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EVIDENCE OF INCREASED PSYCHOLOGICAL STRESS AND SOMATIC DNA DAMAGE AMONG WOMEN WITH RECURRENT ABORTION

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ABSTRACT

Recurrent abortion refers to the loss of two or more pregnancies before 20 weeks of gestation. Recurrent abortion could be because of environmental, physiological, endocrinological or biochemical factors. Perceived stress scale (PSS) is the most widely used psychological tool to scale the perception of stress and is a measure of the degree to which situations in one's life are appraised as stressful. However, the role of psychological stress in recurrent abortions has not been examined properly. Hence, the present study was conducted to evaluate the perceived stress in women with recurrent abortions with no known risk factors for obstetric complications and to determine demographic and pregnancy-related factors associated with stress. In the current study 136 women with recurrent abortions were included in the test group and 110 healthy women with one or two children were selected as control subjects. Perceived stress scale (PSS) was employed to assess the women's stress status and correlate it with various demographic, physiological and clinical characteristics of the study subjects.Cytokinesis-block micronuclei (CBMN) assay was also performed to quantify the extent of somatic DNA damages among subjects with recurrent abortions. Women with recurrent abortion showed a statistically significant increased score of PSS and CBMN frequency than the control subjects.

Keywords:DNA damage,Recurrent abortion, Perceived stress scale, Psychological stress, Cytokinesis-block micronuclei (CBMN) assay

INTRODUCTION

American Society for Reproductive Medicine (2013) defined that, "recurrent abortion refers to the loss of two or more pregnancies before 20 weeks of gestation". Varma and Gupta (2012) estimated that, "pregnancy loss is a common problem, with miscarriage estimated to affect 25% of women who have been pregnant by 39 years of age and ectopic pregnancy occurring in approximately 1% of pregnancies".Adib-Rad et al (2020) denoted that, "the etiology of RPL is often unknown. The loss of a desirable pregnancy is a considerable negative life occurrence and this usual problem may cause notable physical and psychological distress". La et al (2021) suggested that, "the causes of recurrent abortions could be female age, anatomical abnormalities, genetic, endocrinological, placental anomalies, infection, smoking and alcohol consumption, psychological factor, exposure to environmental factors".

Mevorach-Zussman et al (2012) explained that, "pregnancy loss is related to anxiety and distress, especially in women who experience recurrent pregnancy loss (RPL)". Maina et al (2008) pointed out that, "stress, anxiety and depression are known to adversely affect

pregnancy outcomes and are associated with small-for-gestational-age infants and long-term neurodevelopmental adverse outcomes".Stergiakouli et al (2014) mentioned that, "the association between psychological stress and miscarriage could result, at least in part, from activation of the hypothalamic-pituitary-adrenal axis by recruitment of hypothalamic neurons which secrete corticotrophin-releasing hormone, increasing pituitary secretion of adrenocorticotrophic hormone secretion and hence of adrenal cortisol".Engidaw et al (2019) explained that, "a number of biopsychosocial risk factors contribute to perceived stress during pregnancy. Important factors among them were a past history of depression, stressful life events and interpersonal conflicts".

According to Sugiura-Ogasawara et al (2013), "the prevalence of depression varies considerably across studies, ranging from 15% to 33%". Surico et al (2015) suggested that, "stress also inhibits pituitary human chorionic gonadotropin secretion compounding the effect of prolactin on progesterone release from the corpus luteum". Gokoel et al (2021) suggested that, several forms of early life stress can predict elevated levels of inflammation, which in turn plays a key role in the pathogenesis of depression". Parker and Douglas (2010) reported that, "multiple effects of certain hormone contribute to the suppression of maternal immune response to the conceptus".Slavich and Irwin (2014) explained that, "depressed adults who experienced severe forms of early life stress were more likely to have high levels of C-reactive protein (CRP) than depressed adults who did not experience these severe forms of early life stress".

Cohen et al (1994) defined that, "the perceived stress scale (PSS) is the most widely used psychological instrument for measuring the perception of stress and is a measure of the degree to which situations in one's life are appraised as stressful". Lee (2012) explained that, "PSS is an easy-to-use questionnaire with established acceptable psychometric properties". Li et al (2012) defined that, "the PSS is a widely used psychological instrument for measuring the perception of stress. It is a 10-item self-report questionnaire and is designed to reflect how unpredictable, uncontrollable and overloaded respondents find their lives". Cohen et al (1983) described that, "the instrument uses a 5-point Likert scale (0 = never, 4 = very often). Scores can range from 0 to 40, with higher scores indicating a higher level of perceived stress".

Agarwal and Allamaneni (2004) have suggested that, "oxidative stress plays a major role in the normal functioning of the reproductive system and subsequent pathogenesis of female infertility. Large concentrations of ROS can evoke oxidative DNA damage, DNA strand breaks and chromosomal aberrations in sperm and oocytes, that may lead to recurrent miscarriages". According to Carrell et al (2003) and Bernardini et al (2004), "subjects suffering from repeated pregnancy loss may show a significant increase in chromosome aneuploidy, abnormal chromatin condensation, DNA fragmentation, high apoptosis, and abnormal sperm parameters compared with fertile individuals". Furness et al (2011) denoted that, "higher levels of DNA damage are detected among women with complicated pregnancies".Oxidative stress can cause extensive oxidative damage to DNA, DNA strand breaks & chromosomal aberrations. Whenever there is any failure in the normal repair process, and when cellular apoptosis does not occur, irreparable DNA damages may occur. Kurthkoti et al (2008) explained that, "unrepaired DNA damages cannot be tolerated in mammals and may have serious consequences and is vital in maintaining the integrity of genetic blueprint". Fenech (1993) explained that, "technical developments (e.g. chromosome painting) allow the measurement of unequal distribution of chromosomes within daughter nuclei in cytokinesis-blocked binucleate cells". Moreover, Fenech (1993) proposed, "Cytokinesis Block Micronuclei (CBMN) assay for measuring the extent of somatic DNA damage".The cytokinesis-block micronucleus cytome (CBMN cyt) assay is a new and comprehensive technique for measuring DNA damage, cytostasis, and cytotoxicity in

different tissue types, including lymphocytes. DNA damage events are scored specifically in once-divided binucleated cells. the CBMN method has evolved into an efficient "cytome" assay of DNA damage and misrepair, chromosomal instability, mitotic abnormalities, cell death and cytostasis, enabling direct and/or indirect measurement of various aspects of cellular and nuclear dysfunction (Fenech, 2007).

Recurrent abortion could be because of environmental, physiological, endocrinological or biochemical factors. Health behaviors and lifestyle patterns of women with history of recurrent miscarriage are of great concern. For the proper management of a new pregnancy among the couples suffering with recurrent abortion needs a deeper understanding and detailed insight into the etiology especially, in a customized way. However, the role of psychological stress and recurrent abortions has not been extensively studied. Hence the present study was conducted to evaluate the perceived stress in women with recurrent abortions with no known risk factors for obstetric complications and to determine demographic and pregnancy related factors associated with stress.

MATERIALS AND METHODS

A total of 136 women with recurrent abortions were included in the test group and 110 healthy women with one or two children were selected as control subjects. Detailed demographic, clinical and lifestyle characteristics were recorded using well-structured proforma. Perceived stress scale (PSS) was employed to assess the women's stress status. Four ml of venous blood sample was collected by venipuncture and transferred to sodium heparinized vacutainer for quantifying the extent of somatic DNA damages by cytokinesis-block micronuclei (CBMN) assay (ref..)from the study subjects after getting informed consent.

OBSERVATIONS AND RESULTS

The age of the test subjects ranged from 18 to 45 with a mean age of 28.58. The age of the control subjects ranged from 18 to 45 with a mean age of 28.04 ($t=0.472$; $p=0.318$).The observed mean CBMN frequency of test subjects was 12.54±1.61 and for control it was 10.12±0.73. Moreover, a statistically significant difference was observed between the mean CBMN frequency of test and control subjects ($t = 14.56$; $p = <0.05$).

PSS score was categorized into three groups viz. 1) low stress (scale: 0-13), 2) moderate stress (scale: 14-26) and 3) high perceived stress (scale: 27- 40). Majority of the test subjects 63% (n = 86) revealed high perceived stress, followed by 36% (n = 49) with moderate stress and 1% (n=1) revealed low perceived stress score. The mean PSS score of test subjects was 27.47±5.76 and 14.49±3.51for control group. A statistically significant difference of PSS score was observed between the test and control subjects ($t= 20.70$; $p= <0.05$).

Table: 1 Distribution of mean PSS score according to demographic and physiological characteristics among study subjects

Variables	**Category**	**Mean PSS Score**	
		Test subjects (n=136)	**Control subjects (n=110)**
Age (years)	≤20	29.57 (7)	15.57 (7)
	21-30	27.68 (80)	14.46 (66)

	31-40	26.04 (41)	14.36 (36)
	>40	30.87 (8)	13 (1)
Age at menarche (years)	≤13	27.13 (92)	14.36 (85)
	>13	28.20 (44)	14.92 (25)
Obesity	Yes	27.64 (43)	14 (5)
	No	27.11 (93)	14.51 (105)
PCOS	Yes	27.69 (36)	13.92 (14)
	No	26.68 (100)	14.57 (96)
Menstrual periods	Irregular	28.22 (44)	15.33 (3)
	Regular	25.90 (92)	14.46 (107)

The age of the study subjects were categorized as ≤20, 21-30,31-40 and >40 years. Increased PSS score (30.87) was observed among test subjects with >40 years, followed by PSS score of 29.57 among test subjects with ≤20 years. The study subjects were grouped according to their age at menarche, as ≤13 and >13. The study observed that subjects with increased age at menarche showed increased PSS. Among the tested group, subjects with obesity, PCOS and menstrual irregularities revealed increased PSS score.

Table: 2 Distribution of mean PSS score according to clinical parameters and molecular cytogenetic analysis among study subjects

Variables	**Category**	**Mean PSS Score**	
		Test subjects (n=136)	**Control subjects (n=110)**
Major chronic illness	Yes	28.21 (28)	0
	No	27.28 (108)	14.49 (110)
Diabetes Mellitus (DM)	Yes	27.57 (18)	14.28 (16)
	No	26.83 (118)	15.68 (94)
Autoimmune diseases	Yes	28.25 (16)	0
	No	27.37 (120)	14.49 (110)
No. of pregnancies	≤3	26.29 (102)	14.67 (91)
	>3	27.87 (34)	13.63 (19)

No. of abortions	≤3	26.72 (111)	14.49 (110)
	>3	27.64 (25)	0
Mean CBMN frequency	≤12	27.12 (35)	12.33 (107)
	>12	28.48 (101)	14.55 (3)

Subjects with varying degrees of chronic illness like hypothyroidism showed increased PSS score. Out of 136 test subjects, 13.23% (n=18) were reported with diabetes mellitus and these subjects showed increased PSS score than the subjects without Diabetes Mellitus. Autoimmune disease was reported in 11.8% (n=16) of the test subjects and their observed PSS score was 28.25. Test subjects with more than 3 pregnancies showed an increased PSS score. Moreover, test subjects reported with more than 3 abortions also showed an increased PSS score of 27.64. Out of 136 test subjects, 101 subjects were showed an increased mean CBMN frequency. In control subjects only 3 individuals showed an increased mean CBMN frequency and their observed PSS score was 14.55.

DISCUSSION

In present study, 246 study subjects were selected, among these 55.3% (n=136) were test subject with recurrent abortion and 44.7% (n=110) control subjects. Singh and Sidhu (2010) estimated incidence of 5.27%BOH including 1.4%recurrent pregnancy losses and 3.87%with history of unexplained stillbirth or neonatal loss. In the current study, test subjects showed an increased mean PSS score (27.47±5.76) than the control subjects (14.49±3.51). Li et al (2012) reported that the stress is a risk factor for recurrent pregnancy loss(RPL) based on their findings of a significantly higher PSSscore among 45 women with unexplained RPL.

In the current study, test subjects with advanced age (>40 years) and subjects with age range ≤20 years were showed an increased mean PSS score. Bender Atik (2018) defined that, age is a key factor in RPL, which is more common in women above 40 years age. Magnus et al (2019) identified thatthe risk of miscarriage was lowest among women aged 25-29 (10%), rose rapidly after age 30 and reached 53% in women aged 45 or above, concluding that the risk of miscarriage varies greatly with maternal age. In the present study, test subjects reported with age at menarche >13 years showed an increased mean PSS score (28.20) than the control subjects.

Thirty six subjects out of 136 test subjects in the resent study group were reported with obesity and their observed mean PSS score was higher than those without obesity. Omar et al (2020) pointed out 3.6 times higher recurrent miscarriage among obese women as compared with normal weight women and 3.2 times among those aged ≥31 concluding that obesity is significantly associated with increased risk of RPL. Maternal obesity could increase oxidative stress leading to a high risk of maternal complications such as diabetes mellitus in the mother with RPL (Perez et al, 2018).

Current test subjects with major chronic illness reported an increased mean PSS score of 28.21. Magnus et al (2021) reported individuals with illness showed strongest relation to miscarriage risk , preferably with cardio metabolic diseases. The risk of miscarriage was higher if a woman had more than one chronic disease. Significant and consistent association between pregnancy loss and type 2 diabetes was identified by Egerup et al (2020). In the present study, 13.2% subjects reported with diabetes mellitus and their observed PSS score was 27.57. Comparatively an elevated PSS score was observed among the test subjects with diabetic than the others without DM.

PCOS was observed in 36 test subjects and their observed PSS score was 27.69 in the current study.BahriKhomami et al (2019) have observed thatwomen with PCOS have an increased risk of miscarriage compared to those without PCOS. Jakubowicz et al (2002) reported that, "patients with PCOS usually have a high abortion rate of 30– 50% in the first 3 months of pregnancy, a high incidence of recurrent early abortion of 36–82%, and a high incidence of habitual abortion of 58%".

Palm-Fischbacher and Ehlert (2014) reported high level of perceived stress associated with menstrual cycle irregularity. Nagma et al (2015) revealed that, "menstrual cycle irregularity was associated with high chronic stress level. Moreover, past studies that utilized special tools to check the stress level (i.e., >20 in the Perceived Stress Scale or Global Severity Index) of university students also indicated that stress was correlated with irregular menstruation". In the present study, menstrual irregularity was reported in 44 test subjects and their observed mean PSS score was higher than that of others with regular menstrual periods.

Leight et al (2010) estimated the prevalence of depression during pregnancy that can be as high as 16% or more. In the current study, test subjects with no. of pregnancies <3 showed an increased PSS score. Moreover, test subjects with no. of abortions >3 also showed an increased PSS score. Monk et al (2020) reported thatpregnant women were likely to be anxious when there was nobody providing everyday life support. PSS scores 14 was regarded as indicating higher perceived stress. Shangguan et al (2021) have observed thatpregnant women with higher perceived stress (6.87 times more) are likely to be anxious than those with lower perceived stress.

RPL patients are likely to develop depression and anxiety than women with no history of pregnancy loss.Lower education level and multiple pregnancy losses appear to be two independent risk factors of depression and anxiety in women with RPL (He et al ,.2019). Qu et al (2017) pointed out thatthe risk of miscarriage was significantly higher among women with a history of psychological stress. Kolte et al (2015) reported a higher stress level(≥19 on the PSS scale)among women with RPL (41.2%) as compared to controls (23.2%). In the current study, the mean CBMN frequency of test subjects was higher than the control subjects. Moreover, test subjects increased mean CBMN frequency showed a high PSS score (28.48) also.

CONCLUSION

In the current study it was observed that, test subjects with recurrent abortions showed an increased PSS score than the control subjects. These findings revealed that psychological stress also acts as a major factor in recurrent abortion. Moreover, an elevated mean CBMN frequency was also observed among the subjects with recurrent abortions.

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