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RESEARCH ARTICLE

Genetic Instabilities and Oxidative Stress in Congenital TORCH Infected Children

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Abstract

The maternal infections that are transmissible in utero at several stages of the pregnancy can be caused by a group of infectious organisms, namely TORCH. These are associated with multiple abortions, sterility, intrauterine foetal death or malformations because of the foetus's inability to resist infectious organisms and the DNA damages caused by them. The oxidative stress plays a role in the pathophysiology of these abnormalities. The aim of the present study was to evaluate the role of oxidative stress and DNA damages in congenitally TORCH infected children. The study measured the level of oxidative stress by Malondialdehyde (MDA) test and the DNA damages are quantified by CBMN assay. Detailed demographic, lifestyle and clinical characteristics were compared with the results obtained. The mean CBMN frequencies as well as MDA values are observed to be higher in 47 congenitally TORCH infected children than the 16 control children. The present study observed a positive correlation between congenital TORCH infection and the oxidative DNA damage and thereby measures the effect of teratogens in pregnant women. This aids to aware the people about the cytogenetic effects of the environmental factors.

Introduction

During gestation, many microorganisms can infect the foetus, causing severe birth defects. Such organisms and the resulting clinical syndromes have been categorized as TORCH infections (Franca and Mugayar, 2004). The maternal infections that are transmissible in utero at several stages of the pregnancy can be caused by many organisms, of which the members of the TORCH complex, namely Toxoplasma gondii (T. gondii), Rubella virus (RV), Cytomegalovirus (CMV), the Herpes Simplex Virus (HSV) occupy prominent positions. These infections are associated with inadvertent outcomes like multiple abortions, sterility, intrauterine foetal deaths, still births, congenital malformations and other reproductive failures, especially when they are acquired during the first trimester of the pregnancy (Li et al., 2009).

Every year, an estimated 7.9 million children are born with a serious birth defect of genetic or partly genetic origin. Over 1 million more infants are born with serious birth defects of post-conception origin including those that result from maternal exposure to environmental agents (teratogens) such as alcohol, rubella, syphilis, and iodine deficiency that can harm the developing foetus (MOD, 2006).

Congenital anomalies represent a significant cause of premature birth of child morbidity and mortality. From 200,000 new borns per year, over 10,000 presented malformations. Epidemiologic studies have shown that the incidence of malformations is increasing and varies upon geographic features, race and gender. Perinatal mortality is generated in 66.66% of cases by congenital malformations; illnesses from perinatal period and the rest of them are generated by birth (Rodica et al., 2009). Congenital malformations can be divided into broad categories, one being malformations attributed to discrete environmental factors. Environmental causes of congenital anomalies are referred to as teratogenic. These are generally problems with the mother's environment (Gilber, 2002).

Infectious agents can create intrauterine infections leading to birth defects, abortion and stillbirth (Golalipour et al., 2009). 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 (Mladina et al., 2000).

Oxidative stress is a condition in which the delicate balance existing between prooxidant (free radicals) production and their subsequent amelioration via the antioxidant defense system becomes skewed in favor of free radical expression (Cross and Halliwell, 1994). Oxidative stress is manifested at the maternal foetal interface from early pregnancy onwards. It plays a role in both the normal development of the placenta as well as in the pathophysiology of complications such as miscarriage, preeclampsia, intrauterine growth restriction (IUGR), and premature rupture of the membranes (Burton and Jauniaux, 2004).

At molecular level, the oxidative damage to DNA cause polysaccharide ring cleavage, base modification or chain breakage, damage to cellular constituents etc. Damage to lipids leads to formation of lipid aldehydes, lipid peroxides (Kohen and Nyska, 2002; Valko et al., 2007; Lipinski, 2011). MDA is a byproduct of lipid peroxidation; therefore, an elevation in MDA levels may reflect an overproduction of lipid peroxides and/or impaired antioxidant defense mechanism.

These lipid peroxides are produced mainly in the placenta due to membrane disruption by Reactive Oxygen Species (ROS) (Routledge, 2000). ROS are released from phagocytic cells that destroy cells infected with viruses, or bacteria, although surrounding tissue can also be affected leads to DNA damage.

In India, the studies relating oxidative stress and TORCH infection that leads to DNA damage and the resulting congenital anomalies in children, was not carried out. This throw light to the fact that people are unaware about the impact of such infection and the extent of genetic instability it can cause. Hence the present study was undertaken to assess the effect of increased oxidative stress and to quantify the extent of somatic DNA damages in congenitally TORCH infected children. This may aid to aware the people about the effect of the environmental factors and thereby the prevention of congenital anomalies due to TORCH infection.

Materials and Methods

The study was carried out in forty seven children suffering with varying degrees of congenital TORCH infection. Sixteen healthy children were also selected as control for this study. The samples were recruited from various maternity centers of Kerala for genetic testing to Genetika, Centre for Advanced Genetic Studies, Trivandrum, Kerala. Detailed demographic, lifestyle, and clinical characteristics were recorded using proforma. Cytokinesis-Block Micronuclei (CBMN) Assay was performed on each sample by using Cytochalasin B for quantitating the extent of somatic DNA damages.

Eight ml of venous blood was collected aseptically from all the subjects by venepuncture after overnight fasting. 4ml was transferred into the vacuutainer containing sodium heparin to perform the CBMN assay. The remaining 4ml was transferred into plain tube and allowed to clot. With the serum, malondialdehyde test is performed to detect the frequency of oxidative stress in the study subjects. MDA was determined using thiobarbituric acid as main reagent and the values are measured on semi-auto analyser at 540nm.

Two parallel cultures were set up for each sample, culture A & B. The culture A was for detecting constitutional chromosome anomalies by using peripheral blood lymphocyte culture method described by Moorhead et al., (1960), and GTG banded karyotypes were prepared according to ISCN pattern 1995. The culture B was for quantitating the extent of somatic DNA damages by Cytokinesis Block Micronuclei (CBMN) assay.

Lymphocyte cultures were prepared for each subject and were performed in 10 ml RPMI 1640 supplemented with 100 units/ ml penicillin, 100 units/ml streptomycin, 15% foetal bovine serum and 1% phytohemagglutinin. At 44th hr after initiation, cells were blocked in cytokinesis by adding cytochalasin B (Sigma, final concentration, 4.5µg/ml). The total incubation time for all cultures was 72 hr. After incubation, the cells were fixed in 3:1 methanol/glacial acetic acid, dropped onto clean microscopic slides, air dried, and stained with Giemsa stain. For each sample, 1,000 binucleated cells were scored at 100X magnification. The number of micronuclei per 1,000 binucleated cells was recorded.

Results

Forty seven children with varying degrees of congenital abnormalities or clinical abnormalities viz cleft lip, low set ears, webbed neck, dysmorphism, depressed nasal bridge, congenital heart disease, developmental delay, cystic hygroma, etc who were suffering from congenital TORCH infections were selected. The age of these children ranged from 4 days to 2 years. Sixteen, age and sex matched healthy childrens were selected as control study. The maternal age of these children ranged from 20 to 38 years with a mean age of 27.1 and the paternal age was 22 to 41 years with a mean age of 32.04. Various demographic, clinical, lifestyle and physiological condition were recorded and correlated with the extent of DNA damages and oxidative stress.

The Cytokinesis block micronuclei assay revealed that there is a statistical significant increase in the mean CBMN frequency among the study subjects (12.89) than the control subjects (10.16). In the case of MDA value, it was found to be 1.7 in study subjects and 1.35 in control subjects. The present study observed a significantly high level of MDA in study subjects when compared to the control subjects (Table: 1).

TABLE 1:
DISTRIBUTION OF MDA VALUE AND MEAN CBMN FREQUENCY AMONG STUDY AND CONTROL SUBJECTS

Variable	Number	MDA Value	Mean CBMN Frequency
Study	47	1.7	12.89
Control	16	1.35	10.16

The distribution of CBMN frequency and MDA value in study subjects according to age were given in the table 2. The age of the children were ranged from 4 days to 2 years. Among the 47 study subjects, 19.14% above the age of one year showed the highest mean CBMN frequency of 12.96. The study showed significant relationship between the paternal age and the mean CBMN frequency. As the paternal age (32 to 41) and maternal age (>35) increased, the study showed significant relationship with mean CBMN frequency .i.e, the older the parents the greater the mean CBMN frequency. The study observed that there is a significant relationship between the mean CBMN frequencies and increased duration of married life.

TABLE 2:

DISTRIBUTION OF MDA VALUE AND MEAN CBMN
FREQUENCY ACCORDING TO THE AGE OF THE STUDY SUBJECTS

Category	Variable	Number	Percentage	MDA	Mean CBMN Frequency
Age of Children	<1	31	65.95%	1.61	12.48
	1	7	14.89%	1.81	12.85
	>1	9	19.14%	1.89	12.96

Subjects with the history of drug intake and the history of chronic illness among parents showed higher mean CBMN frequency. This clearly indicates a significant relationship between the mean CBMN frequency and the history of drug intake and illness.

Regarding the TORCH investigation 18 (38.29%) of the children reported toxoplasma IgG positivity, 33 (70.21%) showed rubella IgG positivity, 37 (78.72%) showed CMV IgG positivity and 15 (31.91%) showed HSV IgG positivity. Eight out of forty seven study subjects reported IgG positivity in toxoplasma, rubella, CMV, HSV and the rest showed one or two IgG positivity.

The study frankly observed that subjects with CMV IgG positivity showed increased CBMN frequency as well as increased MDA level than the IgG negative subjects. This is also true among subjects with toxoplasma, rubella, CMV, HSV. The mean CBMN frequency was found to be increased with increased severity of the clinical conditions moreover the children born to advance paternal age, history of chronic illness among parents and the history of various drug intake also showed increased mean CBMN frequency.

Discussion

The purpose of the present study was to investigate the oxidative stress and DNA damages in children with congenital TORCH infection. It is well known that the best time to identify and address risk factors for poor reproductive health outcomes for mothers and babies is not after but before conception through preconception care (Johnson et al., 2006). Infections are one of those risks, because certain infectious diseases carry a real threat to mothers and the foetus in utero (Lassi et al., 2014).

The development of the cytokinesis-block (CB) technique has made the human lymphocyte micronucleus assay (MN) a reliable and precise method for assessing chromosome damage. The level of genetic integrity of human populations is increasingly under threat due to industrial activities that result in exposure to chemical and physical genotoxins (Fenech and Morley, 1985).

In the present study it was observed that children above 1 year age had high mean CBMN frequency. It was analyzed that the severity of TORCH infection gets increased when the age of the children increases. They show congenital abnormality with increasing mean CBMN frequency. Paternal age and maternal age had influenced in the TORCH positive babies. The mean CBMN frequency was higher in increasing age compared to others. In the present study the duration of married life also influenced the TORCH infected babies.

Some studies show that foetal infection may manifest in case of an infection in an immunized pregnant woman when she has been immunized for that particular agent (Toxoplasma Gondii, Cytomegalovirus) before pregnancy, although it is much lower in intensity (Guerina, 2005). A reliable diagnosis of acute T. gondii, RV, or CMV infection in pregnant women is the main objective of the TORCH diagnostic assays. Generally, detection of IgM antibodies is a sensitive indicator of an ongoing or recent infection (Bobic et al., 1991; Gras et al., 2004). Improvements in IgM and IgG avidity assays assist the clinician in detecting acute infection and distinguishing between a primary and secondary immune response (Grangeot et al., 1997; Lazzarotto et al., 1997).

Conclusion

TORCH infection cause severe birth defects. The children who had reported TORCH infection showed increased mean CBMN frequency and MDA value. The mean CBMN frequency was increased with increasing paternal age, chronic illness and history of drug intake. The mean CBMN frequency and MDA value was increased in toxoplasma, rubella, CMV, HSV, IgG positivity than the subjects with IgG negativity. Also the TORCH infected children showed increased DNA damages and increased level of oxidative stress that leads to severe congenital anomalies. Avoiding contact with pet animals may help to get rid of the chance of TORCH infection.

References

Bobic, B., Sibalic, D. and Djurkovic Djakovic, O. (1991). High levels of IgM antibodies specific for Toxoplasma gondii in pregnancy 12 years after primary toxoplasma infection, Gynecol. Obstet. Invest, 31182–184.

Burton, G.J. and Jauniaux, E. (2004). Placental oxidative stress; from miscarriage to preeclampsia, J Soc Gynecol Invest, 11, 342–352.

Fenech, M. and Morley, A. (1985). A measurement of micronuclei in lymphocytes, Mutat, Res, 147, 29-36.

Franca, C.M. and Mugayar, L.R. (2004). Intrauterine infections: a literature review Spec Care Dentist, 24(5), 250-3.

Gilbert, G.L. (2002). Infections in pregnant women, Med J Aust, 176(5), 229-36.

Golalipour, M.J., Khodabakhshi, B. and Ghaemi, E. (2009). Possible role of TORCH agents in Congenital malformations in Gorgan, northern Islamic Republic of Iran, Eastern Mediterranean Health Journal, 15(2).

Grangeot-Keros, L., Mayaux, M.J., Lebon, P., Freymuth, F., Eugene, G., Stricker, R. and Dussaix, E. (1997). Value of cytomegalovirus (CMV) IgG avidity index for the diagnosis of primary CMV infection in pregnant women, J. Infect. Dis, 175, 944 –946.

Gras, L., Gilbert, R.E., Wallon, M., Peyron, F. and Cortina-Borja. (2004). Duration of the IgM response in women acquiring Toxoplasma gondii during pregnancy: implications for clinical practice and Cross-sectional incidence studies, Epidemiol. Infect, 132, 541–548.

Guerina, N. (2005). Management of Cytomegalovirus and Toxoplasma Gondii in pregnancy, In: S.D. Craigo, E.R. Baker. eds. Medical complication in pregnancy, Practical pathways in obstetrics and gynecology', McGraw-Hil Medical Publishing Division, , 359-381.

Halliwell, B. and Cross C.E. (1994). Oxygen-derived species, their relation to human disease and environmental stress, Environ Health Perspect, 102(Suppl 10), 5-12.

Johnson, V.A., Brun-Vézinet, F., Clotet, B., Kuritzkes, D.R., Pillay, D., Schapiro, J.M. and Richman, D.D. (2006). Update of the drug resistance mutations in HIV-1: Fall 2006, Top HIV Med, 14(3), 125-130.

Kohen, R. and Nyska, A. (2002) 'Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification', Toxicol Pathol, 30, 620–50.

Lassi Zohra, S., Ayesha, M. Imam., Sohni, V. Dean., Zulfiqar, A. Bhutta., Lassi et al., (2014). Preconception care: preventing and treating infections, Reproductive Health, 11(Suppl 3), S4, 12-16.

Lazzarotto, T., Spezzacatena, P., Pradelli, P., Abate, D.A., Varani, S. and Landini, M.P. (1997). Avidity of immunoglobulin G directed against human cytomegalovirus during primary and secondary infections in immunocompetent and immunocompromised subjects, Clin. Diagn. Lab. Immunol. 4, 469–473.

Li, Z., Yan, C., Liu, P., Yan, R. and Feng Z. (2009). The prevalence of the serum anti-bodies to TORCH among women before pregnancy or in the early period of pregnancy in Beijing, Clinica Chimica Acta, 403, 212-15.

Lipinski, B. (2011). Hydroxyl radical and its scavengers in health and disease, Oxid Med Cell Longev, 809696.

Mladina, N., Mehikic, G. and Pasic, A. (2000). Torch infections in mothers as a cause of neonatal Morbidity, Medical Archives, 54(5–6), 273–276.

MOD (March of Dimes), (2006). The March Of Dimes Global Report on Birth Defects: The Hidden Toll of Dying and Disabled Children, March of Dimes Birth Defects Foundation.

Moorhead, P.S., Nowell, P.C., Wellmann, W.J., (1960). Chromosome preparation of leu-kocytes, Cultured from Human Peripheral Blood, Exp Cell Res, 20: 613-617.

Rodica Radu., Anamaria Molnar., Tudor Mirza., Stefan, I.. and Tigan. (2009). Congenital Malformation Prevalence in Cluj District between 2003-2007, Applied Medical Informatics, 25(4), 37-46.

Routledge, M.N. (2000). Mutations induced by nitrogen species in the supF forward mutation Assay. Mutat. Res, 450, 2000, 95–105.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M. and Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease, Int J Biochem Cell Biol, 39(1), 44-84.