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Androgen Excess and Genetic Instabilities in Anovulatory Infertility

KEYWORDS: Anovulation, Androgen Excess, Genetic Instability and Cytokinesis Block Micronuclei Assay

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ABSTRACT *Anovulation is the failure of the ovaries to release ova over a period of time generally exceeding three months. Anovulation is one of the major causes of infertility. Among the causes of infertility, 20-25% accounts for anovulation. The present study was undertaken to evaluate the androgen excess and genetic instabilities in anovulatory infertility by investigating the various anthropometric and clinical aspects of the subjects. Twenty four subjects with anovulatory infertility and 19 healthy women without any chronic illness were involved in this study. Reproductive hormones namely leutinizing hormone (LH) and follicle stimulating hormone (FSH) were estimated in each subjects after obtaining their informed consent. Cytokinesis-block micronuclei (CBMN) assay was also carried out in the lymphocytes of the subjects to assess the somatic DNA damage. The study demonstrated that the micronuclei frequency significantly elevated in the study subjects than control subjects. Anovulatory women with various risk factors such as history of chronic illness, family history of cancer, increased duration of married life, endometriosis, hypercholesterolemia etc. can lead to increased genetic instabilities and the severity of infertility. Lifestyle management should be used as the primary therapy for the treatment of metabolic complications and improvement in ovulatory function and pregnancy.*

INTRODUCTION

The normal function of ovary to releases one ovum every 25–28 days. This average time between ovulation events is variable, especially during puberty and the perimenopause period (StreeRoga, 2010). Anovulation is the failure of the ovaries to produce, mature and release ova. This condition may result from ovarian immaturity or post maturity, altered ovarian functions, primary ovarian dysfunction or disturbed interaction of the hypothalamus, pituitary gland and ovary, caused by stress or distress (Mosby's Medical Dictionary, 2009).

Anovulation or irregular menstrual cycle is the hallmark of polycystic ovary syndrome (PCOS) and remain a central part of the consensus diagnosis. PCOS is probably the most common cause of anovulatory infertility (Rotterdam, 2004; Jakubowski, 2005). PCOS affects 5–10% of women of reproductive age (Mastorakos, 2006). There is high prevalence of PCOS in women with anovulatory infertility (83%) (Kousta, 1999). Androgen excess is one of the most common endocrine disorders of reproductive aged women, affecting approximately 7% of population (Asuncion et al., 2000). Anovulation among regularly menstruating women may be associated with androgen levels and endocrine changes similar to those seen in women with PCOS (Sunni, 2013). The prevalence of PCOS is excess (5%) with the combination of anovulation and hyperandrogenism (ESHRE, 2012). Such ovulatory dysfunction also frequently represents foetal or perinatal exposure of females to androgen excess in many mammalian species.

Anovulatory Androgen Excess (AAE) is a condition in women that usually develops in adolescence and is diagnosed in about five of every 100 women of any race and any country of origin. It is diagnosed by a combination of abnormal cycles (amenorrhea, oligomenorrhea or irregular cycles) and evidences that male like hormones called androgens are either too high or too active causing hirsutism, acne and androgenetic alopecia (Azziz et al., 2009).

Anovulation is one of the major causes of infertility (Padma, 2013). Among the causes of infertility, 20-25% accounts for anovulation. One of the most common identifiable factors that accounted for female infertility was ovulatory disorders (25%). Other reports describe ovulatory disorders are responsible for more than half of the causes of female infertility (Unuane et al., 2011).

Female infertility is also associated with genomic instability. There is a high incidence of genomic instability in lymphocytes of women with PCOS (Milosevic, 2012). Moreover, couples with a history of spontaneous abortions and idiopathic infertility tend to have an increased micronuclei (MN) frequency in lymphocytes (Trkova et al., 2000).

Antioxidants (including vitamins C and E) and antioxidant cofactors (such as selenium, zinc, and copper) are capable of disposing, scavenging or suppressing the formation of reactive oxygen species (ROS) (Ruder, 2009). ROS is also believed to play a role in the different phases of the endometrial cycle. Late luteal phase is characterized by elevated levels of lipid peroxide and a decrease in the antioxidant, superoxide dismutase. ROS stimulates the secretion of Prostaglandin 2F-α *(PG2Fα)* through activation of nuclear factor-kappaα (NFkα). Disruption in physiological levels of ROS leads to female reproductive dysfunction and in

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some cases, to unexplained infertility. Oxidative stress (OS) in female reproduction has been associated with PCOS and endometriosis. These pathologies negatively affect pregnancy rates and in vitro fertilization (IVF) outcomes (Gupta, 2014).

Management of anovulatory infertility is still a difficult medical task because of the difficulty in the diagnosis and treatment. Anovulation can sometimes be treated with medical or surgical induction, but it is the cause of anovulation that will determine whether ovulation induction is possible (Hamilton et al., 2006). There is now a greater focus on the management of the metabolic consequences of anovulatory infertility, primarily through lifestyle intervention to achieve weight loss and increase physical activity. No systematic studies were reported regarding the genetic instability, especially the DNA damage in anovulatory infertility. Hence, the present study was undertaken to evaluate androgen excess and genetic instabilities in anovulaory infertility.

MATERIALS AND METHODS

Twenty four subjects with anovuatory infertility were selected for this study. The samples were referred from various gynecology departments and infertility centers of Kerala to Genetika, Centre for Advanced Genetic studies, Thiruvananthapuram, Kerala. Nineteen healthy subjects without any chronic illness were also selected as control for this study. Detailed anthropometric and clinical characteristics were recorded using proforma. Both the study and control subjects reported normal Karyotype. In this study Cytokinesis Block Micronuclei (CBMN) Assay was also carried out in each subject. CBMN Assay was performed by using Cytochalasin B for quantitating the extent of somatic DNA damages.

Five ml of blood sample was collected by venipuncture and transferred two ml of blood into sodium heparinized vacuutainers for quantifying the extent of somatic DNA damages by cytokinesis-block micronuclei (CBMN) assay. The remaining three ml of blood was transferred into a plain tube. Blood was allowed to clot, serum was separated immediately. Luteinizing hormone (LH) and Follicle stimulating hormone (FSH) were measured by Chemiluminescence Immunoassay (CLIA).

Two ml blood was added to a culture tube containing 10 mL RPMI 1640 supplemented with 100units/mL penicillin, 100µg/mL streptomycin, 15% fetal bovine serum and 100µg/mL phytohemagglutinin. Cytochalasin B was added to the cultures at a final concentration of 4.5µg/mL (Sigma) after 44thhours of initiation of cells with phytohaemagglutinin. Cells were harvested after 72 hr incubation, and they were treated with a hypotonic solution (0.075M KCl) for 1 min and fixed in fresh fixative solution (methanol: acetic acid, 3:1). The cells were dropped onto slides and the slides were air dried and stained with 10% Giemsa. Micronucleated cells were analyzed under light microscopy at 100X magnification. The number of micronuclei in not less than 1000 binucleated cells were scored and the distribution of micronuclei among binucleated cells was recorded.

RESULTS

The subjects were grouped according to their demographic and anthropometric characteristics such as age, birth order, residence, religion, occupation, parental consanguinity, duration of married life, economic status and BMI. Among the 24 study subjects, 14 subjects (58.33%) were belonged to below the age of ≤25 years and showed a mean CBMN frequency of 12.68. The remaining 10 subjects (41.67%) with the age of >25 years showed a mean CBMN frequency of 13.34. The birth order ranged from 1 to 7 and majority of the subjects were belonged to first birth order. The highest mean CBMN frequency (13.8) showed by subjects with >6 birth order. Majority of the study subjects were belonged to rural area (70.83%) followed by coastal area (16.67%) and urban area (12.5%) and the highest mean CBMN frequency was observed in urban area (13.87). Highest mean CBMN frequency showed in the Muslim religion (13.5) followed by Hindu religion (13.23) and Christian religion (12.5). Sixteen study subjects had non-sedentary type of occupation with mean CBMN frequency of 12.82 and only 8 individuals have sedentary type of occupation with mean CBMN frequency of 13.59. Parental consanguinity was reported only in 2 out of 24 study subjects and showed mean CBMN frequency of 13.19. The duration of married life of these subjects ranged from 1 to 5 years with a mean duration of married life of 3 years. Subjects who have >3 years of married life showed highest mean CBMN frequency of 13.35. Those subjects, with high economic status showed highest mean CBMN frequency 13.65. On the basis of BMI, 20 to 25 Kg/m^2 showed mean CBMN frequency of 12.96 and >30 Kg/m^2 showed mean CBMN frequency of 14.2. Highest mean CBMN frequency showed by subjects with BMI of >30 Kg/m^2.

History of chronic illness was reported in 2 out of 24 study subjects with mean CBMN frequency of 13.5. Only one reported family history of cancer among 24 study subjects with mean CBMN frequency 13.25. Only one had endometriosis with mean CBMN frequency of 14.2. Consumption of contraceptive drugs was reported in 3 out of 24 study subjects and their mean CBMN frequency was 13.2. Majority of study subjects (n=20; 83.33%) attained menarche between 13 to 15 years of age and the remaining 4 subjects attained menarche between 16 to 18 years. Those who attained menarche between 16 to 18 years of age showed highest mean CBMN frequency of 13.28.

Normal serum total cholesterol was reported only in 8 (33.34%) study subjects and the remaining 16 subjects were hypercholestrolimic (>200 mg/dL). The mean CBMN frequency of hypercholestrolimic subjects were13.38. The study subjects showed FSH value 31 to 40 mIU/ml had higher mean CBMN frequency (13.53) compared to 11 to 20 mIU/ml (13.03) and 21 to 30 mIU/ml (13.07). Study subject with LH level >80 mIU/ml showed highest mean CBMN frequency of 13.4.

Table 1:- Distribution of mean CBMN frequency according to various demographic and anthropometric characteristics of the study subjects

Category	Variables	Total	Percentage (%)	Mean CBMN Frequency
Age range	≤25	14	58.33	12.68
	>25	10	41.67	13.34
Birth order	1 to 3	20	83.3	12.2
	4 to 6	3	12.5	13.15
	>6	1	4.17	13.8
Residence	Coastal	4	16.67	13.02
	Rural	17	70.83	13.27
	Urban	3	12.5	13.87
Religion	Christian	2	8.33	12.5
	Hindu	20	83.33	13.23
	Muslim	2	8.33	13.5

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Nature of occupation	Sedentary	8	33.34	13.59
	Non sedentary	16	66.67	12.82
Parental consanguinity	Yes	2	8.33	13.19
	No	22	91.67	13.15
Duration of married life	<3	11	45.83	12.68
	3	5	20.83	13.28
	>3	8	33.33	13.35
Economic status	High	2	8.33	13.65
	Medium	21	87.5	13.17
	Low	1	4.17	12.7
BMI (kg/m^2)	20 to 25	11	45.83	12.96
	26 to 30	12	50	13.32
	>30	1	4.17	14.2

Table 2: Distribution of mean CBMN frequency according to various clinical and endocrinological characteristics of the study subjects

Category	Variables	Total	Percentage (%)	Mean CBMN frequency
History of chronic illness	Yes	2	8.33	13.5
	No	22	91.67	13.16
Family history of cancer	Yes	1	4.17	13.25
	No	23	95.83	11.9
Endometriosis	Yes	1	4.17	14.2
	No	23	95.83	13.15
Contraceptive drugs used	Yes	3	12.5	13.2
	No	21	87.5	13.1
Age at Menarche (years)	13 to 15	20	83.3	12.72
	16 to 18	4	16.67	13.28
Total cholesterol (mg/dL)	<200	8	33.34	13.09
	≥200	16	66.66	13.38
Follicle stimulating hormone (FSH) (mIU/ml)	11 to 20	8	33.33	13.03
	21 to 30	9	37.5	13.07
	31to 40	7	29.17	13.53
Luiteinizing hormone (LH) (mIU/ml)	21 to 40	4	16.67	12.95
	41 to 80	19	79.17	13.23
	>80	1	4.17	13.4

DISCUSSION

Anovulation or irregular menstrual cycles are hallmarks of polycystic ovary syndrome (PCOS) and remain a central part of the consensus diagnoses (Zawadzki and Dunaif, 1992; Rotterdam, 2004). PCOS is one of the most common endocrine disorders amongst women of reproductive age. It is characterized by hyperandrogenism, menstrual disturbances, infertility due to chronic anovulation and polycystic ovaries (Sirmans and Pate, 2013). Cytogenetic studies have shown that women with PCOS have increased damage in their genetic material (Moran et al., 2008; Nersesyan et al., 2006; Yesilada et al., 2006).

The present study illustrated that DNA of anovulatory infertile subjects showed significant damage, by increased mean CBMN frequency in lymphocytes, confirming some reports that deal with this issue (Moran et al., 2008). These reports suggest the presence of genetic abnormality in PCOS subjects (Nersesyan et al., 2006; Yesilada et al., 2006; Hamurcu et al., 2010).

In the present study showed increased mean CBMN frequencies with advancing age were consistent with the results of international collaborative projects (Bonassi et al., 2003). In the current study it was observed that micronuclei frequency (MN) was highest in the age group >25 years, there seems to be relation between age and micronuclei frequency.

The increase in genomic damage in obese subjects and the positive correlation between genomic damage and BMI in total over-weight/obese subjects indicate that obesity increases genomic damage (Donmez-Altuntas, 2014). The present study showed BMI >30 kg/m^2 have highest mean CBMN frequency of 14.2.

According to Balen, (2007) the endocrine abnormalities in women with polycystic ovary syndrome include raised concentrations of luteinising hormone (LH; seen in about 40% of women), testosterone and androstenedione in association with low or normal concentrations of follicle stimulating hormone. The present study observed an increased FSH value, increased LH value as well as an increased total cholesterol level. All these characteristics showed a significant increase with mean CBMN frequency among the anovulatory subjects.

Genetic instability can have very serious consequences for PCOS patients. It has been proved that chromosomal abnormalities (structural, numerical) are associated with increased risk of cancer and early miscarriages (Migliore and Coppede, 2002). There are studies supporting the existence of these two phenomena in PCOS patients (Gadducci et al., 2005). In this study a positive correlation exists between the increase in mean CBMN frequency and subjects with family history of cancer. The study subjects with family history of cancer showed mean CBMN frequency of 13.25. It is generally accepted that PCOS is associated with gynaecological malignancies such as endometrial cancer and less with ovarian cancer. The present study showed increased mean CBMN frequency in subjects with endometriosis (14.2).

The cytokinesis-block micronuclei assay revealed increased micronucleus frequency in couples with infertility or two or more spontaneous abortions, suggesting a possible role of chromosomal instability in reproductive failure (Trkova et al., 2000). The current study also observed a higher micronuclei frequency among study subjects than the control subjects.

CONCLUSION

In short, the present study involves androgen excess and genetic instabilities in anovulatory infertility. The distribution of mean CBMN frequency according to demographic, biochemical and endocrinological factors of the study subjects was observed. Age, birth order, parental consanguinity, BMI etc. showed increased level of CBMN frequency. The level of mean CBMN frequency was higher among those who have the family history of cancer and history of chronic illness. Total cholesterol, FSH and LH were also found to be significantly elevated in study subjects. These findings suggest that the women with anovulatory infertility have a high incidence of genomic instability and androgen excess does not cause morbidity or mortality, but it was associated with insulin resistance, dyslipidemia, hypertension, and vascular diseases; therefore, it is a forerunner of cardiovascular disease. The deep rooted origin and detrimental effects of PCOS on the biochemical, reproductive and metabolic functions of the body and its life-threaten-

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ing consequences warns the early recognition and management of this syndrome. It may be impossible to reverse the genetic programming of the syndrome. It can be managed by modifying various lifestyle factors. A wider range of treatment options have become available to infertile couples like medical treatment, surgical treatment and different assisted reproduction techniques.

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