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ORIGINAL RESEARCH

Endocrine and Non-Endocrine Consequences in Women with Polycystic Ovary Syndrome

Mudra H KANSARA[1], Mandava V RAO[1, 2*], Himanshu BAVISHI[3]
[1]Gujarat Genetic Diagnostic Center (GenDiCe), Department of Zoology, University School of Sciences, Gujarat University
[2]Supratech Micropath Laboratory and Research Institute, Ahmedabad, Gujarat
[3]Bavishi Fertility Institute, Nr. Adani CNG Station, Opp Municipal Garden, Ahmedabad-380007, Gujarat
*Corresponding Author email: manvrao@gmail.com

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ABSTRACT
Polycystic ovarian syndrome (PCOS) is the most common hormonal abnormality in reproductive age of women. The features of PCOS include increased androgen production and disordered gonadotropin secretion leading to menstrual irregularity and infertility. In addition, 40-80% of women with this condition are reported to be overweight or obese. In our study of 50 cases with closely matched age groups, we evaluated hormonal levels and non-endocrine factors like serum lipid peroxidation (LPO), superoxide dismutase (SOD), obesity and age. Alteration in hormones in different age groups (20-30, 31-40 and 41-50 years) was detected with changes in LPO and SOD levels comparatively. Similarly, obesity is an indicative of higher BMI values. These altered endocrine, oxidative stress and elevated BMI indicators are associated with this pathological condition. Among preventive measures/strategies, such cases are suggested to undergo regular exercises and promoting healthy lifestyles, based on diet on long term basis as effective therapies or to have metabolic surgery to restore fertility.

KEY WORDS: *PCOS subjects, Age, Hormones, Obesity, Oxidative stress*

Introduction

Female infertility affects an estimated 48 million women with the highest prevalence of infertility affecting people in South Asia, Sub-Saharan Africa, North Africa/Middle East, and Central/Eastern Europe and Central Asia (Mascarenhas *et al*., 2012). There are few medical conditions such as polycystic ovarian syndrome (PCOS), endometriosis, bad obstetric history (BOH), ovarian failure (OF), fibroids, tubal defect etc., which can lead to female infertility in addition to genetic and environmental factors (Papalou *et al*., 2016).

Amongst all these disorders, PCOS is one of the most common disorders among females. It is a complex and heterogeneous endocrine disorder with well-established metabolic abnormalities. The World Health Organization estimates that it affects 116 million women (3.4% of women) worldwide (Vos *et al*., 2012). The prevalence of PCOS in India is from 2.2% to 26% (Shirsath *et al*., 2015). It is an anovulatory cause of infertility affecting 6-10% of premenopausal women (Antoaneta *et al*., 2015). Hyperandrogenism, oligomenorrhea and chronic anovulation are common clinical manifestations of PCOS women (Norman *et al*., 2007). Alterations in several metabolic pathways have been implicated in the pathophysiology of it, including abnormalities in steroid hormone regulation and insulin signaling pathways (Azziz, 2002). Although there is no consensus as to an explanation of the biological mechanisms behind PCOS, its altered hypothalamic-pituitary-ovarian (HPO) axis brings about irregularities of normal hormonal regulation and folliculogenesis (Jonard *et al*., 2004). This condition is able to create

hyperandrogenism, hyperestrogenism and altered gonadotropin levels followed by other related factors like hyperinsulinemia triggering a reduction of sex hormone binding globulin (SHBG) (Barontini *et al*., 2001 ; Lim *et al*., 2016).

Obesity has been linked to abnormal function of the hypothalamic-pituitary-ovarian (HPO) axis through multiple mechanisms that contribute to a development of PCOS. Multiple other growth factors and inflammatory conditions are increased in obese women (Legro, 2012). The imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system produces the oxidative damage leading to oxidative stress (OS). Reactive oxygen species can affect a variety of physiological functions in the reproductive tract, when ROS/RNS increase to pathological levels like in PCOS (Zuo *et al*., 2016). These are capable of inflicting significant damage to cell structures inducing this condition. Hence, we have undertaken biochemical and endocrine analysis in three age groups of PCOS in Gujarat as these people adopt various western lifestyles in addition to other stress factors.

Materials and Methods

Selection of patients

Patients diagnosed as PCOS at Bavishi Fertility Institute, Ahmedabad were selected for the study. The consents were taken from the patients prior to study and the objectives of the study were also explained. A specific proforma was designed to collect the details of personal, medical and reproductive history. In personal history age, age at marriage, information of menstrual cycle, medical history of family, pedigree, weight, height and other information were recorded. These subjects (50) within the age of 20 to 50 years were selected and divided into three age groups i.e. 20-30 yrs, 31-40 yrs and 41-50 yrs. The blood samples were collected and centrifuged at 2000 rpm for 10 min, clear serum was used for the analysis of endocrine and antioxidant indices along with BMI values. This work was approved by local Human Ethical Committee (HEC) of Gujarat University. (GU./HEC001/2015), Ahmedabad.

Hormonal studies

The hormones evaluated in the patients were follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), progesterone (P) and testosterone (T) hormones. This analysis was carried out by Chemiluminescence technique using Architect instrument. The units of these gonadotropins are mIU/ml (Veeresh *et al*., 2015) and units of steroids are pg/ml for estradiol (E2), ng/dl for testosterone (T) (González *et al*., 2012) and ng/ml for progesterone (P) (Phipps *et al*., 2000).

Analysis of SOD and lipid peroxidation (LPO)

The activity of superoxide dismutase (SOD) was assayed by the modified spectrophotometric method of Kakkar *et al*. (1984). Its levels were expressed as units of enzymatic activity per mg of protein contained in the samples (U/mg protein). The thiobarbituric acid reactive species (TBARS) levels were determined by the modified method of Buege and Aust (1978) and the values were expressed in units of nmol/ml.

BMI values

The body mass (weight) and height of an individual were recorded. We calculated the BMI by dividing the body mass by the square of the body height and expressed in units of kg/m^2 (Seleem *et al*., 2014). All these values were statistically analyzed using GraphPad Prism software 5.03. The value of $P<0.05$ is considered significant.

Results

We enrolled 50 PCOS cases followed by 40 subjects in control group ranging in age from 20-50 yrs. and divided them in 20-30, 31-40 and 41-50 yrs in our study. BMI values were increased significantly ($P<0.05$) in our data. Similarly the anti-stress factors like SOD levels were decreased ($P<0.05$), whereas LPO levels were increased ($P<0.05$) markedly in our study, but no difference in average age was noticed (Table 1).

Table 1: BMI and anti-stress indices in control and PCOS groups

Parameter	Control (40)	PCOS (50)
Age (yrs)	29.22 ± 1.55	32.13 ± 1.87 ^{ns}
BMI (kg/m^2)	21.06 ± 0.51	26.66 ± 0.72 *
LPO (nmol/ml)	2.72 ± 0.18	5.45 ± 0.26 *
SOD activity (U/mg protein)	5.75 ± 0.38	2.35 ± 0.14 *

All the values are Mean ± SE. * = p< 0.05; ns = Non Significant

Gonadotropin profiles

The gonadotropin (LH and FSH) levels were altered in three

age groups (20-30, 31-40 and 41-50 yrs) of patients. FSH levels were more in old aged groups, which were significant ((P<0.05) in our study. Similarly, LH levels were also found to be increased (P<0.05) (Figs. 1 and 2).

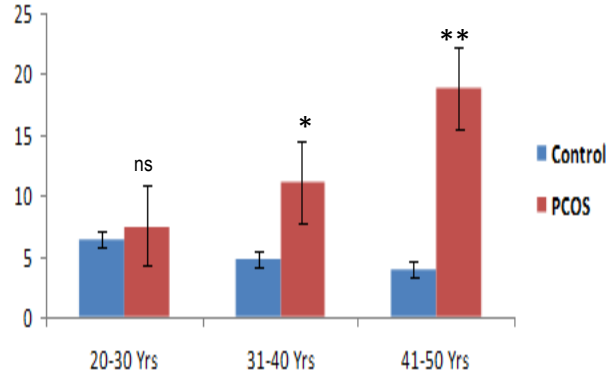

Figure 1: FSH (mIU/ml) profile in controls and PCOS age groups (All the values are Mean ± SE. * = p< 0.05; ** = p< 0.005; ns = Non Significant)

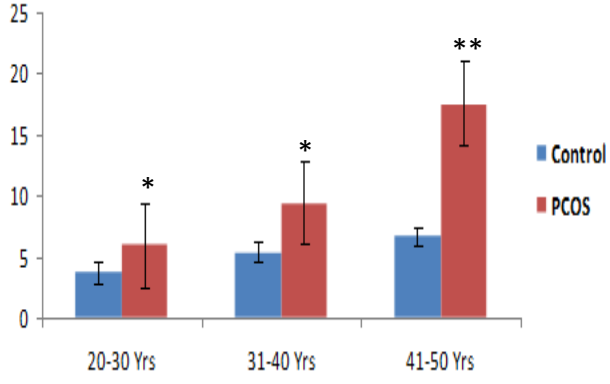

Figure 2: LH (mIU/ml) profile in age groups of controls and PCOS (All the values are Mean ± SE. * = p< 0.05; ** = p< 0.005)

Steroid hormone profiles

Serum E2 levels were higher (P<0.005) in old aged patients. Testosterone levels were also higher (P<0.05) in all age groups. Serum progesterone (P) levels however were not raised in our study (Figs. 3-5).

Discussion

Our study indicated that hormonal milieu was changed, as circulating gonadotropin levels were increased with age, indicating a direct correlation with age in our study, though mean age was not significant with control subjects. Similarly E2 and T levels followed the same condition, where hyperandrogenism and hyperestrogenism were correlated with a function of age. It is a well established fact that HPO axis undergoes changes with an altered secretion of hypothalamic neurons (GnRH) leading to elevated LH and FSH levels which in turn bring about higher levels of steroid hormones in blood of PCOS women. Due to this condition folliculogenesis is affected creating anovulation and menstrual irregulation (Bungum *et al*., 2013; Dasgupta *et al*., 2013). Majority of women with PCOS also experience ovarian dysfunction presenting oligomenorrhoea and amenorrhoea (Teede *et al*., 2010). Hyperandrogenism is a predictor for hyperinsulinemia where glucose metabolism is affected. As a result, insulin resistance brings about disturbed metabolic effects (Duleba, 2012) to support our data. Increased estrogen levels in our study are also explained by conversion of hypothalamic and adipose tissue aromatase from androgen to estrogen in PCOS patients (Legro, 2012 ; Papalou *et al*., 2016) in support of our report.

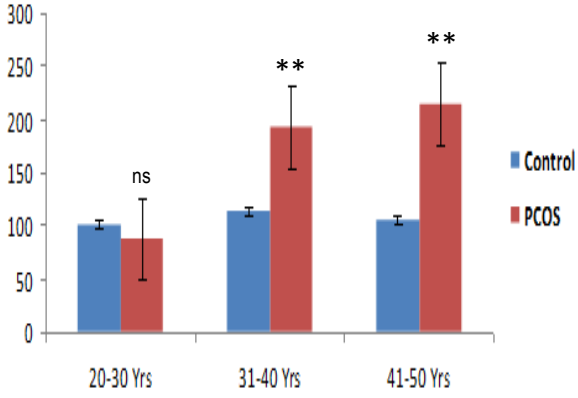

Figure 3: Estradiol (E2) levels (pg/ml) in age groups (All the values are Mean ± SE. ** = p< 0.005; ns = Non Significant)

Further hyperandrogenism initiates metabolic syndrome, hirsutism, cardiovascular risk and obesity in PCOS patients (Benson *et al*., 2008 ; Lim *et al*., 2016). In our population, obesity is also confirmed by higher values of BMI, which is not a cause of PCOS induction. But it is able to exacerbate PCOS along with insulin resistance, metabolic risks, hyperandrogenism and cardiovascular disturbances and reduction in SHBG with cellular inflammation (Teede *et al*., 2010 ; Legro, 2012) in light of our data. PCOS etiology is much unclear, though several factors are involved in its

induction to cause infertility. One of the factors is an increasing oxidative stress in PCOS that induces genomic and mitochondrial DNA damage leading to sterility in these cases. The superoxide dismutase (SOD) is able to catalyze the conversion of superoxide to elevated O_2 and H_2O_2. The latter further gets converted to H_2O by catalase to provide protection to the cells (Tejasvi *et al*., 2014). In our case, its levels were reduced indicating its role in prevention of oxidative stress (OS) in our obese cases

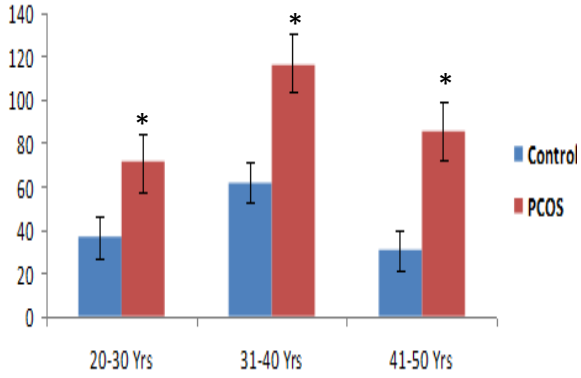

Figure 4: Testosterone (T) levels in all age groups (ng/dl) (All the values are Mean ± SE. * = p< 0.05)

Our results were in agreement with Zhang *et al*., (2008), who support systemic that OS is contributory for this dysfunction. Further OS is substantiated by continuous increase in lipid peroxidation (LPO) levels. LPO levels also called TBARS complexes, a marker of malonyldialdehyde dominated oxidation of polyunsaturated fatty acids (PUFS) in endocrinocytes of obese cases (Shirsath *et al*., 2015 ; Zuo *et al*., 2016 ; Papalou *et al*., 2016).

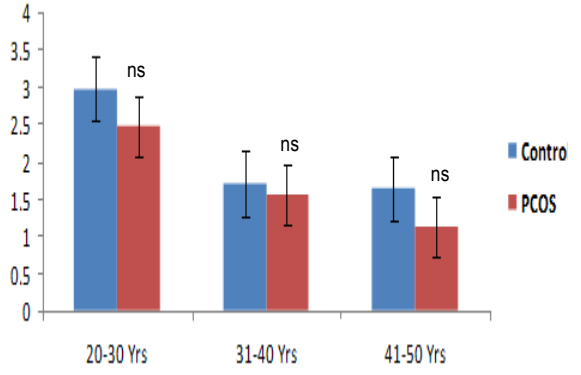

Figure 5: Progesterone (P) profile in all age groups of controls and PCOS (ng/ml) (All the values are Mean ± SE. ns = Non Significant)

This elevated oxidative stress could lead to continuous production of ROS/ RNS that creates cellular damage affecting hypothalamic-pituitary-ovarian (HPO) axis function in subjects of PCOS in comparison to age matched control women in our study (Seleem *et al*., 2014 ; González *et al*., 2012 ; Escobar-Morreale, 2012). Papalou *et al*. (2016) also argued that OS in conjunction with the rest etiologic mechanisms of PCOS results in an adverse redox status and stigmatizes the process of this syndrome.

Our study hence concludes that PCOS is caused by altered endocrine status notably with age, obesity, and systemic oxidative stress in females affecting fertility. This condition is controlled by adopting healthy lifestyles, restricted diet, proper sleep and daily exercises on long term basis (Zuo *et al*., 2016) or to undergo surgery for restoration of fertility depending on severity of obesity in these women.

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