Genotoxic and Teratogenic Effect of Congenital TORCH Infection

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Abstract— TORCH is a group of infectious organisms which causes malformations in new born foetus. There is good evidence that some maternal infections, especially during the early gestation, can result in foetal loss or malformations because of the foetus's inability to resist infectious organisms and the DNA damages caused by them. The goal of the present study was to evaluate the somatic DNA damage in TORCH positive children by CBMN assay and to correlate the results with various socioeconomic and demographic characteristics. The study was carried out in 45 study and 16 control subjects. The study clearly demonstrated that the mean CBMN frequency with respect to the demographic and clinical risk factors such as maternal age, paternal age, duration of married life, cytogenetic analysis, history of illness, history of drugs intake, etc had increased level of somatic DNA damage in CBMN assay. Thus the study indicates a positive correlation between congenital TORCH infection and the extent of somatic DNA damage i.e. increased levels of somatic damages were observed in subjects with more risk factors associated with genetically based conditions and demographic variables.

Index Terms — CBMN Assay, Congenital anomalies, DNA damage, Genotoxicity, Karyotype, Teratogen, TORCH infection.

1 INTRODUCTION

Some maternal infections, especially during the early gestation, can result in foetal loss or malformations because the ability of the foetus to resist infectious organisms is limited and the foetal immune system is unable to prevent the dissemination of infectious organisms to various tissues [1]. The foetus and/or neonate are infected predominantly by viral but also by bacterial and protozoan infection. Infections with various pathogens cause miscarriage or may lead to congenital anomalies in the foetus while others are associated with neonatal infectious morbidity [2].

Congenital malformations can be divided into broad categories, one being malformations attributed to discrete environmental factors. Infectious agents, as environmental factors, can create intrauterine infections leading to birth defects, abortion and stillbirth. The TORCH agents are Toxoplasma, other agents; Rubella virus, Cytomegalovirus (CMV) and Herpes simplex virus (HSV). These are the most important infectious agents that can cause congenital malformations. The infections in women are usually asymptomatic and chronic. Prevalence varies from one geographical area to another. The social and reproductive issues, pregnancy wastage, cost of treatment and morbidity to the infant make TORCH agents a major cause of concern [3].

It is estimated that approximately 10-15% of congenital structural anomalies are the result of the adverse effect of environmental factors on prenatal development. This means that approximately 1 in 250 newborn infants have structural defects caused by an environmental exposure and presumably, a larger number of children have growth retardation or functional abnormalities resulting from non-genetic causes, in other words, from the effects of teratogens [4].

A teratogen is defined as any environmental factor that can produce a permanent abnormality in structure or function, restriction of growth, or death of the embryo or foetus. A dose-response relationship should be demonstrated in humans so that the greater the exposure during pregnancy, the more severe the phenotypic effects on the foetus. Teratogenic factors comprise medications, drugs, chemicals, and maternal conditions or diseases including infections [4].

A recent survey of pregnant women showed that unintended pregnancies are associated with a higher risk for teratogenic exposures during pregnancy than planned pregnancies [5].

Infections acquired in utero or during the birth process are a significant cause of foetal and neonatal mortality and an important contributor to early and later childhood morbidity. Any infection with the TORCH group in

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pregnant women will adversely affect the foetal outcome. In India, TORCH screening and its cytogenetic impact study was not much carried out. This throw light to the fact that people are unaware about the impact of such infections and the extent of genetic instability it can cause. Hence the present study was undertaken to aware the people about the cytogenetic effects of teratogens and its genotoxic effect in TORCH infected Children.

2 MATERIALS AND METHODS

Forty five children suffering with varying degrees of congenital TORCH infection were selected for this study. The samples were referred from various maternity centres of Kerala for genetic testing to Genetika, Centre for Advanced Genetic Studies, Trivandrum, Kerala. Sixteen healthy children were also selected as control for this study. Detailed demographic, social and clinical characteristics were recorded using proforma after getting informed consent from parents. The institutional ethics committee of Genetika was approved the same. Chromosome preparation was done by peripheral blood lymphocyte culture method [6] and banded with GTG banding technique [7]. Cytokinesis-Block Micronuclei (CBMN) Assay was performed on each sample by using cytochalasin B for quantitating the extent of somatic DNA damages.

The fresh blood collected by venepuncture was transferred into vacuutainer containing sodium heparin as anticoagulant. Added 5 to 6 drops of whole blood samples to a vial containing 10ml of RPMI 1640 medium supplemented with 20% foetal bovine serum. Then phytohaemagglutinin (PHA, 10µg/ml) was added to proliferate the lymphocyte cells and incubated at 37^0C for 72 hrs. At the 70^{th} hour to the culture added a drop of colchicine (0.04µg/ml) to arrest the cell division at metaphase, then mixed gently and kept in incubator at 37^0C for 2 hours. Then transferred the whole content into a sterile centrifuge tube and centrifuged at 1000 rpm for 10 minutes. Discarded the supernatant, mixed the contents using cyclomixer, and re-suspend the pellet in pre-warmed hypotonic KCl (0.075M) and incubated for 20 minutes at 37^0C. 2-3 drops of Methanol: Acetic acid fixative (3:1 ratio) which was freshly prepared was added to the culture and centrifuged at 1000 rpm. Supernatant was discarded and the cell contents were mixed in a cyclomixer. 10ml of fresh fixative was added to the pellets present at the bottom of the centrifuge tube and kept for at least 30 minutes in refrigerator. Again the preparation was centrifuged at 1000 rpm for 10 minutes and the supernatant was discarded. Added fresh fixative centrifuged and repeated the process 3 or 4 times until the supernatant appeared clears. Clear cell suspension was prepared and dropped the cell suspension, drop by drop on to precleaned labelled and chilled slides from a particular height so as to get good quality metaphases. The slides were air dried and the slides were stained with 10% Giemsa staining solution for 10 minutes. Washed the slides in distilled water and observed under a research microscope through 100x objective. For karyotyping and detecting the structural anomalies, GTG banding technique was performed. To detect numerical and structural abnormalities 20-25 metaphases were analyzed and 5-6 metaphases were karyotyped [8].

3 RESULT

The demographic characteristic findings are given in the table 1. The age of the children were ranged from <1 to 3 years. The age of the control subjects ranged from <1 to 2 years. Among the 45 study subjects, 66.67% below the age of one year showed the highest mean CBMN frequency of 12.822. The study showed significant relationship between the paternal age and the mean CBMN frequency. As the paternal age (>40) increased the mean CBMN frequency (13.7) also increased. In the case of maternal age also the study showed significant relationship with mean CBMN frequency i.e. as the age of the mother (>35) increased the CBMN frequency (12.9) also increased. The duration of married life of the parents were observed and among them those which had duration >10 years showed highest CBMN frequency of 12.9. This indicates that there is a significant relationship between the mean CBMN frequencies and increased duration of married life. Subjects with the history of drug intake showed higher mean CBMN frequency of 13.188 and subjects which had the history of chronic illness also showed a higher CBMN frequency of 12.865. This clearly indicates a significant relationship between the mean CBMN frequency and the history of drug intake and illness.

The clinical characteristic findings are given in the table 2. Among the 45 study subjects, 40% subjects showed normal karyotype and 60% subjects showed abnormal karyotype. The mean CBMN frequency was found to be higher (13.144) in those subjects who had an abnormal karyotype. In the case of clinical condition 67% had congenital anomalies, 5% had dysmorphism, Down phenotype, 11% had facial dysmorphism, 4% had congenital heart problems, development delay, 4% had cleft palate and 9% had multiple anomalies and ambiguous genitalia. Among the clinical features analysed, higher percentage of the study subjects had congenital anomalies. The mean CBMN frequency of control subjects was 10.161 and the study subjects was 12.725. The mean

CBMN frequency of the study subject was found to be higher than the control subjects and moreover it was observed that there is a positive correlation between the demographic and clinical characteristics with the extent of DNA damages.

TABLE 1: DISTRIBUTION OF DEMOGRAPHIC CHARACTERISTICS OFTHE STUDY SUBJECTS

Distribution of demographic characteristics of the study subjects

	Variable	Number	(%)	Mean CBMN frequency
Children Age	<1	30	66.67	12.822
	1	7	15.55	12.455
	2	5	11.11	12.71
	3	3	6.67	12.42
Paternal Age	≤25	4	8.89	12.395
	26-30	17	37.78	12.49
	31-35	13	28.89	13.06
	36-40	6	13.33	13.145
	>40	5	11.11	13.7
Maternal Age	≤20	4	8.89	12.49
	21-25	18	40	12.538
	26-30	8	17.78	12.608
	31-35	10	22.22	12.666
	>35	5	11.11	12.9
Duration of Married Life	1 to 3	28	62.22	12.68
	4 to 6	6	13.33	12.688
	7 to 9	4	8.88	12.848
	>10	11	24.44	12.91
H/o Drug Intake	Yes	9	20	13.188
	No	36	80	12.61
H/o Illness	Yes	10	22.222	12.865
	No	35	77.78	12.686

TABLE 2: DISTRIBUTION OF CLINICAL CHARACTERISTICS OFTHE STUDY SUBJECTS

Distribution of clinical characteristics of the study subjects

	Variable	Number	(%)	Mean CBMN Frequency
Cytogenetic Analysis	Normal Karyotype	18	40	12.098
	Abnormal Karyotype	27	60	13.144
Clinical Conditions	Congential Anomalies	30	66.67	12.96
	Dysmorphism Down Phenotype	2	4.44	13.33
	Facial dysmorphism	5	11.11	12.438
	Congential Heart Problems, Developmental Delay	2	4.45	12.07
	Cleft Palate	2	4.44	11.12
	Multiple Anomalies, AG	4	8.89	12.155

4 DISCUSSION

It is estimated that approximately 10-15% of congenital structural anomalies are the result of the adverse effect of environmental factors on prenatal development. This means that approximately 1 in 250 newborn infants have structural defects caused by an environmental exposure and, presumably, a larger number of children have growth retardation or functional abnormalities resulting from non-genetic causes, in other words, from the effects of teratogens [4].

Grag et al [8] observed a high occurrence of congenital abnormality among women who are between 33 and 39 years of age. Tennat et al [9] also observed that high pregnancy rates among mothers in this age range could account for congenital anomalies. In the present study it was observed that the distribution of mean CBMN frequency gradually increased as the age of the mother increased. The maternal age below 25 years showed a mean CBMN frequency of 12.49 and above 35 years showed 12.88. Similarly it was observed that the paternal age also had influence in the TORCH positive babies. The mean CBMN frequency was higher in those above 40 years of age and comparatively lower for those below 25 years.

From these findings it was analysed that as age of the parents increased, the severity of the TORCH infection also increased. In the current study, along with the characteristics such as the paternal age and the maternal age, the duration of married life also influenced the mean CBMN frequency. In the case of duration of married life, the mean CMBN frequency increased with increased duration.

A number of chronic illnesses in the mother are linked with an increased risk of congenital abnormalities, fetal growth restriction, or certain diseases in the unborn baby. In some maternal conditions, the risk lies with the drugs used for treatment, rather than the illness itself. It is important to get these conditions under control before becoming pregnant. In some cases, a change in treatment may be needed before pregnancy begins. Some drugs may induce teratogenic effects that are not clinically evident until many years after birth, such as the reproductive tract abnormalities associated with DES exposures in utero [10]. Human teratogens generally increase rates of specific defects or a spectrum of defects. For example, thalidomide causes limb, spine, and central nervous system (CNS) defects; isotretinoin causes ear, CNS, and cardiac defects; valproic acid causes neural tube defects; warfarin causes artilage defects; and angiotensin II converting enzyme (ACE) inhibitors cause renal functional effects [11]. In the present study, it was observed that the distribution of mean CBMN frequency was higher in those study subjects with the history of drug exposure and similarly, the mean CBMN frequency was higher for those which had the history of chronic illness.

In a cross-sectional study conducted by Rizk et al [12], the newborns were assessed for congenital anomaly (CA). Neonatal data were extracted from medical records of the nursery. The classification of malformations was based upon WHO classification of CA. Of the all 1000 single births, 24 (2.4%) were diagnosed as being congenitally malformed. Cardiovascular system defects and limbs anomalies (4/1000) were mostly detected, followed by genitourinary system (2/1000), nervous system (2/1000), respiratory system (2/1000) malformations and chromosomal anomalies (1/1000). In the present study, it is analysed that among 45 study subjects, 30 study subjects had congenital anomalies, 5 study subjects had facial dysmorphism, 4 study subjects had multiple anomalies with ambiguous genetilia and 2 subjects had dysmorphism, down phenotype, cleft palate, congenital heart problems, developmental delay, etc. Among these clinical conditions, the highest proportion was subjects with congenital anomalies.

5 Conclusion

The mean CBMN frequency with respect to the demographic and clinical risk factors such as maternal age, paternal age, duration of married life, cytogenetic analysis, history of illness, history of drugs intake, etc. showed increased level of somatic DNA damage in CBMN assay. The study demonstrated a positive correlation with congenital TORCH infection and the extent of somatic DNA damages in subjects with more risk factors associated with genetically based conditions and demographic variables. The prevention is the only way to escape from the great threat of congenital TORCH infection. These infections were caused as a result of unhygienic conditions of the couples and also direct or indirect contact with the pet animals such as cats especially during the pregnancy period. There are some vaccinations available such as rubella vaccinations as preventive measure for infection. Prenatal analysis or other antenatal screening tests can be done to detect the presence of maternal infections and thus can avoid abnormal foetal or prenatal abnormalities. The couples with increased age had the chances of severe infections and this may lead to abnormalities to the foetus. Hence timely marriage as well as planned and safe pregnancy is also a right decision to escape from the threats of congenital TORCH infections.

6 References

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