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CYTOGENETIC AND MOLECULAR CYTOGENETIC STUDIES ON ABORTED FOETUSES

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ABSTRACT Miscarriage is spontaneous or induced interruption of pregnancy until 20 complete weeks. Miscarriages occur in approximately 15% of diagnosed pregnancies. A chromosomal abnormality derived from one parent or the recurrence of a numerical abnormality might be a cause recurrent abortion. The present study was conducted on thirty two medically terminated foetuses with gestational week between 14 to 20 week were included as test group and eighteen healthy children of the age ranged from 2 months to 2 years as control subjects. All these aborted foetuses were referred from various infertility clinics and maternity centers of Kerala to Genetika, Centre for advanced genetic studies, Trivandrum for chromosome analysis. Various demographic, physiological, clinical and life style characteristics of the couple (parents) were collected using proforma. Karyotype analysis was performed using intracardiac puncture blood sample of the aborted foetuses and venous blood samples were collected from the control subjects to detect chromosome abnormalities, if any. Cytokinesis-Block Micronuclei (CBMN) Assay was also performed on each sample by using cytochalasin B for quantitating the extent of somatic DNA damages. Regarding the foetal karyotype analysis, 52.94% (n=18) of the study subjects showed abnormal karyotype and 47.06% (n=16) showed normal karyotype. The mean CBMN frequency of study subject was greater than that of control subjects. The higher incidence of abnormal foetal karyotype and increased mean CBMN frequency was found among subjects with advanced maternal and paternal age. Chromosomal analysis is an important etiological investigation in couples with repeated spontaneous abortions as it helps in genetic counseling and deciding about further reproductive options. Modifying lifestyle habits and proper medication will help to avoid future pregnancy loss.

KEYWORDS : Recurrent abortion, Foetal karyotype, Chromosome abnormalities, DNA damage

INTRODUCTION

Miscarriage is spontaneous or induced interruption of pregnancy until 20 complete weeks. Miscarriages occur in approximately 15% of diagnosed pregnancies (Stephenson et al 2002) and although they are common, most women who have miscarriages give birth to a healthy child in later life. The probability for a couple to have two consecutive miscarriages ranges from 2.2 to 4%. A chromosomal abnormality derived from one parent or the recurrence of a numerical abnormality might be a cause recurrent abortion (Pflueger 1999).

Globally over 42 million abortions are performed annually and 10-15 percent of it takes place at second trimester that is between 13-28 weeks and third trimester abortions relatively less. The complication rate of abortions (second and third trimester abortions) is 13 times higher than that of first trimester abortions. Abortion-related complications account for approximately 13% of maternal deaths worldwide, roughly estimated as 47000 deaths per year. Second trimester abortion carries a higher risk of morbidity and mortality as compared to first trimester abortion especially in developing countries. Currently, there are conflicting reports regarding the rates of chromosomal abnormalities between recurrent and sporadic pregnancy losses. Some studies related to second trimester abortion are done previously still the genetic reason behind the second and third trimester was remaining unclear. It is necessary to understand the genetic reason behind spontaneous abortion for the diagnostic and therapeutic approaches of assisted reproduction.

Second trimester abortion is the termination of pregnancy in a period from 13 to 28 weeks of gestation, which again is subdivided into early period between 13 and 20 weeks and late period between 20 and 28 weeks (Lalitkumar et al 2007). Globally, over 42 million abortions are performed annually and 10–15% of the cases take place in second trimester period, over half of which are considered unsafe and disproportionately contribute to maternal deaths (Facts on Induced Abortion Worldwide 2007). In 2008, there were 29 abortions per 1,000 women aged 15–44 years in developing countries, compared with 24 per 1,000 in the developed world (Sedgh et al 2012). As researches showed, the prevalence of induced second trimester abortion was as high as 25%–30% in India (Mulat et al 2015). Chromosomal abnormalities in the foetus are the major causes of abortions. Detailed investigations are necessary to rule out the genetic basis of these types of abortions, so that proper solutions can be instituted. Hence the present study was undertaken to evaluate the cytogenetics and molecular cytogenetics study on aborted foetus. The specific objectives of the study are to evaluate the chromosomal abnormalities, if any, present in aborted foetuses by Karyotyping and to measure the extent of somatic DNA damage, if any, in aborted foetuses by Cytokinesis Block Micronuclei (CBMN) assay.

MATERIALS AND METHODS

The present study was conducted on thirty two medically terminated foetuses with gestation week between 14 to 20 week were included as test group and eighteen healthy children of the age ranged from 2 months to 2 years as control subjects. All these aborted foetuses were referred from various infertility clinics and maternity centers of Kerala to Genetika, Centre for advanced genetic studies, Trivandrum for chromosome analysis. Various demographic, physiological, clinical and life style characteristics of the couple (parents) were collected using proforma. Karyotype analysis was performed using intracardiac puncture blood sample of the aborted foetuses and venous blood samples were collected from the control subjects to detect chromosome abnormalities, if any. Cytokinesis-Block Micronuclei (CBMN) Assay was also performed on each sample by using cytochalasin B for quantitating the extent of somatic DNA damages.

OBSERVATIONS AND RESULTS

The karyotype of 32 study subjects were analyzed, among them paternal age of study subjects were ranged from 26-45 years with a

mean age of 33.5. The maternal age was ranged from 22-40 with a mean age of 29.58. The gestational weeks of study subjects were ranged from 14-20 weeks and most of them were of 14 weeks old at the time of abortion.The duration of married life of the study subjects was ranged from 2-11 with a mean duration of 4.79 years and number of gestations was ranging from 2-5 years. Among the 34 subjects, 19 subjects had family history of abortions and 15 subjects without family history of abortion. The number of spontaneous abortions among study subjects was grouped into two as ≤1 and ≥2. Majority of the study subjects belonged to ≥2.

The physiological characters included gestational weeks, H/o illness, no. of previous abortions, number of gestations and family history of abortions. Majority of the study subjects showed congenital abnormalities. The torch infections were observed among the study subjects. The Cyto Megalo Virus (CMS), Toxoplasmosis, Rubella Virus, Herpes Simplex Virus (HSV) infections and other infections were observed among the study subjects. History of illness and history of infection have significant role in this study so those are taken as another variable. The lifestyle characters included drug intake. Drug intake was taken as lifestyle variable. The clinical characters are included in this study are cleft lip with no cardiac pulsation, congenital anomalies, dysmorphism, depressed nasal bridge, multiple anomalies and down syndrome.

Regarding the karyotype analysis, 52.94% (n=18) of the study subjects showed abnormal karyotype and 47.06% (n=16) showed normal karyotype. While considering the foetal karyotype analysis, 53% showed abnormal foetal karyotype and 47% showed normal foetal karyotype. The mean CBMN frequency of 34 study subjects was 12.0. Among them 16 (47.06%) had normal chromosomal pattern and 18 (52.94%) had abnormal chromosomal pattern. The mean CBMN frequency of 18 control subjects was 9.98. The mean CBMN frequency of study subject was greater than that of control subjects.

The higher incidence of abnormal foetal karyotype was found among subjects with advanced maternal and paternal age. Moreover, the results showed significant relationship between paternal age and the mean CBMN frequency. As the paternal age increased the mean CBMN frequency has also increased. The abnormal karyotype was higher among subjects with increased duration of marriage and the mean CBMN frequency also increased along with increased duration of marriage. The abnormal karyotype was higher among subjects with higher number of gestation and increased gestational weeks and the mean CBMN frequency also increased along with increased number of gestations and increased gestational weeks. This is true regarding the incidence of abnormal karyotype was higher among subjects with increased number abortions and the mean CBMN frequency also increased along with increased number of abortions.

The study subjects with history of family abortions, infection, illness and drug intake showed high incidence of abnormal karyotype and the mean CBMN frequency was also increased on study subjects that had history of infection, illness and drug intake. The mean CBMN frequency was increased on study subjects having TORCH group of infections.

DISCUSSION

According to the study done by Tavokina et al (2006) it was observed that, "the frequency of chromosomal abnormalities among spontaneous miscarriages of the first trimester of pregnancy makes 50-60%". In 2005 Pflueger reported that, "pregnancy loss is quite common, with 15–20% of recognized pregnancies resulting in failure. The majority of these occur early in gestation, although losses in the second and third trimester are not rare. Approximately 2–5% of women will experience two or more losses. The majority of pregnancy failures are associated with cytogenetic abnormalities, with over 50% of early miscarriages and as many as 5% of stillbirths exhibiting abnormal karyotypes". In the current study it was observed that, the incidence of abnormal foetal karyotype was 52.94%. Moreover the incidence of abnormal foetal karyotype was more among subjects with increased number of previous abortions.

Stephenson et al (2002) suggested that, "miscarriage may occur in approximately 15% of diagnosed pregnancies and, although they are common, most women who have miscarriages give birth to a healthy child later in life". In a previous study done by Gardo (1993) reported that, "the probability for a couple to have two consecutive miscarriages ranges from 2.2 to 4%. Among the main causes for miscarriage are chromosome anomalies (whether numerical or structural), mostly represented by trisomies, by polyploidies and by the monosomy of sex-determining chromosome X". Morton et al (1987) observed that, "most miscarriages occur in the first trimester, generally between eight and 12 weeks, and half of these are caused by chromosome anomalies. On the other hand, approximately 99% of pregnancies with chromosome anomalies evolve to miscarriage".

Capalbo et al (2015) observed that, "birth defects have been observed in 3% of the live births, with a significant proportion of these defects (20%) related to chromosomal abnormalities or gene mutations. Some of the aneuploidy, including anioplose, 13, 18, 21, X, and Y chromosomes can lead to the birth of the baby alive and abnormal". Marquard et al in 2010 reported that, "more than 50% of couples who have a history of recurrent abortions are referred to as unspecified or idiopathic causes". Studies done by Handyside et al (2010) and Feichtinger et al (2015) showed that, "chromosomal analysis can determine the cause of 80% of cases of repeat unexplained abortions in women over 35 years of age". In previous study done by Hassold et al (2001), "mother's age is the most important factor that directly affects the frequency of chromosomal abnormalities in the embryo". In the present, study subjects with history of family abortions, infection, illness and drug intake showed high incidence of abnormal karyotype and the mean CBMN frequency was also increased on study subjects that the rest.

Maternal age also plays a significant role in the incidence of recurrent miscarriage. This suggests that pregnancy abnormality is a significant contributory factor to miscarriage given that the incidence of pregnancy abnormality increases with maternal age (Quenby and Farquharson, 1993). The risk of miscarriage for women younger than the age of 24 years is 9.5%. With age this risk rises, it increases to 11% by the age of 30 and reaches 33% in women aged 40. The incidence increases dramatically to 53% in those women over the age of 44 (Quenby and Farquharson, 1993). In the present study, the incidence of abnormal foetal karyotype was more among foetuses having increased maternal and paternal age. Moreover, it was also revealed that as paternal age increases there was increase in abortion rates and the mean CBMN frequency was high in higher age group.

In 2013 Hoffman et al reported that, "Congenital heart disease (CHD) is the most common congenital birth defect. CHD accounted for nearly one third of all birth defects, and the prevalence rate reached to 8 to 12 per 1000 live births worldwide". Jenkins et al (2007) have demonstrated that, "a number of genetic and environmental factors have been associated with the development of CHD in the foetus. Furthermore, some risk factors have been identified, such as phenylketonuria, rubella, retinoic acid, and the use of certain specific drugs. Moreover, mothers infected with a virus during pregnancy may be at a higher risk of developing CHD in offspring". Ye et al (2019) suggested that, "the risk of CHD in offspring was significantly increased among mothers with viral infections, the reasons are not clear, which was rarely discussed in previous studies". In a previous study by Waldorf et al (2013) have shown that, "rubella virus, herpesvirus, and cytomegalovirus were human teratogens that could cause a spectrum of birth defects, including blindness, deafness, CHDs, mental retardation and central nervous system complications, if the viral infection is acquired in the early months of pregnancy". In the present study also it was observed that, maternal infection showed an important role in abnormal growth of foetus.

Griffin et al (1995) reported that, "advancing paternal age has been recognized as a contributing factor for increasing the risk of producing aneuploid gametes. Abnormal DNA fragmentation may be seen in the setting of advanced paternal age or may result from correctable environmental factors, such as exogenous heat, toxic exposures, varicoceles, or increased reactive oxygen species in semen". According to the study by Sartorelli et al (2001), "the probability of producing aneuploid offspring is increased in older men and there are higher frequencies of sperm chromosome aberrations". In the present study an increased micronuclei frequency was observed among study subjects with increased paternal age.

SUMMARY AND CONCLUSIONS

Various lifestyle and environmental factors are directly responsible for higher extent of DNA damage that causes abnormal foetal development. The present study concluded that molecular, cytogenetic and immunological factors play an important role in spontaneous

abortions. Chromosomal analysis is an important etiological investigation in couples with repeated spontaneous abortions as it helps in genetic counseling and deciding about further reproductive options. 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 abnormalities can be reduced to a certain extent in the society. Modifying lifestyle habits and proper medication will help to avoid future pregnancy loss.

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