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ROLE OF CYTOGENETICS IN FIRST TRIMESTER ABORTION USING PRODUCT OF CONCEPTION AND EXTENT OF SOMATIC DNA DAMAGES IN MATERNAL BLOOD

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ABSTRACT

Spontaneous abortion (miscarriage) is most common during the first trimester of pregnancy. A fetal loss is occurring when the fetus has died within 8-12 weeks of gestation without heart activity has been recorded. Miscarriages affect 15% of women, primarily in the first trimester. Forty two women suffering with first trimester abortion were referred from various maternity centres of Kerala to Genetika, centre for advanced genetic studies, Trivandrum for chromosome analysis were taken as study subjects and twenty age sex match healthy women are selected as control subjects. The present study was undertaken to evaluate the role of cytogenetics in first trimester abortion using product of conception and extent of somatic DNA damages in maternal blood using Cytokinesis Block Micronuclei (CBMN) assay.

The results were computed and correlated with various other demographic, physiological, clinical and lifestyle characteristics. Regarding the karyotype of POC, 69% showed abnormal karyotype and 31% showed normal karyotype. The mean CBMN frequency of study subjects was 12.05 and control was 10.02 and showed a statistical significance. The study revealed that, the chromosomal abnormalities play an important role in first trimester pregnancy losses and these abnormalities were associated with various demographic, physiological, clinical and lifestyle characteristics. Thus, cytogenetic study should be offered to all couples especially for mother with two or more abortions. Patients who have had an unexplained pregnancy loss should be offered genetic counseling with an option for karyotype analysis for finding the role of cytogenetic in pregnancy losses. There by proper management of pregnancy losses in the future can be avoided.

INTRODUCTION

Spontaneous abortion (miscarriage) is most common during the first trimester of pregnancy. A fetal loss is occurring when the fetus has died within 8-12 weeks of gestation without heart activity has been recorded (Quenby, 2007). Miscarriages affect 15% of women, primarily in the first trimester (Clark et al., 2001). Among the heterogeneous causes of miscarriage, chromosomal abnormalities, mainly aneuploidies, occur most frequently. Although in the great majority of cases they are *de novo* mutations and their impact on the risk in subsequent pregnancies is compound. Identification of the cause of miscarriage plays an important role in genetic counseling (Massalska et al., 2017).

There are many reasons associated with higher rate of pregnancy loss. This include chromosomal abnormalities which are found in more than half of embryos miscarried in the first 13 weeks (Stephenson and Kutteh, 2007). A chromosomal abnormality derived from one parent or the recurrence of a numerical abnormality might be a cause of recurrent abortion. In about 50–70% of abortion, a chromosomal abnormality is identified in the products of conception (Hogge et al., 2003; Shawky and Kamal, 2012). Percentage of prenatal loss of chromosomally abnormal fetus is different according to type of aberration and it was estimated to be 100% loss for autosomal monosomy and tetraploid, 96.5% for autosomal trisomy and structural rearrangement constitutes up to 53.4% (Pflueger, 1999). Cytogenetic evaluation of product of conception (POC) is essential to determine the cause of pregnancy loss and aid the prenatal diagnosis of subsequent pregnancies. POC helps to profile cytogenetic abnormalities, their relationship with maternal and gestational age.

Globally chromosomal abnormalities have been rapidly increasing. Unexplained and spontaneous abortion affects approximately 1% of women; unexplained miscarriage remains a frustrating problem. There are several reasons may leads to an abortion but the genetic/core reason behind spontaneous abortion still remain unknown. Genetic study with product of conception on first trimester abortion can help to find out the reason behind spontaneous abortion. Due to high incidence and complex etiology of spontaneous abortion, it is of great importance to investigate the subsequent pregnancy outcomes in order to ensure an effective perinatal care.

More than one third of the approximately 205 million pregnancies that occur each year around the world is unplanned and about 20% of them end in induced abortion (Geetha Balsarkar et al., 2015). Miscarriages affect 15% of women, primarily in the first trimester period of pregnancy (Clark et al., 2001). Approximately 50% of all cases of early pregnancy loss are due to fetal chromosomal abnormalities (Alijotas-Reig et al., 2013). The frequency of clinically recognized early pregnancy loss for women aged 20–30 years is 9–17% and this rate increases sharply from 20% at age 35 years to 40% at age 40 years and 80% at age 45 years

(American Society for Reproductive Medicine, 2012). Several types of genetic problems like maternal structural chromosomal abnormalities and recurrent aneuploidies may be associated with recurrent miscarriage (Elghezal et al., 2007). Many risk factors are thought to be associated with early pregnancy loss is still unclear. Hence the present study was undertaken to evaluate the role of cytogenetics in first trimester abortion using product of conception and extent of somatic DNA damages in maternal blood using Cytokinesis Block Micronuclei (CBMN) assay.

MATERIALS AND METHODS

Forty two women suffering with first trimester abortion were referred from various maternity centres of Kerala to Genetika, centre for advanced genetic studies, Trivandrum for chromosome analysis were taken as study subjects and twenty age matched healthy women were selected as control subjects. Demographic, physiological, clinical and life style characteristics were collected using proforma. In this study, Cytokinesis Block Micronuclei (CBMN) assay was carried out in each subject for quantitating the extent of somatic DNA damages using maternal blood samples. Chromosome analysis was performed using product of conception. Chromosome preparation was done by taking tissue from product of conception and banded with GTG banding techniques (Seabright, 1971).

OBSERVATION AND RESULTS

Forty-two women with first trimester abortion and their products of conception were selected as study subjects and twenty age matched healthy subject were selected as control subjects. The maternal age was ranged from 20-45. The gestational weeks of study subjects were ranged from 7-15 weeks and most of them were of 12 weeks old at the time of abortion. The duration of married life of the study subjects was ranged from 1-19 with a mean duration of 4.79 years and number of gestations was ranging from 2-7 years. Among the 42 subjects, 34 subjects had number of spontaneous abortions. There are 11 study subjects reported with family history of thyroid disorder and 25 study subjects with history of illness and 18 subjects with a history of infection.

The results were computed and correlated with various other demographic, physiological, clinical and lifestyle characteristics. Regarding the karyotype of POC, 69% showed abnormal karyotype and 31% showed normal karyotype. The higher incidence of abnormal karyotype was found among subject with advanced maternal age. The incidence of abnormal karyotype was higher among subjects belonged to urban area.

The mean CBMN frequency of study subjects was 12.05 and for control subjects it was 10.02 and this showed a statistical significance difference with $p<0.05$. The physiological characters such as height, weight, BMI shown to have a direct correlation with mean CBMN frequency. Subjects with regular exercise and good physical activity showed decreased mean CBMN frequency as compared to subject with irregular exercise and poor physical activity. The study subject with history of infection showed higher incidence for abnormal karyotype and mean CBMN frequency. The abnormal karyotype and mean CBMN frequency was higher in subject with increased duration of married life. The abnormal karyotype and mean CBMN frequency was higher among subject with increased number of gestations.

DISCUSSION

Abortion is primarily a health concern of women but it is increasingly being governed by patriarchal interests which more often than not curb the freedom of women to seek abortion as a right. Findings indicate that, the abortion rate declined significantly in the developed world, but not in the developing world, between 1990 and 2014 (Sedgh et al., 2007). The present study also observed that, the increase in maternal age shows a high risk of pregnancy loss that is, the higher incidence of abnormal foetal karyotype were found among subjects with increase maternal age.

In the present study, it was observed that, the incidence of abnormal foetal karyotype was increased with increasing gestational weeks. In a study conducted by Kashanian et al (2006), it has been reported that, women whose first pregnancy resulted in miscarriage are at a higher risk of having miscarriage in the second pregnancy when, compared with women who had a live birth. Kiss et al (2009) found that, chromosome abnormalities were found in 5% of the couples with a history of two abortions, in 10.3% with three abortions and in 14.3% with four or more abortions. In a Patient with a history of two miscarriages, the subsequent risk of pregnancy loss was estimated about 25%, whereas subjects with history of 3 abortions, the subsequent risk of miscarriage were estimated to 33% (Dubey, 2005). The present study also demonstrated that, the incidence of abnormal karyotype was increased with increase in number spontaneous abortions and MTPs.

Infection may affect the placenta and there by harm the developing baby - cause premature labor, or lead to birth abnormalities. According to the National Institutes of Health (NIH), infections that are known to harm the developing baby including, bacterial vaginosis, which might cause preterm labor, contagious diseases, such as hepatitis, syphilis, herpes and HIV - which can infect the fetus. Chlamydia can cause eye infections. Pneumonia and gonorrhea, which can contaminate the amniotic fluid and cause preterm labor there by leads to eye infections and possible blindness. Fifth disease, which can trigger a miscarriage or cause fetal anemia, group B streptococcus, which can cause severe complications in newborns and in rare cases can be fatal, toxoplasmosis, which can cause birth abnormalities and intellectual disabilities. *Listeria* which can cause miscarriage, stillbirth and birth abnormalities. Cytomegalovirus which is often harmless but can also cause birth abnormalities and intellectual disabilities (Villines, 2019). In the present study also demonstrated that the incidence of abnormal karyotype was observed higher among subjects with history of infection.

Maternal obesity has been associated with adverse pregn ancy outcomes. Increased BMI increases the incidence of induction of labor, caesarean section, pre-term labor and macrosomia. The BMI of women in the first trimester of pregnancy is associated with the risk of adverse pregnancy outcome (Shahla Yazdani et al., 2012). In the present study also demonstrated that, the incidence of abnormal karyotype was observed among subject with increase BMI.

Drugs intake by the pregnant mother can affect the fetus in several ways. They can act directly on the fetus causing damage or abnormal development leading to birth defects or death. About 2-3% of all birth defects results from use of drugs. Risk of miscarriage almost doubles for the women who drink alcohol in any form during pregnancy. And birth weight of the babies is substantially below normal. This syndrome includes inadequate growth before or after birth, facial defects, a small head, mental retardation and abnormal behavioral development (Sullivan, 2004). The current study also observed that the prolonged use of drugs by women were leads to miscarriage and also showed that the incidence of abnormal karyotype were higher among subjects with history of drug intake.

To assess the various environmental exposures require data or assumptions regarding mother's location throughout pregnancy (Bell et al., 2012). In the present study it was demonstrated that, the incidence of abnormal karyotype was observed higher in urban area than rural and costal area. Environmental conditions in the area of mother's residence

during pregnancy have direct association with pregnancy and childhood health outcomes. These include exposure to ambient air pollutants during pregnancy that affects fetal growth and risk of birth defects.

CONCLUSION

The current study concluded that the chromosomal abnormalities play an important role in first trimester pregnancy losses and these abnormalities were associated with various demographic, physiological, clinical and lifestyle characteristics. Thus cytogenetic study should be offered to all couple especially for mother with two or more abortions. Patient who have had an unexpected pregnancy loss should be offered genetic counseling there by an option for karyotype analysis for finding the role of cytogenetics in pregnancy loss. The diagnosis of chromosomal anomalies at the exact time can lead to the prevention of future birth of affected baby and hence the incidence of chromosomal abnormality can be reduced to a certain extent in the society.

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