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OXIDATIVE STRESS AND DNA DAMAGES IN BAD OBSTETRIC HISTORY

KEY WORDS:

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ABSTRACT

Bad obstetric history (BOH) implies previous unfavourable foetal outcome in terms of two or more consecutive spontaneous abortions, history of intrauterine foetal death, intrauterine growth retardation, stillbirth, early neonatal death and/or congenital anomalies. Twenty couples suffering from bad obstetric history were selected as study subjects. Fifteen healthy age matched couples with one or two live children were selected as control subjects for the present study. The study was undertaken to assess the effect of increased oxidative stress and DNA damages in couples experiencing BOH. Malondialdehyde (MDA) test was performed to detect the oxidative stress in patients with BOH and the extent of somatic DNA damage was quantified by Cytokinesis Block Micronuclei assay. The results of mean MDA concentration and the mean CBMN frequency showed a statistically significant difference between the study and control subjects. Subjects with increased hormone level (FSH and LH) also showed increased MDA value and mean CBMN frequency. Similar result was observed among subjects with TORCH infections. Male study subjects having the unhealthy lifestyle habits and abnormal semen parameters showed increased MDA value and mean CBMN frequency. The results were correlated with various demographic, lifestyle and clinical aspects of the patients. Modification of life style, by changing the dietary habit and sedentary life style will help to reduce the oxidative stress. Moreover, the diagnosis of chromosomal anomalies at the exact time can lead to prevention of future birth of affected babies and also pregnancy loss due to suspected genetic loss can also be reduced in the society to an extent.

INTRODUCTION

Recurrent miscarriage (RM), also known as recurrent pregnancy loss, is a distressing condition affecting around 1% of couples trying to conceive. It can be very frustrating for both clinicians and patients as, despite intensive workup, no clear underlying pathology is forthcoming in at least 50% of couples (Homer, 2019). Bad obstetric history (BOH) implies previous unfavourable foetal outcome in terms of two or more consecutive spontaneous abortions, history of intrauterine foetal death, intrauterine growth retardation, stillbirth, early neonatal death and/or congenital anomalies (Namrata Kumari et al., 2011). The causes of BOH may be genetic, hormonal, abnormal maternal immune response, maternal infection and anatomical (Nickerson et al., 2012). Common established causes include uterine anomalies, antiphos pholipid syndrome, hormonal and metabolic disorders and cytogenetic abnormalities. Other etiologies have been proposed but are still considered controversial, such as chronic endometritis, inherited thrombophilias, luteal phase deficiency and high sperm DNA fragmentation levels (Hachem et al., 2017). However, an increasing risk of fetal loss with increasing maternal age has been documented in women aged more than 30 years (Andersen et al., 2000). Primary infection caused by TORCH (Toxoplasma Gondi, Rubella virus, Cytomegalo virus and Herpes simplex virus) are the main cause of BOH (AlHilli et al., 2014). Gestational diabetes mellitus (GDM) forms the most common medical complication of pregnancy (Ambroise et al., 2000).

Oxidative stress (OS), which is defined as an imbalance between pro-oxidants and antioxidant capacity, has been implicated in suboptimal reproductive performance from the earliest stages of development to labour and delivery. Oxidative stress occurs when there is an imbalance between the production of free radicals and the body's ability to counteract their damaging effects through neutralization with antioxidants (Poston et al., 2011). Oxygen radicals and Reactive Oxygen Species (ROS) play both a physiologic and pathologic role in the female reproductive tract. It includes various mechanism like lipid damages, inhibition of protein synthesis and depletion of ATP. There by it affects physiological function in female reproduction such as oocyte maturation, ovarian steroidogenesis, ovulation, implantation and embryo development (Ruder et al., 2008).

ROS generation is mainly due to electron leakage from the mitochondrial membrane, but also due to exogenous exposures such as smoke and environmental pollutants. At higher levels, OS can cause indiscriminate damage leading to loss of function and even cell death. Several structures such as membranes, proteins, lipids and nucleic acid are prone to damage by superoxide ions (Kong et al., 2010). Lipid degradation occurs, forming products such as malondia ldehyde (MDA) and ethane that are commonly measured as end products of lipid peroxidation. Lipid peroxidation is of particular significance in miscarriage which plays a key role in the pathogenesis of subfertility in both males and females (Alahmar et al., 2019). Pregnancy complications such as spontaneous abortion, recurrent pregnancy loss and preeclampsia, can also develop in response to OS (Agarwal et al., 2012). Recently, OS has been reported to have an important role in the normal functioning of the female reproductive system and in the pathogenesis of female infertility (Bedaiwy et al., 2002). Due to the formation of large number of ROS, breaking of DNA (double strand breaks) in sperm and oocytes occurs which may leads to BOH.

BOH is the common complication of pregnancy, affecting

approximately 15% of all clinically recognized pregnancies in the general population. Perinatal mortality remains a challenge in the care of pregnant women worldwide, particularly for those who had history of adverse outcome in previous pregnancies. The risk factor for pregnancy is increasing day by day. Recent studies showed that environment, genetic and life style factors contribute to BOH, still the relation between DNA damage related BOH is unclear. Hence the present study was undertaken to assess the evidence of increased oxidative stress and DNA damages in couple experiencing BOH. The specific objectives are to estimate the level of oxidative stress and the extent of somatic DNA damages, if any, in couples experiencing BOH by, Cytokinesis-block micronuclei (CBMN) assay in couples experiencing BOH.

MATERIALS AND METHODS

Twenty couples suffering from varying degrees of bad obstetric history were selected as the test subjects and fifteen healthy age matched couples with one or two live children were selected as control subjects for the present study. Study subjects were referred from various infertility centres of Kerala to Genetika, Centre for Advanced Genetic studies, Trivandrum. Detailed demographic, physiological and life style characteristics of the subjects were recorded using proforma. Eight ml of venous blood was collected aseptically from all the subjects by venepuncture. Cytokinesis Block Micronuclei (CBMN) assay to quantifying the extent of somatic DNA damages and Peripheral blood lymphocyte culture (PBLC) was carried out to evaluate the chromosome aberrations, if any, among these subjects. Malondialdehyde (MDA) was performed based on the reaction of malondial dehyde (MDA) with thiobarbituric acid (TBA); forming a MDA- tba2 adduct according to a modified version of Sato (1979) methods. The physiological characters like height, weight, BMI, obesity, etc were recorded and other clinical parameters like Family history of (FH/o) infertility, H/o Diabetes, H/o Hypertension, H/o Dyslipidemia, H/o Chronic illness, H/o Thyroid disorder, H/o Endometriosis, H/o UTI and H/o infections were also evaluated. History of X-ray exposure, H/o drug intake, consanguinity, smoking habit, alcohol consumption, water intake per day and dietary pattern were also included as lifestyle characteristics.

OBSERVATIONS AND RESULTS

The age of the female study subjects ranged from 22 to 41 years with a mean age of 28.1. The age of the male study subjects ranged from 29 to 51 years with a mean age of 35.95. The duration of married life of these sub-fertile subjects ranged from 1 to 16 years with a mean duration of 4.6 years. The number of previous abortion of study subjects reported as 1 to 5 with a mean of 2.65.

Oxidative stress marker (MDA), showed a statistically significant increase among the study subjects than the control subjects. Subjects with increased age, birth order, increased duration of married life, delayed menarche, irregular menstruation, uterine abnormalities, etc. showed increased MDA value than the subjects without these characteristics. The mean CBMN frequency was also found higher in couples with increased age, increased duration of married life, increased number of pregnancies, increased number of previous abortions, PCOS, uterine abnormalities, smoking, alcohol consumption etc.

Out of the 20 female study subjects 3 showed abnormal karyotype and out of 20 male study subjects, 4 showed abnormal karyotype. Subjects with abnormal karyotype showed increased MDA value and mean CBMN frequency. Moreover, subjects with increased hormone level (FSH and LH) also showed increased MDA value and mean CBMN frequency. Similar result was observed among subjects with TORCH infections. Male study subjects having the unhealthy lifestyle habits and abnormal semen parameters showed increased MDA value and mean CBMN frequency. The incidence of abnormal karyotypes was also higher among subjects with abnormal semen parameters.

DISCUSSION

Overall incidence of BOH in literature showed large etiological heterogeneity. Age, obesity and high parity have been shown to be independent risk factors for RPL and stillbirth and incidence of BOH was found to be 5.27%. Another term related to BOH is RPL (recurrent pregnancy loss) or Habitual Abortion is a distinct disorder defined by two or more failed clinical pregnancies, and up to 50% of RPL will not have a clearly defined aetiology.

A large prospective study conducted by Andersen et al., (2000) reported that, the risk of a spontaneous abortion was 8.9% in women aged 20-24 years and 74.7% in those aged 45 years or more. High maternal age was a significant risk factor for spontaneous abortion irrespective of the number of previous miscarriages. The risk of an ectopic pregnancy and stillbirth also increased with increasing maternal age (Andersen et al., 2000). The present study was conducted in 20 couples and it was observed that the mean CBMN frequency gradually increases with increased maternal age as well as paternal age. The mean CBMN frequency of women with below the age of 30 was 11.6 and above the age of 40 showed a mean CBMN frequency of 12.79. Paternal age is also a risk factor for recurrent fetal loss. Highest mean CBMN frequency showed in subjects above 33 years of age (13.24) and comparatively lower for those below 33 years (11.59).

An increasing risk of fetal loss with increasing maternal age has been documented in women aged more than 30 years in a prospective study by Singh (2010). At 42 years of age, more than half of all pregnancies resulted in a spontaneous abortion, ectopic pregnancy or stillbirth. After three or more spontaneous abortions, the proportion of pregnancies ending in spontaneous abortion increased to 44.6% in nulliparous women and 35.4% in parous women. In the present study number of abortions increased with increased age in the study subjects.

Cytogenetic analysis of previous miscarriages is an important component in the assessment of the couples with BOH. Identifying a cytogenetic cause for BOH can be psychol ogically important to overcome grief and loss. According to Anderson et al., 2000 there are many reasons for spontaneous abortion including chromosomal abnormalities, maternal age, medical-illness, infections etc among these chromo somal abnormalities it is believed to be the most common etiological factors behind spontaneous abortion and may account for up to 50% of miscarriages.

In a study conducted by Cigaril et al., (2005) reported that, catalase (CAT), superoxide dismutase (SOD) and lipid peroxide (LPO) levels were increased in pregnant women compared with non-pregnant women. CAT, SOD activities and LPO levels were increased from the rst trimester to the third trimester in pregnancy without UTI. However, CAT and SOD activities were decreased; LPO levels were increased from the First trimester to the third trimester in pregnancy with UTI. Pregnancy causes oxidative stress and also UTI during pregnancy may aggravate oxidative stress (Ciragil et al., 2005). In the current study an increased mean CBMN frequency along with peaked percentage of karyotype was observed in subjects with history of UTI.

According to a study done by Ghneim (2016), the levels of MDA production, were significantly increased in RM patients when compared to those in healthy patients (HP) women. Similar trend was observed in the placental tissue MDA levels of RM patients were significantly increased when compared to those obtained for HP women (Ghneim et al., 2016). MDA levels increased significantly in the women with spontaneous

abortion compared to the healthy pregnant women (Torkz ahr ani et al., 2019).

Generation of ROS is a consequence of metabolically active cells and it is likely that threshold levels of oxidative stress exist for promoting conception. The best available evidence suggested a varied diet with regular use of multivitamins, limited in caffeine and alcohol and maintenance of a healthy body weight may promote fertility (Ruder et al., 2008).

OS plays in modulating a range of physiological functions and its role in pathological processes affecting the female reproduction. OS modulates a host of reproductive pathol ogies affecting natural fertility in a woman's life and also menopausal transition and post-menopausal years. The role of OS is becoming increasingly important as there is new cumulative evidence suggesting that oxidative stress is involved in conditions such as abortions, preeclampsia, hydatidiform mole, fetal teratogenicity, preterm labor and intrauterine growth retardation, all of which lead to an immense burden of maternal and fetal, morbidity and mortality (Gupta et al., 2009). In the present study, MDA levels increased significantly in the study subjects compared to control subjects. Thus significant MDA level indicates increased lipid peroxidation which leads to increased oxidative stress in the study subjects.

It has been reported that hyper-secretion of basal LH with or without polycystic ovaries is a risk factor for miscarriage. Raised follicular phase serum LH levels increase the risk of miscarriage following either spontaneous conception or assisted conception. Elevated urinary LH excretion has been reported in 57% of women with recurrent miscarriage. Deleterious effects of high LH can be reversed by LH suppression using gonadotropin-releasing hormone analogs (Kaur et al., 2016). Endocrine disorders play a major role in approximately 8% to 12% of recurrent pregnancy loss (RPL). Endocrine abnormalities, including thyroid disorders, luteal phase defects, polycystic ovary syndrome, hyperpro lact inaemia and diabetes have to be evaluated in any case of RPL. Moreover, elevated androgen levels and some endocr ino logical aspects of endometriosis are also factors contributing to RPL (Pluchino et al., 2014).

According to Trout (2000), women with unexplained RPL have a greater incidence of elevated serum FSH and E (2) -Estradiol levels. When combined, FSH or E (2) levels, or both, were elevated in 58% of the unexplained RPL group and 19% of the control group. According to the present study FSH and LH levels are observed to be higher in study subjects with increased percentage of abnormal karyotype than control subjects.

CONCLUSIONS

It is important to understand the ways in which lifestyle behaviours may benefit or harm fertility in order to minimize complications and to maximize fertility outcomes. By understanding the impact of lifestyle on reproductive health, and by actively modifying lifestyle behaviours, men and women are capable of controlling their own fertility potential. The study demonstrated a positive correlation with karyotypic abnormality and various risk factors associated with BOH. The diagnosis of chromosomal anomalies at the exact time can lead to prevention of future birth of affected babies also pregnancy loss due to suspected genetic loss can also be reduced in the society to an extent. Further research is required into the mechanisms responsible for and also preventing the DNA damage including antioxidant therapy.

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