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ROLE OF TORCH INFECTION, OXIDATIVE STRESS AND SOMATIC DNA DAMAGE IN HIGH-RISK PREGNANT WOMEN LEADING TO CONGENITAL ANOMALIES

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ABSTRACT: An increased complication rate during or after pregnancy and birth leads to a condition known to the mother or child when High Risk Pregnancy (HRP) and may be for both. An attempt was made here to find the role of TORCH infection in high-risk pregnant women; oxidative stress and somatic DNA damage, leading to congenital anomalies(CAs).The patients were referred from various infertility clinics and maternity centers of Kerala to Advanced Genetic Study Centre (Genetika), Trivandrum, Kerala. Study population includes 170 study subjects and 105 healthy controls. Blood sample was collected for TORCH IgG (ELISA), oxidative stress evaluation (Malondialdehyde by thibarbituric acid assay) and somatic DNA damage (CBMN assay). Among 275 subjects, 170 (61.81%) had the history of high-risk pregnancy with a mean age of 35.65±6.30 years. The control group included 105 women with previous normal pregnancy with a mean age of 34.03±6.10 years. TORCH infections had a significant role in HRP (p-value<0.001). Study group population showed a mean MDA value of 2.95 ± 2.09 and that of control group was1.67 ± 0.70. The mean value of CBMN frequency was 12.44 ± 1.01 in study subjects and that of controls was 9.98 ± 0.58. Study group showed a higher CBMN frequency and MDA value than control group. Proper diagnosis and intervention during early stage can be done by routinely screening of sample for TORCH agents, oxidative stress and somatic DNA damage even if the case is asymptomatic which shows high risk in postnatal periods. This can manage and reduce adverse foetal outcomes efficiently, thus reducing morbidity and mortality.
Keywords: congenital anomalies, TORCH infection, CBMN, Oxidative stress

INTRODUCTION

A higher risk of complications during or after pregnancy and delivery refers to a condition known as High-Risk Pregnancy by the mother or infant and may be for both [1]. A pregnant

mother or foetus is in high risk whether it is asymptomatic or mild infections is due to TORCH agents (toxoplasmosis), Cytomegalovirus (CMV), rubella virus, herpes simplex virus (HSV) [2]. Any primary infection is deemed severely to be a chronic infection because it can lead to foetal intrauterine death, intrauterine growth retardation, congenital defects, spontaneous abortions, and early stillbirth, and signs of sickness in liveborn babies [3]. In developed countries, studies suggest that infection with any of these agents can lead to apparent morbidity and mortality [4].

Toxoplasmosis is a human disease caused by the Toxoplasma gondi parasite, an intracellular protozoan that spreads through infected fruit, water, or cooked meat. For the cysts after ingestion, there is an incubation time of 5–23 days.Infected women are usually asymptomatic, although breastfeeding is difficult, leading to foetal intrauterine malformations, loss of pregnancy and stillbirth [5,6].

Rubella virus is spread by droplets from person to person and by placental transmission from mother to infant. This illness lasts for 5 days and the incubation period is 2-3 weeks [7]. In children and adults, it typically occurs as a moderate or asymptomatic infection. Whether the mother becomes infected during the first trimester of pregnancy, the probability of transmission to the foetus and the severity of congenital infection are maximal. Congenital rubella syndrome (CRS) is the most severe result of infection with the rubella virus [8].

CMV is a species-specific and omnipresent species that is spread horizontally, through sexual contact or through contaminated saliva and urine, during the organ donation or blood transfusion process. In most cases, in the case of pregnant mothers, infection is due to close interaction with young children or sexual intercourse with contaminated urine or saliva [10]. The incubation time for CMV infection ranges from 4 to 12 weeks. Symptoms of newborn babies include hepatosplenomegaly, retardation of intrauterine development, jaundice, intracranial calcification microcephaly, anaemia, chorioretinitis, and thrombocytopenic purpura [11]. Disabilities such as visual loss, learning disability and hearing in infancy are also attributed to infection with CMV [12].

The most common sexually transmitted viral disease (STD) worldwide is HSV.HSV1 and HSV2 are two categories that are the main cause of genital herpes, transmitted either through non-sexual contacts or sexually during childhood [13,14]. It has an incubation period that varies from 4 to 21 days. The primary genital HSV infection remains asymptomatic in more than 75% of cases [15], while it remains a major cause of mortality and morbidity in newborns (Brown et al., 1997). Infection with genital herpes during pregnancy can lead to prematurity, miscarriage, neonatal herpes, and congenital herpes [17].

In the whole reproductive period of women's lives is influenced by Oxidative stress (OS) [18], which is shown by the formation of free radical that bring forth birth defects and abortions situations [19]. In implantation and egg fertilization, reactive oxygen species (ROS) concentrations play an important role [20]. The natural workings of the female reproductive system and the pathogenesis of female infertility play a significant role [21]. Even larger number of ROS formation leads to the breakage of double strands DNA in sperm and oocyts, causing fetus outcome. These DNA damage when increased may be associated with many medical disorders, which includes pregnancy complications. Modern factors in lifestyles such as proximity to contaminants, lack of exercise contribute to increased inflammation, unhealthy nutrition, oxidative stress, and eventually DNA harm.These lifestyle changes increase the risk of pregnancy loss, miscarriage, and congenital malformations has been reported in growing literatures [9].

The present study is therefore taken up as a challenge to evaluate the effects of increased oxidative stress, damage to DNA and also the role of TORCH infection in pregnant women at high risk. This helps to raise people's awareness of the factors that cause birth defects, and screening high-risk cases that can reduce adverse foetal outcomes, decrease morbidity and mortality is the role of cytogenetic analysis.

MATERIALS AND METHODS

Samples

Among 275 subjects, One hundred seventy women who are having clinically diagnosed pregnancy with high risk for congenital anomalies / abnormal USS findings and suspected to have infections are included in the study. One hundred and five age matched women with 2 or more live children were selected as control group. These women were referred from various infertility clinics and maternity centers of Kerala for genetic studies to Advanced Genetic Study centre (Genetika), Trivandrum, Kerala.

Inclusion criteria for study and control group

Study group

Clinically diagnosed pregnancy with high risk for congenital anomalies / abnormal USS findings and suspected to have infections are included in the study.

Control group

- Normal healthy subjects without present or previous history of chronic diseases were used as control.
- Age and sex matched controls with 2 or more live children fulfilling the above criteria are included in this study.

Exclusion Criteria

Subjects

- without any acute or chronic illness.
- without any type of cancer.
- undergoing prolonged medication.
- above the age of 45 and below the age of 18.

Collection and processing of blood sample

By venepuncture, eight ml of venous blood was extracted aseptically from all study subjects. 4ml was transferred into the vacuutainer containing sodium heparin to perform CBMN assay (Fenech in 1993). The remaining 4 ml wasused for other investigations (ELISA, MDAbythiobarbituric acid Assay). By using 10 ml of RPMI 1640 mediathe lymphocytes were cultured in sterile bottles supplemented with 15% of fetal bovine serum and 10µg/ml phytohaemagglutinin (PHA). Cytochalasin B was applied to the crops after the 44th hour of incubation of cells with PHA at a final concentration of 4.5µg/ml. After 72 hours of incubation, the cells were extracted and treated with a hypotonic solution (0.075M KCl) for 3 minutes and put in a fresh fixative solution (methanol: acetic acid, 3:1). The cells were lowered and air dried on microscopic slides and then stained with 10 percent Giemsa. At 100X magnification under a microscope, micro nucleated cells were analysed. The number of micronuclei was scored at no less than 1000 binucleated cells and the distribution of micronuclei between binucleated cells was recorded.

Statistical analysis

Version 17.0.0 of the Statistical Package for Social Sciences (SPSS) for Windows was used to evaluate the results. Descriptive characteristics of group variables were expressed as mean ± standard deviation (SD) and the importance of sample variables within groups was checked by unpaired students' non-tests. The number (frequency) of categorical variables was expressed and analyzed by chi-square tests. The relationship of CBMN frequency with maternal risk factors and MDA with maternal risk factors was analyzed using Pearson's correlation. By $p<0.05$, the statistical significance was defined.

RESULTS

Comparison of study and control subjects based on TORCH infections

In the present study included two seventy-five females aged between 18 and 45 years. Among 275 subjects, 170 (61.81%) are having the history of high-risk pregnancy with a mean age of 35.65±6.30 years. The control group includes 105 women with previous normal pregnancy with a mean age group of 34.03±6.10 years. Among the study group, 105 (61.7%) subjects had the toxoplasma infection, whereas 65 (38.2%) had no toxoplasma infection. Among the control group, 5(5%) had toxoplasma infection and 100 (95%) had no toxoplasma infection. Observable statistical difference of p value $p<0.001$ was seen in both groups based on toxoplasma infection. In study group, 88 (51.7%) had rubella infection, 82 (48.2) had no rubella infection. Among the control group, 3(3%) had rubella infection and 102 (97%) had no rubella infection. Both groups showed a statistical significant p value $p=<0.001$ based on rubella infection. CMV infection was observed in 94 (55.2%) study subjects, whereas 76 (44.7%) had no CMV infection. Among the control group, 6(6%) had CMV infection and 99 (94%) had no CMV infection ($p<0.001$). In study group, 90 (52.9%) subjects had HSV infection, whereas 80 (47%) had no HSV infection. Among the control group, 2(2%) subjects had HSV infection and 103 (98%) had no HSV infection. Both groups showed statistically significant p value $p<0.001$ based on HSV infection. According to the data obtained, TORCH infection is a significant risk factor among women with HRP (Table 1, Figure 1).

Table 1: Comparison of TORCH infections between study and control subjects

TORCH infection	Category	Study		Control		χ^2	p
		N	%	N	%		
Toxoplasma	Yes	105	61.7	5	5	11.04	<0.001
	No	65	38.2	100	95		
Rubella	Yes	88	51.7	3	3	79.9	<0.001
	No	82	48.3	102	97		
CMV	Yes	94	55.2	6	6	88.2	<0.001
	No	76	44.7	99	94		
HSV	Yes	90	52.9	2	2	82.6	<0.001
	No	80	47	103	98		

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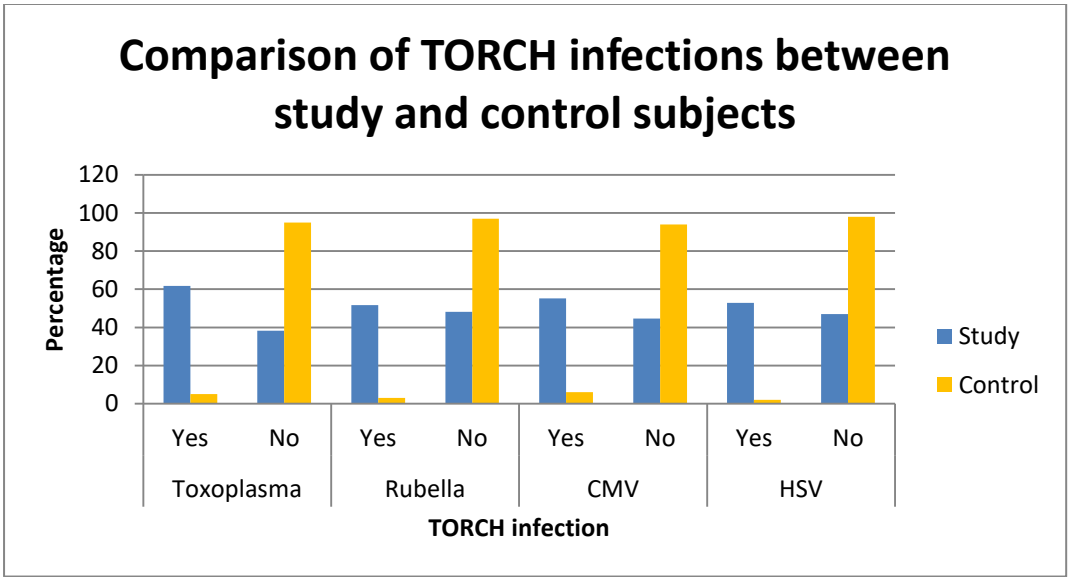

Figure 1: Comparison of TORCH infections between study and control subjects

Distribution of risk markers among study and control subjects

The comparison of risk markers like MDA and mean CBMN frequency was done among study and control group population. Among the study subjects, mean MDA was 2.95 ± 2.09 and that of control was 1.67 ± 0.70. The mean of CBMN frequency was 12.44 ± 1.01 among study subjects and that of controls was 9.98 ± 0.58. Theextent of somatic DNA damage and oxidative stress washigheramong study subjects than control group. Both groups showed statistically significant p value (p <0.001) (Table 2).

Table 2: Distribution of risk markers among study and control subjects

Risk markers	Study		Control		t	P
	Mean	sd	mean	Sd		
MDA (U/L)	2.95	2.09	1.67	0.70	6.08	<0.001
Mean CBMN frequency	12.44	1.01	9.98	0.58	22.77	<0.001

DISCUSSION

A traumatic event for the mother and for society is the birth of an abnormal child. Worldwide, about within 4 weeks of birth, 303,000 newborns die every year, according to the WHO, due to congenital anomalies [22]. Long-term disability can contribute to congenital anomalies, which can have a significant impact on individuals, families and societies. This research aims to ascertain the role of TORCH infection and to evaluate the degree of somatic DNA damage and oxidative stress in high-risk pregnancy subjects.

The results of the present study confirm that sero-positivity for T. GondiiIgG for women with a history of high-risk pregnancy is 61.7 percent. In a research conducted by Sarkar et al., 2012 [23], it was recorded that the sero-positivity of toxoplasmosis was substantially greater in antenatal women with Poor Obstetric History than in antenatal women who had previous regular deliveries. In this research, 105 women with BOH and 105 women without BOH were compared. Sero prevalence of toxoplasmosis was seen in 52 (49.52 percent) women and in women without BOH among antenatal women with BOH; sero prevalence was seen in 13(12.38 percent) women.

Sero positivity for rubella IgG in women with a history of high-risk pregnancy is 51.7 percent in the latest report. That means they were resistant to infection with rubella and the remaining 48.3% of sero negative cases were vulnerable to infection with rubella. Ramana et al., 2013 [24], found a strong correlation in women between rubella infection and BOH. According to this report, 19 (12.67 percent) were positive for rubella IgM antibodies in 150 pregnant women with BOH, and 2 (6.67 percent) were positive for rubella IgM antibodies in 30 women without BOH. In wasteful pregnancy, it is apparent that maternal infections such as rubella play a crucial role.

In this study, CMV specific IgG antibody was detected in 55.2% women having the history of high-risk pregnancy. Padmavathy et al 2013 have reported CMV seropositivity rate of IgM/IgG was 9.2%/96.4% in 87 pregnant women with bad obstetric history. Tiwari et al.,2016 showed that CMV primary infection was found to be 12.6% in 63 abortion cases while it was only 2% in normal pregnancy.

In the current research, HSV IgG sero positivity was 52.9 percent in women with a history of high-risk pregnancy compared with control subjects. Padmavathy et al2013 reported a seropositivity rate of2.3%/5.8% for HSV IgM/IgG among87 pregnant women with BOH was [25]. In a study of 380 pregnant women with BOH, Turbakar et al.,2003 reported seroprevalence in 145 (33.58%) womenfor HSV IgG [26].

The present research found that in women with high-risk pregnancy, oxidative stress (OS) increased compared with women with normal pregnancy. A significant association ($p<0.001$) was found between women with high-risk pregnancy (2.95 ± 2.09) and women with normal pregnancy (0.70 ± 6.08). Leal et al.,in 2011, during the third trimester of gestation, MDA levels increased in women, which alters the placental circulation during this time, leading to increased OS [27].

In this study, the extent of somatic DNA damage in women with high-risk pregnancy and women with normal pregnancy were quantified. Somatic DNA damage is statistically higher ($p<0.001$) among women with high-risk pregnancy (12.45 ± 1.01) than in women with normal pregnancy (9.98 ± 0.59). This result is consistent with a study conducted by Trková et al., 2000 [28], which found that micro nucleated cells were increased in women with infertility lymphocytes and in women with two or more spontaneous abortions.

CONCLUSION

This study found that TORCH infections, oxidative stress and somatic DNA disturbance play a role in the adverse foetal outcome of high-risk pregnancy. We recommend that proper diagnosis and intervention during early stage can be done by routinely screening of sample for TORCH agents, oxidative stress and somatic DNA damage even if the case is asymptomatic which shows high risk in postnatal periods. This can control and decrease adverse foetal effects efficiently, while reducing morbidity and mortality. In addition, in order to reduce the risk of congenital abnormalities and poor obstetric outcomes, in order to immunize all young girls and/or women of childbearing age before birth, vaccination plans must be updated. For more management, screening of the foetus during the antenatal phase and the newborn at birth is required.

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