Original Research Article

Polycystic ovarian syndrome (PCOS)- Expression of inflammatory cytokine markers in south Indian population

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Abstract

Polycystic ovarian syndrome (PCOS) has become most common endocrine disorder in the younger women and its etiology is still not clear. PCOS is generally characterized by altered metabolism and irregular menstrual cycles. It is believed that inflammation has a pivotal role to play in PCOS. The present study investigates the expression levels of few inflammatory proteins in PCOS women (normal weight, obese and over weight) including C-reactive protein (CRP), interleukin-6 (IL6), and tumor necrosis factor-alpha (TNFa). The results show chronic low-grade inflammation present in PCO women especially who are obese and overweight. Hence, treating these PCOS women with anti-inflammatory drugs will be ideal way to counter the chronic inflammation process.

Key Word: Polycystic ovarian syndrome (PCOS), C-reactive protein (CRP), interleukin-6 (IL6), tumor necrosis factor-alpha (TNFa)

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INTRODUCTION

Polycystic ovarian syndrome (PCOS) is an endocrine disorder seen in female with multiple clinical manifestations but the etiology remains unknown. PCOS women are subjected to increased visceral adiposity, insulin resistance (IR) and related metabolic. Numerous studies have reported of an inflammatory response in mononuclear cells (MNC) of women with PCOS due to the dietary habits. Gonzalez *et al* (2005) reported about the association between inflammation and insulin resistance in PCOS[1]. Literature review on rise in number of circulating pro-atherogenic inflammatory mediators in PCOS have been independently reported varied scientific groups[2-3] Ross *et al* (1999) suggested the presence of low-grade chronic inflammation in PCOS women and this inflammation plays an important role in endothelial dysfunction and atherosclerosis[4]. C-reactive protein (CRP) is considered as an important marker for inflammation (i.e. Local or systemic). Castell *et al*, (1989) described CRP as an acute-phase reactant produced by hepatocytes in the presence of pro-inflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor-alpha (TNFα)[5]. Interleukin 6 (IL-6) is an endocrine cytokine produced by mononuclear cells and adipose tissue which enhances the synthesis of hepatic C-reactive protein (CRP)[6-9]. In asymptomatic individuals CRP also produced by adipose tissue which is a major predictor of metabolic dysfunction [10-11] and as a mediator of inflammatory processes[12-13]. This study assessed the concentrations of circulating hs-CRP, IL-6 and TNF-α as markers of inflammation in serum of PCOS women and healthy controls.

MATERIALS AND METHODS

Study design: The study was conducted on 300 PCOS and 75 healthy control women, recruited from patients visiting tertiary care hospital. PCOS participants were selected based on observation of oligoamenorrhea/ an-ovulation, clinical or biochemical evidence of hyper-androgenism and/or polycystic ovaries on ultra

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sonography (The Rotterdam Citeria, 2003). Normal, unaffected, age-matched fertile women with regular menstrual cycles (interval of 28-35 days) and with normal ovaries from the same geographical region were included in the study as controls. Exclusion criteria were women with galactorhea, hyperthyroidism, any systemic disease that affects their reproductive physiology, or any medication which interferes with the normal function of the hypothalamic-pituitary-gonadal axis. Participants' age group was in the range from 16-40yrs. The study was approved by the Naithika Independent Ethical Committee (ECR/42/Indt/AP/2013/RR-2016). A written informed consent was collected from all the subjects enrolled in the study. Participant's history and other anthropometric assessments were carried out.

Sample collection and requirement: Blood samples were collected from participants by simple venipuncture and processed within two hours by centrifuging at 3000 rpm for 10-15 minutes at 20°C for isolating serum and later stored at -20°C until further use.

Estimation Of Inflammatory Markers

High sensitive C-reactive protein: hs-CRP is an alpha globulin and plays a role in inflammation. hs-CRP in the serum was measured using the CRP Human ELISA Kit (Thermofisher Scientifics, Cat # KHA0031) by adopting the sandwich ELISA principle.

Interleukin 6: To study the role of IL-6 in the PCOS progression, IL-6 in the serum sample was measured using IL6- human ELISA kit (Thermofisher Scientifics, Cat # EH2IL6). A highly specific IL-6 antibody was coated in the microtitre plate. The IL-6 present in the sample would bind to the specific antibody and subsequently bind to the biotinylated anti-IL-6 secondary antibody. After incubation, the unbound antibody was washed and incubated with Streptavidin-HRP solution for 30 minutes. The samples were further incubated with TMB Substrate solution for 15 minutes and H_2SO_4: stop reagent was added. Absorbance was measured using 450 nm as the primary wavelength and optionally 620 nm as the reference wave length

Tumor necrosis factor α: Tumor necrosis factor α (TNF α) is an important cytokine and plays an important role in the progression of many inflammatory disorders. In patient serum, TNF α was measured using the TNFa-human ELISA kit (Thermofisher scientifics, Cat # BMS223HS) for both natural and recombinant human TNF α.

Statistical analysis: All the data is expressed as mean ± SE. The mean were analyzed by one way ANOVA (with student T-test for comparison with controls). Pearson correlation test was done to see the relationship between control subjects and PCOS (normal weight, obese and overweight)

OBSERVATIONS AND RESULTS

The serum inflammatory markers such as high sensitive C reactive protein (hs-CRP), IL-6, TNF-α were compared between cases and control subjects and are shown in Figures below (1, 2 and 3). The mean hs-CRP levels was higher in 34% of PCOS woman and its level were 7.9 ± 0.05, 9.9 ± 0.04, 9.9 ± 0.6 and 12.3 ± 0.7 among PCOS (normal weight, obese and over weight) and control subjects respectively. The levels differed significantly between the groups ($p<0.001$, Figure 1).

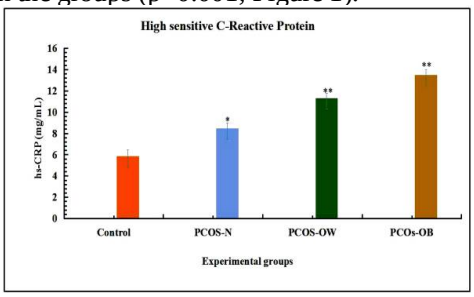

Figure 1: The level of hs-CRP in Control and PCOS normal (N), overweight (OW) and obese (OB). The values were expressed as Mean ± SE (n – control = 75; N = 150; OW= 90; OB = 60). The values were analyzed using one way ANOVA with Dunnet's T- test for comparison with the controls (*p<0.05, **p<0.01, significantly different in comparison to the controls).

The IL6 levels was elevated in 15% of PCOS women and its levels among normal weight, obese, over weight and control subjects were 11.3 ± 1.6, 32.9 ± 8.1, 14.5 ± 6.9 and 9.9 ± 0.4 respectively. The IL 6 levels were considerably increased in all the PCOS cases, with the highest in over weight PCOS women. The IL 6 levels differed significantly between the groups ($p<0.01$, Figure 2).

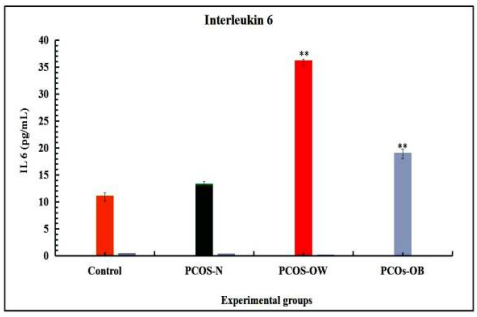

Figure 2: The level of IL6 in Control and PCOS normal (N), overweight (OW) and obese (OB). The values were expressed as Mean ± SE (n – control = 75; N = 150; OW= 90; OB = 60). The values were analyzed using one way ANOVA with Dunnet's T- test for comparison with the controls (**p<0.01, significantly different in comparison to the controls).

Nearly 32% of PCOS women showed raised TNF-α levels and its mean were 159.3 ± 21.3, 302.0 ± 28.9, 202.7 ± 42.5 and 120.0 ± 14.7 among the PCOS (normal weight, obese and overweight) and controls respectively

Figure 3. There was no significant difference among the normal as well as the overweight patients ($p>0.05$), while the levels differed significantly between obese PCOS and controls (*$p<0.05$,**$p<0.01$, Figure 3).

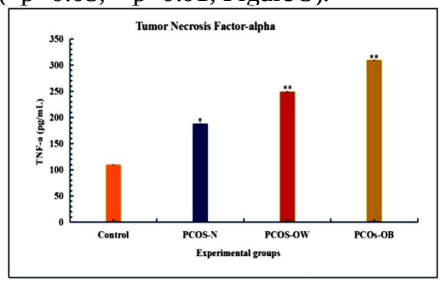

Figure 3: The level of TNFα in Control and PCOS normal (N), overweight (OW) and obese (OB). The values are mean ± SE (n – control = 75; N = 150; OW= 90; OB = 60). The values were analyzed using one way ANOVA with Dunnet's T- test for comparison with the controls (**p<0.01, significantly different in comparison to the controls)

DISCUSSION

PCOS is the common endocrine disorder among the women of fertile age. In women with PCOS, there is evidence of a low-grade chronic inflammation reflected by an increase in circulating levels of these inflammatory mediators. These inflammatory mediators were linked to factors such as metabolic and ovarian dysfunctions, type 2 diabetes mellitus, insulin resistance, cardiovascular diseases, hyper-androgenism and an-ovulation in PCOS women. Atherogenesisis has been linked with chronic local inflammation as a result of increase production of pro-inflammatory mediators into the circulation such as TNF-*α* and IL-6. Van Lente *et al*, (2000) and Yudkin *et al*, (2000) reported about the co-relation between the hs-CRP concentrations and existence of low-grade chronic inflammation in PCOS women[14,15]. Over production of hs-CRP levels are considered as a characteristic feature of obesity[15]. The present study was designed for assessing and quantifying the levels of inflammatory markers such as hs-CRP, IL-6 and TNF-*α* in the serum of PCOS women. The findings from our study reveal that the levels of all three inflammatory markers were elevated in PCOS women in comparison to controls. A statistical significance was observed in the levels of hs-CRP between PCOS and control participants. However, numerous studies demonstrated that there is no association of hs-CRP in PCOS with obesity. While other studies have demonstrated that there is elevation in hs-CRP levels with increases body mass index and the waist circumference in PCOS[13-16]. Our findings do correlate with the existing the literature. Additionally, a positive correlation has been reported among the levels of Dehydroepiandrosterone sulfate (DHEAS) and testosterone with the levels of hs-CRP. The androgens are believed to influence the metabolic and reproductive outcomes in PCOS women. Hence, it would be pivotal in treating the PCOS women by quantifying the levels of testosterone (ovarian) and DHEA-S (adrenal) androgens and predicting the phenotype or severity of the syndrome. A negative correlation has been established between elevated hs-CRP levels and diminished levels of HbA1C, sex hormone binding globulin (SHBG), Follicle stimulating hormone (FSH) and vitamin D. Diminished levels of FSH hormone ceases the growth, maturation of follicles, causes an-ovulation and ultimately infertility in PCOS women. Yang *et al*, (2014) have study in Chinese population and reported about the significant elevation in serum TNF-α levels in PCOS compared to controls, and our findings also correlate with their studies [16]. Piontek *et al*., (2016) suggests positive association between TNF-α and leptin levels and it is further elaborated that increased levels of TNF-α levels directly enhances the levels of fasting blood sugar, HbA1C, DHEAS, SHBG and decreases the levels of Anti-mullerian hormone (AMH), luteinizing hormone (LH), estradiol and vitamin D levels in PCOS women[17]. Interleukin-6 (IL-6), which is one of the major proinflammatory cytokine responsible for chronic inflammation, is believed to be associated with insulin resistance (IR) and cardiovascular dysfunctions[18]. Earlier in vivo studies demonstrated that infusion of human recombinant IL-6 could induce gluconeogenesis, subsequent hyperglycemia, and compensatory hyperinsulinemia. Elevated IL-6 levels and Obesity are associated and considered as a major risk factor for T2DM, therefore, IL-6 must be considered as a biomarker in diagnosis of PCOS, T2DM and cardiovascular diseases PCOS and in there management in PCOS women. But, there are reports which suggest non-correlation between IL-6 levels in PCOS patients[17,18]. There are studies that compared and quantified the levels of IL-6 in PCOS and control women. The present study also compared the levels in overweight, obese and normal PCOS women and the results from our study support the existing literature. Our results are similar to the findings of Tarkun *et al*, 2006 wherein the serum level of IL-6 in PCOS patients was higher than controls[19]. A rise in the high density lipoprotein levels were seen with a rise in the IL-6 levels in PCOS women compared to the controls. When the inflammatory markers were correlated with each other we found that the CRP marker correlated positively with IL-6 marker showing a link between these two markers. CRP has emerged as a major predictor of metabolic dysfunction in asymptomatic individuals, and is also produced by adipose tissue[10,11]. IL-6 is an endocrine cytokine produced by MNC and adipose tissue that is directly responsible for stimulating hepatic C-reactive protein synthesis[6-9].

CONCLUSION

The present study was done in south Indian population and the study reports the positive correlation between PCOS women (Obese and over weight) and inflammatory markers (hs-CRP, TNFa and IL-6). Elevation in inflammatory markers in PCOS women who are obese and weight show high incidence of PCOS associated complication in them. hs-CRP, TNFa and IL-6 levels were higher in women with PCOS compared with BMI-matched controls and that a high serum hs-CRP, TNFa and IL-6 concentrations were related to IR and androgen levels. Therefore, high levels of hs-CRP, TNFa and IL-6 must be considered as biomarkers in the management of PCOS.

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