ORIGINAL RESEARCH PAPER
Volume-9 | Issue-1 | January-2020 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

OXIDATIVE STRESS AND DNA DAMAGES IN POF SUBJECTS AND ITS ASSOCIATION WITH ANTHROPOMETRIC FACTORS

Biological Science

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ABSTRACT

The concept of premature ovarian failure (POF) is the cessation of ovarian function before the age of 40, associated with elevated gonadotropins serum levels (FSH ≥ 40 UI/l) and at least 1%–3% of women of reproductive age were affected and this leads to infertility. The POF etiology is highly disparate including genetic, autoimmune, metabolic and infectious causes, but in most cases the etiology remains as an open question. The role of oxidative stress in the pathogenesis of POF has not studied extensively and there is no such corresponding data that states the association of anthropometric variables and DNA damages in POF subjects. The study was taken to observe the association of anthropometric variables with oxidative stress leading to DNA damage in POF subjects. The present study comprised of 100 subjects with POF and 100 healthy age and sex matched control subjects. Their anthropometric measurements such as height, weight and abdominal circumference were measured and the Body Mass Index (BMI) was calculated. The mean malondialdehyde (MDA) value was taken as the marker for oxidative stress. The DNA damages were analyzed using Cytokinesis Block Micronuclei (CBMN) Assay. The results of the present study support the close association of anthropometric variables in POF subjects with oxidative stress. The study clearly indicates that the study subjects have higher mean value of MDA than the control subjects. Moreover, the DNA damage was also found to be higher among the study subjects. Thus the study explains the strong association of anthropometric variables with oxidative stress leading to increased DNA damages. Therefore the findings of the study confirm the importance of maintaining a healthy life along with physical activity and improved diet providing the antioxidants as well as a proper medication in order to reduce the risk of DNA damage by lowering the oxidative stress levels in POF subjects.

KEYWORDS

Body Mass Index, CBMN Assay, Infertility, Malondialdehyde, Micronuclei frequency, Obesity

INTRODUCTION

Female infertility is an important health and social disorder and one of its causes is premature ovarian failure (POF) which is becoming an increasingly appealing research subject due to its high incidence rate and the absence of an effective treatment [1]. POF is defined as an early ovarian dysfunction characterized by amenorrhea and elevated gonadotropin serum levels before the age of 40 [2]. The median age of natural menopause is around 50, but 9.7% of women experience menopause before 45 (early menopause) and 1.9% under 40 years of age [3].

The increased prevalence of obesity and overweight in the recent years has highlighted the importance of obesity and its associated chronic diseases, such as type2 diabetes mellitus (T2DM), hypertension, and cardiac diseases [4]. In addition, obesity has negative impacts on multiple organs and systems of the body, such as the reproductive system, and is associated with increased risk of disorders in this system [5]. The pathogenetic mechanisms leading to POF are complex and heterogeneous; the causes may be genetic, autoimmune, infectious or iatrogenic, but a large proportionof POF cases still remain idiopathic [6-8].

Oxidative stress had been proposed to be a determinant for apoptosis in a reproductive cell system [9] Increased reactive oxygen species (ROS) levels inhibit follicle growth in antral follicles and antioxidants such as N-acetyl sistein restores ROS levels and protect ovaries from damaging mediated by free oxygen radicals [10]. The production of ROS, although important for physiologic processes, may also induce pathologic conditions. The cyclical production of ROS may result in cumulative over long periods and contributes to increased risk of ovarian diseases such as primary ovarian insufficiency [11]. The outcome of this dynamic equilibrium is usually the induction of oxidatively induced DNA damage and a variety of lesions of small to high importance and dangerous for the cell i.e. isolated base lesions or single strand breaks (SSBs) to complex lesions like double strand breaks (DSBs) and other non-DSB oxidatively generated clustered DNA lesions (OCDLs) [12].

Recently, research works are going on obesity related problems and its association with oxidative stress (OS), but the role of oxidative stress in the pathogenesis of POF has not studied extensively and there is no such corresponding data that states the association of anthropometric variables with oxidative stress and DNA damages in POF subjects. Hence the present study was undertaken to observe the association of anthropometric variables with oxidative stress leading to DNA damage in POF subjects. Therefore in the present study the oxidative stress among the study and control subjects were analyzed by estimating the levels of Malondialdehyde (MDA) and the somatic DNA damages were evaluated by Cytokinesis Block Micronuclei Assay (CBMN Assay).

MATERIALS AND METHODS

The anthropometric measurements, oxidative stress and DNA damages of 100 study subjects diagnosed with POF were compared with 100 healthy control subjects. The age of the study subjects ranged from 18 to 45 years with a mean age of 35.4 years. The samples were referred from various centers of Kerala to Genetika, Centre for Advanced Genetic Studies, Thiruvananthapuram, Kerala. The inclusion & exclusion criteria are as follows:

Inclusion criteria:

- **Study subjects:** Clinically diagnosed POF patients by a Gynecologist were included in the study.
- **Control subjects:** The subjects with any liver or renal diseases, auto immune diseases, cancer and any chronic or acute infections were excluded.

Exclusion criteria:

- **Study subjects:** Those receiving previous treatment, patients above the age of 45 and below the age of 18 years and those diagnosed with ovarian cancer were excluded.
- **Controls:** Those suffering from any acute or chronic illness, cancer or on prolonged medication such as corticosteroids were excluded. Subjects above the age of 45 and below the age of 18 were also excluded.

Ethical Clearance:

- The study was approved by Institutional Ethics Committee

(Human studies) meeting conducted on March 19, 2014 (Reg no. 05/IEC/GTKA) at Genetika, Centre for Advanced Genetic Studies, Trivandrum, Kerala.

Detailed demographic and anthropometric characteristics were recorded using proforma. 2 ml of blood was transferred into a plain tube. Blood was allowed to clot, and the serum was separated immediately. The level of serum biomarker for oxidative stress, malondialdehyde was determined using thiobarbituric acid [13] as main reagent and the values are measured on a semi-autoanalyser at 540nm. In this study, Cytokinesis Block Micronuclei (CBMN) assay by Michael Fenech [14] was carried out in each subject. CBMN assay was performed by using Cytochalasin B for quantifying the extent of somatic DNA damages. 3 ml of blood was transferred into sodium heparinized vacutainers for quantifying the extent of somatic DNA damages. The number of micronuclei of not less than 1000 binucleated cells were scored and the distribution of micronuclei among binucleated cells was recorded. Statistical analysis was performed using the Statistical Package for the Social Science (SPSS) software, version 16.0.

RESULTS:

Table 1: Distribution of Malondialdehyde (MDA) levels among study and control subjects

Distribution of MDA levels in cases and controls are shown in Table 1.The mean values of MDA are significantly higher in cases (3.2 U/L) compared to controls (1.17 U/L) and the difference is statistically significant ($p<0.05$). The mean MDA value of study subjects was greater than healthy control subjects.

Table 2: Distribution of Mean CBMN frequency among study and control subjects

Category	Number	MDA levels (U/L)
Study subjects	100	3.2
Control subjects	100	1.17

The distribution of mean CBMN frequency of the study subjects and control subjects was shown in table 2. The mean CBMN frequency of study subjects and control subjects was 12.88 and 9.83 respectively. The results shows that study subjects have highest mean CBMN frequency than control subjects.

Table 3: Distribution of MDA levels and Mean CBMN frequency according to anthropometric variables among study subjects

Category	Number	Mean CBMN frequency
Study subjects	100	12.88
Control subjects	100	9.83

Distribution of MDA levels and mean CBMN frequency according to anthropometric variables of the study subjects was given in Table 3. While considering the height of the study subjects, the highest mean CBMN frequency (12.78) and MDA levels (3.3 U/L) was found in those with height <1.5 meters. Majority of the subjects have weight ≥60 Kg and they having the highest mean CBMN frequency of 12.9 and increased levels of MDA (3.4U/L). Subjects with BMI >30 Kg/m^2 showed highest mean CBMN frequency of 12.94 and an MDA level of 3.5U/L. Over weight and obese subjects were showed a higher frequency of somatic DNA damage and increased rate of oxidative stress than normal ones. Most of the subjects were having an abdominal circumference ≥88 cm. Subjects with increased abdominal circumference showed highest mean CBMN frequency of 12.97 and increased MDA levels of 3.44U/L.

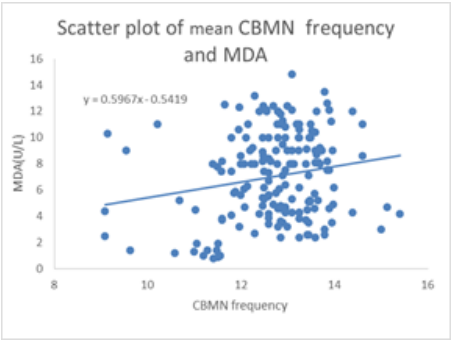
Table 4:Correlation of anthropometric variable with MDA levels and Mean CBMN frequency among study subjects

Variables	Category	Number	MDA levels (U/L)	Mean CBMN frequency
Height (Meters)	<1.5	71	3.3	12.78
	≥1.5	29	3.2	12.65
Weight (Kg)	<60	43	3.23	12.76
	≥60	57	3.4	12.9
BMI (Kg/m^2)	<25	50	2.95	12.81
	25-30	27	3.13	12.88
	>30	23	3.5	12.94
Abdominal Circumference (Cm)	<88	43	3.09	12.76
	≥88	57	3.44	12.97

In table 4, the anthropometric variables of study subjects were correlated with MDA and mean CBMN frequency using Pearson correlation (correlation coefficient) and p value was calculated. A p-value of <0.05 were considered as the level of significance. From the above table; BMI and abdominal circumference showed a positive correlation with MDA and mean CBMN frequency and therefore the study shows that the anthropometric factors are statistically significant.

Anthropometric variables	MDA levels (U/L)		Mean CBMN frequency	
	Correlation coefficient (r)	p	Correlation coefficient (r)	p
BMI (Kg/m^2)	0.397	0.005	0.167	<0.001
Abdominal Circumference (Cm)	0.524	<0.001	0.369	<0.001

Graph 1: Scatter plot of mean CBMN frequency and MDA



Graph 1 demonstrates that as MDA increases mean CBMN frequency will also increases.

DISCUSSION

The exact pathophysiologic mechanism underlying POF still remains unclear. There is no doubt that further investigations are needed for early detection and treatment of POF. There has not been extensive investigation into the role of oxidative stress in POF pathogenesis. Although in the South Indian states, particularly in Kerala, the association between anthropometric variables and oxidative stress leading to DNA damage in POF subjects has not been yet established. The present study was conducted in 100 study subjects diagnosed with POF and in 100 healthy control subjects. The samples were referred from various centers of Kerala to Genetika, Centre for Advanced Genetic Studies, Thiruvananthapuram, Kerala.

POF is a confounding disorder as its aetiology is very heterogeneous and most cases are still idiopathic. However, the incidence of POF patients is estimatedto exceed 30% [15, 16], suggesting a genetic basisfor some cases of idiopathic POF. Cytogenetic analysis on blood lymphocytes derived fromPOF patients is an important tool in the detection of cytogenetic abnormalities that lead to premature ovarian insufficiency [17]. In the present study the findings confirm that the subjects with premature ovarian failure have higher mean CBMN frequency which indicates that the somatic DNA damages were found to be higher among them.

Oxidative stress induces accumulation of lipid peroxide products (MDA, acetaldehyde, *etc*.) and may also alter protein structure which may change its function and cause chemotaxis of inflammatory cytokines. Though physiological free radicals promote oogenesis and follicle formation, excess ROS levels adversely affects female reproductive events [18].

In a study conducted by Manoj et al [19], overall median reactive oxygen species (ROS) range was found to be significantly higher in POF patients when compared to controls. However, 50 per cent of the POF patients had very high ROS levels, 20 per cent had medium elevation and 30 per cent were found to have normal values comparable to controls. In the present study also the MDA levels were found to be higher among the POF subjects than the control subjects. Since a large number of idiopathic cases have raised ROS levels, such cases if diagnosed at an early stage, can be administered antioxidants which may prevent nuclear DNA damage and can also delay or slow down germ cell apoptosis.

Adiposity is associated with reproductive function and potentially also

timing of menopause, yet findings of studies examining overall adiposity (e.g. body mass index [BMI]) and age at menopause have been inconsistent. Some have found higher BMI associated with earlier menopause [20] or ovarian insufficiency [21]. A recent meta-analysis found being overweight or obese and later age at menopause [22].

Adiposity at different ages (e.g. early versus mid-adulthood) may have different effects on early menopause risk, because of changes in reproductive function over time, yet, few studies have directly examined this [23]. In addition, weight distribution is important to consider as it is more closely related to metabolic and cardiovascular conditions than overall adiposity [24]. Furthermore, weight change and weight cycling may be associated with menopause timing, though few studies have considered these [25-27], especially in the context of overall adiposity. In the present study the POF subjects with increased BMI have showed increase in oxidative stress and somatic DNA damages. Thus the present study clearly represents the role of weight and BMI as a risk factor for POF.

It is now well recognized that abdominal fat is a major risk for obesity-related diseases: indeed, visceral fat accumulation contributes to pro-oxidant and pro-inflammatory states, as well as to alterations in glucose and lipid metabolisms [28, 29]. Epidemiological, clinical and animal studies have shown that obesity is coupled with altered redox state and increased metabolic risk [30–35]. Oxidative stress can be a consequence, but also a trigger of obesity. It has also been demonstrated that obesity can induce systemic oxidative stress: indeed, fat accumulation increases NOx activity and endoplasmic reticulum (ER) stress in adipocytes that lead to increased ROS production [36, 37]. At low concentrations, Reactive Oxygen Species and Reactive Nitrogen Species (ROS/RNS) exert a multitude of biological effects, including immune-mediated defense against pathogenic microorganisms and intracellular signaling; conversely, high levels of these extremely reactive species can damage DNA, lipids, and proteins, thus leading to tissue injury and cell death [38]. The present study also clearly demonstrates that the POF subjects with obesity i.e., those subjects with BMI >30 Kg/m^2 have shown an increase in MDA levels and their micronuclei frequency were also found to be higher. Moreover when considering the abdominal circumference, the subjects with abdominal circumference > 88cm showed an increased level of MDA and mean CBMN frequency. Thus the findings of the present study confirmed that the anthropometric parameters are closely related to oxidative stress resulting to DNA damages in POF subjects.

CONCLUSION

Approaches for reducing oxidative stress in obesity involve weight loss, physical activity, diet rich in antioxidant and appropriate medication. Weight reduction decreases malondialdehyde levels and increases antioxidant defenses and thus reduces the risk associated with human obesity. A healthy diet rich in whole grains, fruits, vegetables, legumes, fish and dairy fermented foods will help to maintain weight. Furthermore, women with POF should undergo cytogenetic and ROS level analysis at their early stages. In the present study POF cases have increased levels of MDA and if diagnosed at an initial stage, antioxidants can prevent the DNA damage. The present study therefore shows that anthropometric variable in POF subjects are closely associated with oxidative stress and their predisposition to DNA damage. Thereby, anthropometric variables, oxidative stress and cytogenetic analyses are essential tools in the evaluation of idiopathic POF cases.

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