RESEARCH PAPER | Medical Science | Volume : 6 | Issue : 6 | June 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

Evidence of Teratogenicity and DNA Damage among Women with Second Trimester Abortions

KEYWORDS: Second trimester pregnancy loss, Teratogenicity, Cytokinesis-block Micronuclei (CBMN) assay

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ABSTRACT *Second trimester pregnancy loss is uncommon, but it should be regarded as an important event in a woman's obstetric history. The exposure of various teratogen lead to DNA damage and subsequent chromosomal abnormalities. These factors play a major role in second trimester abortion. The study consists of 40 women with second trimester abortion/s and 15 healthy subjects without any chronic illness were selected as control for the study. The goal of the present study was to evaluate the effect of teratogenicity and extent of somatic DNA damage in subjects with second trimester abortion. The extent of somatic DNA damage is quantified by Cytokinesis Block Micronuclei (CBMN) assay. Detailed demographic and clinical characteristics were recorded and compared. The present study demonstrated that micronuclei frequency was significantly elevated in the study subjects than control subjects. Maternal problems such as history of infection, increased duration of married life, thyroid disorders, diabetes etc can leads to foetal loss. Modification of lifestyle along with proper preventive measures against teratogenic infection and awareness of the role of genetics in the etiology of recurrent pregnancy loss will help in reducing the risk for miscarriage.*

INTRODUCTION

Second trimester pregnancy loss is defined as pregnancy loss after the 14th week of gestation and before the 24th week of gestation (Wyatt et al., 2005). It is uncommon, but it should be regarded as an important event in a woman's obstetric history. Foetal abnormalities including chromosomal problems, maternal anatomic factors, immunologic factors, and teratogenic factors should be considered. However a cause and effect relationship may be difficult to establish (Thomas et al., 2007). 12–15% of conceptions result in clinically recognized pregnancy loss. The majority of these are first trimester miscarriages and fewer than five percent of pregnancies are lost after 10 weeks of gestation (Robert and Silver, 2007). The incidence of miscarriage in the second trimester varies depends on the gestational weeks. In low risk women the risk of miscarriage in the second trimester is approximately 0.5% (Westin et al., 2007).

Various indications for the termination of pregnancy are foetal demise, risk to the pregnant woman, such as severe preeclampsia, eclampsia, renal disease and uncontrolled gestational diabetes, severe foetal congenital anomalies, intrauterine infection such as rubella, premature rupture of membrane, malignant diseases and other medical disorders like severe heart diseases (Chia et al., 2002).

Teratogenic infection can create intrauterine infections leading to birth defects, abortion and stillbirth. The common teratogenic infectious agents are toxoplasmosis, other agents, rubella virus, cytomegalovirus (CMV) and herpes simplex virus (HSV), etc. These are the most important infectious agents that can cause congenital malformations (Golalipour, 2009).

Several drugs and chemicals are known to be teratogenic to the human embryo when administered during pregnancy, especially during the period of organogenesis. The evidence for their teratogenicity has been shown by human epidemiologic and clinical studies. These teratogenic insults occurring during embryonic life may be present immediately after birth, at infancy or even later in life, especially if the damage involves the central nervous system (CNS) (Ornoy, 2003) such damage can lead to aberrant gene expression and apoptosis. Higher levels of DNA damage are detected among women with complicated pregnancies (Furness et al., 2011; Harma et al., 2005). Hence the present study was undertaken to evaluate the effect of teratogenicity and DNA damages in couples experiencing second trimester abortions.

MATERIALS AND METHODS

Forty subjects suffering with second trimester abortion were selected as study subjects and 15 normal healthy subjects without any chronic illness were selected as control for the present study. Detailed demographic and clinical characteristics were recorded using profoma. They were referred from various infertility clinics and maternity centers of Kerala to Genetika, Centre for Advanced Genetic studies, Trivandrum.

Seven ml of blood sample was collected by venepuncture. Two ml of blood was transferred into sodium heparinized vacuutainers for quantifying the extent of somatic DNA damages by Cytokinesis-Block Micronuclei (CBMN) assay. The remaining five ml of blood was transferred into a plain tube, allowed to clot, serum separated immediately. Blood

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sugar and lipid profile were estimated using semi-automated clinical chemistry analyzer.

Two ml blood was added to a culture tube containing 10 mL RPMI 1640 supplemented with 100units/mL penicillin, 100µg/mL streptomycin, 15% fetal bovine serum and 100µg/mL phytohemagglutinin. Cytochalasin B was added to the cultures at a final concentration of 4.5µg/mL (Sigma) after 44th hours of initiation of cells with phytohaemagglutinin. Cells were harvested after 72 hr incubation, and they were treated with a hypotonic solution (0.075M KCl) for 1 min and fixed in fresh fixative solution (methanol: acetic acid, 3:1). The cells were dropped onto slides and the slides were air dried and stained with 10% Giemsa. Micronucleated cells were analyzed under light microscopy at 100X magnification. The number of micronuclei is not less than 1000 binucleated cells were scored and the distribution of micronuclei among binucleated cells was recorded.

RESULTS

Table1- Distribution of mean CBMN frequency according to various demographic characteristics

Category	Variables	Number (Percentage)	Mean CBMN frequency
Age of husband (Years)	25 to 35	19 (47.5%)	12.70
	36 to 45	19 (47.5%)	12.76
	>45	2 (5%)	13.01
Age of wife (Years)	<30	26 (65%)	12.70
	30 to 40	12 (30%)	12.80
	>40	2 (5%)	13.01
Duration of married life (Years)	1 to 5	23 (57.5%)	12.74
	6 to 10	13 (32.5%)	12.47
	11 to 15	1 (2.5%)	12.61
	16 to 20	3 (7.5%)	12.82

Table 2- Distribution of mean CBMN frequency according to various clinical characteristics

Category	Variables	Number (Percentage)	Mean CBMN frequency
Number of gestations	<3	5 (12.5%)	12.42
	3 to 6	33 (82.5%)	12.52
	>6	2 (5%)	12.81
Number of spontaneous abortions	0 to 2	18 (45%)	12.52
	3 to 5	2 (5%)	12.59
	6 to 8	20 (50%)	12.91
Number of MTPs	<2	5 (12.5%)	12.5
	≥2	35 (87%)	12.78
History of infection	Yes	6 (15%)	12.77
	No	34 (85%)	12.59
History of illness	Yes	11 (27.5)	12.75
	No	29 (72.5%)	12.72
History of drug intake	Yes	7 (17.5%)	12.75
	No	33 (82.5%)	12.70
Ultrasound Scan (USS) findings	Cardiac anomalies	5 (12.5%)	12.25
	Maternal abnormalities	2 (5%)	12.61
	Growth retardation	3 (7.5%)	12.12
	Congenital anomalies	30 (75%)	12.90
Cytogenetic Analysis	Abnormal karyotype	24 (60%)	13.13
	Normal karyotype	16 (40%)	12.15

The demographic and clinical characteristic findings are given in the table 1 and table 2. The age of husbands were grouped into 25 to 35, 36 to 45 and >45 years. The highest mean CBMN frequency 13.01 was showed by the age of husband >45 years. Age of wives were grouped into <30, 30 to 40 and >40 years. The highest mean CBMN frequency of 13.01 were showed in the age group >40 years. The age of the couples was increased and the mean CBMN frequency was also increased. The duration of married life, the couples those who had duration of 16 to 20 years of married life were showed mean CBMN frequency of 12.82. The number of gestations of the study subjects was observed and the highest mean CBMN frequency (12.81) was showed in subjects with more than 6 times of gestations. Moreover the subjects with more than six times spontaneous abortions were showed an increased mean CBMN frequency of 12.91. Simultaneously subjects with ≥2 times medical termination of pregnancies (MTP) was showed a mean CBMN frequency of 12.78. In short, increase in number of gestations, spontaneous abortions and MTPs were showed an increased incidence of mean CBMN frequency among the study subjects. Subjects with history of infection, history of illness and history of drug intake were showed increased incidence of abortions and their mean CBMN frequencies are 12.77, 12.75 and 12.75. More over 75% of Ultrasound Scan (USS) findings were showed congenital anomalies. Cytogenetic analysis was showed 60% abnormal karyotype and a mean CBMN frequency of 13.13.

DISCUSSION

In a retrospective study (De La Rochebrochard et al., 2002) estimate of the spontaneous abortion among women aged 35–44 years was higher when paternal age was 40–64 years. The interpretation of this increase in terms of the paternal age effect is uncertain, because the distribution of female age between 35 to 44 years may be shifted toward higher values when comparing partners of men aged 35–39 years with partners of men aged 40 or more years. In the present study, the paternal age >45 years and maternal age >40 years were observed with increased mean CBMN frequency. The result from the current study suggests that increased age of couples plays an important role in second trimester abortions.

Infections play a critical role in pregnancy wastage and their occurrence in patients with bad obstetric history (BOH) or complicated pregnancy is a significant risk factor (Stegmann et al, 2002; Kishore et al, 2003; Kishore et al, 2000). All viral pathogens usually cause a primary maternal viremia which may infect the placenta and thereby the foe-

Volume : 6 | Issue : 6 | June 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

tus with the exception of HSV-I or II, which causes an ascending infection via the genital tract to foetal membranes and then to the foetus (Ajayi et al., 2010). In the present study, among the 40 study subjects, 15% had the history of infection and they were observed with high micronuclei frequency indicating an increased DNA damage in them.

In addition to their role in first trimester miscarriage, chromosomal abnormalities also cause pregnancy loss in the second trimester. About 24 percent of pregnancy losses in the second trimester are caused by chromosomal abnormalities, and about 12 percent of late second trimester losses are attributed to this cause (Warburton et al., 1986). Chromosomal abnormalities found in second trimester losses are similar to those found in live births; the most common are trisomies 13, 18, and 21, monosomy X (i.e., Turner syndrome), and sex chromosome polysomies (Simpson et al., 1996). In the present study, it is analysed that 24 study subjects had abnormal karyotype and 16 study subjects had normal karyotype. Subjects with chromosomal abnormalities or abnormal karyotype showed an increased mean CBMN frequency.

Proximity to commercial pesticide applications was associated with an elevated risk of foetal death due to congenital anomalies. Furthermore, a consistent pattern was found with respect to timing of exposure; the largest risks for foetal death due to congenital anomalies were from pesticide exposure during the 3rd to 8th weeks of pregnancy (Erin et al., 2001). In the present study, it was observed that the mean CBMN frequency was highest in those who had congenital anomalies suggesting an increased DNA damage in them.

Human teratogens generally increase rates of specific defects or spectrum of defects. For example, thalidomide cause limb, spine, and central nervous system defects; isotretinoin causes ear, CNS, and cardiac defects; valproic acid causes neural tube defects; and angiotensin II converting enzyme (ACE) inhibitors cause renal functional effects (Mitchell, 2000). The present study is in agreement with above mentioned statement. Subjects with increased exposure to drugs during their pregnancy period were observed with increased mean CBMN frequency. Thus it can be suggested that lowering the exposure to various drugs among pregnant mothers can be avoided in order to prevent the teratogenic effect of all these drugs, chemicals, toxins towards the developing foetus.

CONCLUSION

The present study involves teratogenicity and DNA damage in second trimester abortions. The distribution of mean CBMN frequency according to demographic and clinical factors of the study subjects was observed. Age of the couples, duration of married life, number of gestations, number of spontaneous abortions, number of MTPs, etc. were showed an increased level of mean CBMN frequency. The level of mean CBMN frequency was highest among those who have the family history of infection, history of illness, history of drug intake. Abnormal karyotype of the study subjects showed increased mean CBMN frequency. The main preventive measures of second trimester pregnancy loss including vaccination and folic acid supplementation are recommended regardless of risk. The fruits and vegetables contain pesticides are the main teratogenic factor so should be well washed prior to consumption. Pregnant women should be advised to avoid contact with soil, pet animals and also avoid with toxic substances like chemicals, radiations, heavy metals, pesticides etc. These preventive measures may help from exposure to various teratogenic agents and thus reduce the risk of DNA damage and subsequent pregnancy loss/congenital anomalies.

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