burden of congenital rubella syndrome (CRS) [15] by introducing rubella-containing vaccines (RCV) into the public-sector childhood immunization program. Similar research from north India foundexistence of anti-RV antibodies in 82.6 percent of cases after vaccination [16].

The seroprevalence of cytomegalovirus infection is reported 45 percent in developed nations and 100 percent in developing nations; while inIndia,it is reported 80-90 percent in females of child bearing age. Findings of current study are similar, where anti-CMV IgG antibodies detected in 67.09 percent of pregnant female, and these findings are consistent as reported 85.93 percent [11].Elevated seropositivity in the current study can be because ofearliercontamination or reinfection of alternative strain.Therefore, prenatal CMV diagnosiscan assistphysicianfor the infection status and feasible pregnancy consequences. Centers for Disease Control and Prevention too endorse prenatal consulting for expecting motherregarding cleaning of hands, minimizingcontact with body fluids of children in preventive part [17].

Spread of herpes infection is acute and is linked with an increased neonatal morbidity and mortality rate. As reported, around 80 percent of herpesinfection gets at the time ofdelivery and its linked mortality was more than 75 percent in untreated patients [18].The seroprevalence of HSV-I and HSV-II infectionsin the current study were 42.3% and 29.9% respectively.High prevalence of herpes (57 to 64%) in India has been reported by few studies [19, 20,and 21]. However in developed countries, herpes prevalence is evaluated to be about 7 percent to 22 percent [17]. Since the majority (>80%) of neonatal herpes is transmitted during delivery, elective cesarean section and prophylactic acyclovir to infected mothers are recommended to control neonatal herpes [22].

TORCH infectiongenerallyfirst cause a maternal viremia and thatmovesacross placenta and possess teratogenic effects, except for herpes where, ascending kind of infection through the genital tract to fetal membranes and then to the fetus is more common [23].Various studies investigated that the prevalence of congenital deformity and prematurebirth possibility will increase in TG positive expecting mother. Moreover, amount of unplanned abortion, cardiac, auditoryor ocular conditions are linked more with Rubella, Cytomegalovirus, and HSV positive cases[6,7].Investigation of IgM positive patientsshows high neonatal mortality and other congenital disorder in newborns. As, multiple TORCH infections in pregnant women show severe adverse outcome on foetal growth so, obstetrician mustacknowledgewhileconsulting the patient.

Conclusion:

The magnitude of TORCH complex infections was more in Eastern India region, and these are linkedwith neonatal mortality, IUFD and congenital anomalies. Additionally, thecurrent study demonstrates the significance of prenatal detection of TORCH complex infections in pregnant women for

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rightcounseling, where higher neonatal mortalities reported.

Because of lack of a government screening programme in India for TORCH complex infections during pregnancy, serological detection for TORCH infections remains the only means for detecting such infections. The pattern of investigations for pregnant women has to be changed by incorporating TORCH screening tests also in their routine antenatal check-ups. We suggest that all the antenatal cases with BOH must be routinely examined for TORCH agentseven though they do not experience any symptoms. This helps in the timely detection and propermedication in managing the cases. The data reported in current study will be a contribution to the obstetricians and paediatricians of our geographical area to make appropriate management protocols. This knowledge will bebeneficial for the clinicians to consult the mothers on preventive action taken to avoid such infections.

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