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Original Research Paper

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EVIDENCE OF INCREASED OXIDATIVE STRESS AND GENETIC ABNORMALITIES AMONG INFERTILE WOMEN WITH POOR OVARIAN RESERVE

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ABSTRACT Poor ovarian reserve (POR) indicates a reduction in the quantity of ovarian follicular pool in women of reproductive age group and is an important cause of infertility. In the present study 40 subjects who were suffering from POR were selected as test subjects and 22 age and sex matched healthy subjects were selected as control. Detailed demographic, physiological, genetic and clinical characteristics were recorded. Out of the 65% of test subjects 85% had normal and 15% had an abnormal karyotype. Lipid peroxidation product, Malondialdehyde (MDA) in the serum was estimated and the observed mean MDA value was significantly elevated in test subjects as compared to control subjects. Biochemical variables (FBS, TC, TG, LDL and HDL) and hormonal parameters (FSH and LH) showed a statistically significant difference between test and control subjects. POR cannot be cured but can be brought into control to an extent by life style modifications, regular exercise and diet along with proper medical support.

KEYWORDS : Poor ovarian reserve, Infertility, Oxidative stress, Genetic abnormalities

INTRODUCTION

"Ovarian reserve defines the quantity and quality of the ovarian primordial follicular pool (Jirge 2016). Poor ovarian reserve (POR) indicates a reduction in the quantity of ovarian follicular pool in women of reproductive age group and is an important cause of infertility in many couples". POR, on the other hand, is a condition that seems closer to Diminished ovarian reserve (DOR). DOR is characterized by poor fertility outcomes even when assisted reproductive techniques are used and represents a major challenge in reproductive medicine. POR may associate with low pregnancy rates irrespective of age and a high pregnancy loss (Khader et al 2013). The incidence of POI (Primary ovarian insufficiency) is 1 in 1,000 women younger than 30 years of age (Jankowska 2017).

5–10% of women with POI could conceive and deliver a child; this ratio lacks confirmation in large-scale population studies; in addition, since POI or POF (premature ovarian failure) describes a spectrum of declining oocyte quality, oocyte quantity, or reproductive potential with different outcomes (Torrealday et al 2017). Esimai et al (2002) reported that 0.3% of women of reproductive age had premature ovarian failure.

The risk factors for the POR can be in various numbers but most of the cases of POF are idiopathic, accounting for 60-80% of total cases [Goswami and Conway (2007)]. According to Agarwal et al (2005), "Oxidative Stress (OxS) influences the entire reproductive lifespan of a woman and even thereafter (i.e. menopause). In another studies by Tranquilli et al (2004) and Loeken (2004) reported that, "OxS plays a role in the etiopathogenesis of endometriosis, polycystic ovarian disease, hydatidiform mole, tubal factor infertility and unexplained infertility". According to Webster et al (2008) "pregnancy complications such as miscarriage, recurrent respiratory loss (RPL), preeclampsia and intrauterine growth restriction, IUGR, are all linked to OxS". The causes of POR may be genetic, autoimmune, environmental factors, nutritional habits, menstrual periods, conditions of hormonal imbalance, endometriosis and many more. There are number of risk factors associated with POR which can cause miscarriages, multiple abortions and is considered to be a cause of infertility in many women. Even though the pathophysiology behind the POR condition is still remains unclear. The association of OxS and genetic abnormalities in POR has not been studied extensively. Therefore, an attempt is made to investigate the role of oxidative stress and genetic abnormalities among subjects with POR.

MATERIALS AND METHODS

In the present study, 40 women who were suffering from POR were selected as test subjects and 22 age matched healthy women were selected as control group. Detailed demographic, physiological, genetic and clinical characteristics were recorded using proforma, after getting informed consent. These subjects were referred from various infertility clinics and maternity centers of Kerala to Genetika, Centre for Advanced Genetic Studies, Trivandrum, Kerala. The venous blood (5ml) was collected by venepuncture and 2 ml of blood was transferred into vacuutainer containing sodium heparin as anticoagulant for Chromosome analysis (karyotype). And the remaining blood was allowed to clot, serum separated immediately. Biochemical investigations (FBS, Lipid Profile), Hormonal analy1sis (FSH, LH) and Malondialdehyde (MDA) were quantified.

RESULTS

Among the test group, 85% (n=40) subjects had normal karyotype and 15% (n=6) showed an abnormal karyotype. The abnormal karyotype include 46,XX/45X, 46,XX,der(14;21) and 45,X/47,XXX. Ages at menarche of the subjects were grouped into three categories as <13 years, >13 years and not attained menarche. All subjects with primary amenorrhea (not attained menarche) reported abnormal karyotype and 33.4% of abnormal karyotype was observed among subjects who attained menarche at the >13 years. Increased incidences of abnormal karyotypes were also observed among test subjects with irregular menstrual periods.

The observed MDA concentration among test subjects was 2.77μmol/L and for control it was 1.24μmol/L ($p<0.05$; $t=9.64$). Biochemical variables (FBS, TC, TG, LDL and HDL) and endocrinological parameters (FSH and LH) showed a statistically significant difference between test and control subjects. The distribution of MDA according to various biochemical and hormonal parameters were described in Table 1.

Table 1: Distribution of MDA according to biochemical and hormonal parameters

Variables	Category	Number	MDA
Fasting Blood Sugar (FBS)	≤110	23	2.43
	>110	17	3.24
Triglyceride (TG)	≤150	11	2.74
	>150	29	2.78
HDL-C	≤40	37	2.80
	>40	3	2.40
LDL-C	≤100	17	2.66
	>100	23	2.93
Triglyceride (TC)	≤200	24	2.45
	>200	16	3.24
Follicle Stimulating Hormone (FSH)	≤21.5	16	2.74
	>21.5	24	2.79
Luteinizing hormone (LH)	≤15	4	2.48
	>15	36	2.80

An increased MDA concentration was noted among test subjects who not yet started menstrual period. Test subjects without regular exercise showed an increased MDA concentration (2.92μmol/L) than that of test subjects with regular exercise. POR women with characteristics like irregular menstruation, H/o Thyroid disorder and obesity showed increased MDA concentration.

DISCUSSION

Poor ovarian reserve (POR) indicated a reduction in the quantity of ovarian follicular pool in women of reproductive age group and is an important cause of infertility in many couples. Gu and Xu in 2020 described that, "POF is a condition of primary or secondary amenorrhea with elevated level of serum gonadotropin and decreased level of estrogen". The condition of DOR is affects 10% of women seeking fertility treatment and is much more prevalent than condition of POR (Ashish et al 2016). In the present study 40 subjects who were suffering from POR were selected as test subjects among them 15% of study subjects showed abnormal karyotype.

Veena Bhaskar et al (2008) suggested that, "MDA is an end product of lipid peroxidation which can be used as a biomarker to measure the level of OxS". Veena Bhaskar et al (2008) reported that, "an elevated MDA levels in serum of infertile women, than in fertile women signifies that the oxidative damage in infertile women". In the current study, an elevated MDA concentration was observed among test subjects (2.77μmol/L) when compared to the control group (1.24μmol/L).

Sun et al (2008) explained that, "ovarian insufficiency varies significantly for women aged 30-45 years". Another study conducted by Bentov et al (2010) reported that, "aging and age-related pathogenesis are associated with loss of mitochondrial function, mainly due to accumulation of mt-DNA mutations and deletions and these may also lead to POF". The present study also observed a positive correlation between advancing age and concentration of MDA. In 2017, Verit et al reported that, "advanced maternal age, diminished ovarian reserve, endometriosis, endocrine abnormalities and OxS have been identified as possible etiological factors for unexplained infertility".

Collins and Rossi (2015) suggested that, "Body mass index (BMI) and weight are closely related to reproductive function". Buyuk et al (2011) stated that, "overweight and obese women with DOR as defined by high day 3 serum FSH levels have lower serum AMH levels and number of oocytes retrieved compared with non-obese women with DOR". In the current study, 30% of test subjects were reported with obesity. Moreover, the test subjects with obesity had an increased MDA level than the subjects without obesity.

Chavarro et al (2007) also reported that, "The Nurses' Health Study found that vigorous exercise for a minimum of 30 minutes a day was also associated with a decrease in ovulatory disorder infertility". The result of present study test subjects without regular exercise showed an increased MDA concentration (2.92μmol/L) than that the test subjects with regular exercise (2.45μmol/L).

Dong et al (2001) suggested that, "OxS biomarkers have been found in various sites in the female reproductive tract, suggesting their role in various physiological functions". In the present study, test subjects reported with clinical parameters (H/o Diabetes, H/o Dyslipidemia and History of chronic illness) showed an increased MDA concentration than the rest.

Ashish Sharma et al (2016) study proved that, "Deletion X-chromosome may leads to POF. These POF patients having, FSH: 93.0±31.91 and when compare with Turner syndrome, both have almost same height, very high FSH levels as compared to other patients of POF". Van Asselt et al (2004) in his Dutch study have suggested that, "the involvement of the X chromosome may not be limited to POF but may influence the broader spectrum of menopausal age". In the present study, out of the 40 test subjects 85% had normal and 15% had an abnormal karyotype. Among the test subjects, individual having 46,XX/45X, 46,XX,der (14;21) and 45,X/47,XXX abnormal metaphase patterns were observed. Holland in 2001 mentioned about, "the association between trisomy X and POF". Goswami et al (2003) estimated that, "3.8% of patients with POF had the triple X syndrome". POF has also been reported in a girl with 48XXXX in work done by Rooman et al (2002). Goswami and Conway (2005) reported about the association of POF and X chromosome deletions or X-autosomal translocations.

CONCLUSIONS

Women with POR are at higher level of infertility risks. Although, most of the lifestyle factors, demographic parameters and physiological characteristics discussed in this study have variable effects on POR. An increased incidence of abnormal karyotype was observed in female subjects with POR. Increased OxS was observed in female infertile subjects with POR. OxS resulting from diversified factors internally or externally leads to severity of the problem and subsequent inflammation. By understanding of various underlying molecular mechanisms among POF women may help to develop more precise ways to reduce the magnitude of OxS. Healthy lifestyle factors, including exercise, are associated significantly with reduction of OxS. Even though, the condition cannot be cured but can be brought into control to an extent by life style modifications, regular exercise and diet along with proper medical support.

REFERENCES:

[1] Agarwal, A., Gupta, S. and Sharma, R., 2005. Oxidative stress and its implications in female infertility–a clinician's perspective. Reproductive biomedicine online, 11(5), pp.641-650.

[2] Ashish Sharma, Tarsem kumar, Rima dada., 2016. Cytogenetic Analysis of Premature Ovarian Failure Patients. International Journal of Anatomy and Research, Int J Anat Res 2016, Vol 4(4):3030-35. ISSN 2321-4287.

[3] Bentov, Y., Esfandiari, N., Burstein, E. and Casper, R.F., 2010. The use of mitochondrial nutrients to improve the outcome of infertility treatment in older patients. Fertility and sterility, 93(1), pp.272-275.

[4] Buyuk, E., Seifer, D.B., Illions, E., Grazi, R.V. and Lieman, H., 2011. Elevated body mass index is associated with lower serum anti-mullerian hormone levels in infertile women with diminished ovarian reserve but not with normal ovarian reserve. Fertility and sterility, 95(7), pp.2364-2368.

[5] Chavarro, J.E., Rich-Edwards, J.W., Rosner, B. and Willett, W.C., 2007. A prospective study of dairy foods intake and anovulatory infertility. Human reproduction, 22(5), pp.1340-1347.

[6] Dong, Minyue., Shi, Yifu., Cheng, Q. and Hao, M., 2001. Increased nitric oxide in peritoneal fluid from women with idiopathic infertility and endometriosis. The Journal of reproductive medicine, 46(10), p.887.

[7] Esimai, O.A., Orji, E.O. and Lasisi, A.R., 2002. Male contribution to infertility in Ile-Ife, Nigeria. Nigerian journal of medicine: journal of the National Association of Resident Doctors of Nigeria, 11(2), pp.70-72.

[8] Goswami D, Conway GS., 2007Premature ovarian failure. Horm Res. 2007;68(4):196–202.

[9] Goswami, D. and Conway, G.S., 2005. Premature ovarian failure. Human reproduction update, 11(4), pp.391-410.

[10] Goswami, R., Goswami, D., Kabra, M., Gupta, N., Dubey, S. and Dadhwal, V., 2003. Prevalence of the triple X syndrome in phenotypically normal women with premature ovarian failure and its association with autoimmune thyroid disorders. Fertility and sterility, 80(4), pp.1052-1054.

[11] Gu, Y. and Xu, Y., 2020. Successful spontaneous pregnancy and live birth in a woman with premature ovarian insufficiency and 10 years of amenorrhea: A

case report. Frontiers in medicine, 7.
[12] Holland, C.M., 2001. 47, XXX in an adolescent with premature ovarian failure and autoimmune disease. Journal of Pediatric and Adolescent Gynecology, 14(2), pp.77-80.
[13] Jankowska, K., 2017. Premature ovarian failure. Przeglad menopauzalny= Menopause review, 16(2), p.51.
[14] Jirge, P.R., 2016. Poor ovarian reserve. Journal of human reproductive sciences, 9(2), p.63.
[15] Khader, A., Lloyd, S.M., McConnachie, A., Fleming, R., Grisendi, V., La Marca, A. and Nelson, S.M., 2013. External validation of anti-Müllerian hormone based prediction of live birth in assisted conception. Journal of ovarian research, 6(1), pp.1-7.
[16] Loeken, M.R., 2004. Free radicals and birth defects. The Journal of Maternal-Fetal & Neonatal Medicine, 15(1), pp.6-14.
[17] Rooman, R.P., Van Driessche, K. and Du Caju, M.V., 2002. Growth and ovarian function in girls with 48, XXXX karyotype-patient report and review of the literature. Journal of Pediatric Endocrinology and Metabolism, 15(7), pp.1051-1056.
[18] Sun, W., Stegmann, B.J., Henne, M., Catherino, W.H. and Segars, J.H., 2008. A new approach to ovarian reserve testing. Fertility and sterility, 90(6), pp.2196-2202.
[19] Torrealday, S., Kodaman, P. and Pal, L., 2017. Premature Ovarian Insufficiency-an update on recent advances in understanding and management. F1000Research, 6.
[20] Tranquilli, A.L., Bezzeccheri, V., Giannubilo, S.R., Scagnoli, C., Mazzanti, L. and Garzetti, G.G., 2004. Amniotic vascular endothelial growth factor (VEGF) and nitric oxide (NO) in women with subsequent preeclampsia. European Journal of Obstetrics & Gynecology and Reproductive Biology, 113(1), pp.17-20.
[21] Van Asselt, K.M., Kok, H.S., Putter, H., Wijmenga, C., Peeters, P.H., van der Schouw, Y.T., Grobbee, D.E., te Velde, E.R., Mosselman, S. and Pearson, P.L., 2004. Linkage analysis of extremely discordant and concordant sibling pairs identifies quantitative trait loci influencing variation in human menopausal age. The American Journal of Human Genetics, 74(3), pp.444-453.
[22] Veena, B.S., Upadhya, S., Adiga, S.K. and Pratap, K.N., 2008. Evaluation of oxidative stress, antioxidants and prolactin in infertile women. Indian journal of clinical biochemistry, 23(2), pp.186-190.
[23] Verit, F.F., Zeyrek, F.Y., Zebitay, A.G. and Akyol, H., 2017. Cardiovascular risk may be increased in women with unexplained infertility. Clinical and experimental reproductive medicine, 44(1), p.28.
[24] Webster, R.P., Roberts, V.H.J. and Myatt, L., 2008. Protein nitration in placenta-functional significance. Placenta, 29(12), pp.985-994.