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Serum C-Reactive Protein and Alpha-1- Acid Glycoprotein levels in women with polycystic ovary syndrome

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Abstract
Background: Polycystic Ovary Syndrome (PCOS) is a common cause of infertility in women of reproductive age group. PCOS is associated with an increased risk of metabolic syndrome, hyperinsulinemia, oxidative stress, inflammation, cardiovascular disease, and cancer. Several adipokines are produced by tissues, such as adiponectin, α1 -acid glycoprotein and leptin. These adipokines regulate glucose and lipid metabolism and insulin action and play important roles in the pathogenesis of IR, obesity, T2DM and cardiovascular diseases.
Aim: To investigate the connection of alpha-1 acid glycoprotein inflammatory biomarker with metabolic characteristics in women with polycystic ovary syndrome (PCOS) and normal cycling controls.
Methods: A cross-sectional study was conducted on 55 women with PCOS and 55apparently healthy controls attended between January 2021 and December 2021. Serum C-Reactive protein and Alpha-1 acid glycoprotein levels were estimated and compared in women with PCOS and controls.
Results: Alpha-1 acid glycoprotein levels were higher in women with PCOS (20.1 ± 5.2) when compared to controls (18.5 ± 5.1). Further, it was positively correlated with waist circumference, fat mass, body adiposity index, and lipid accumulation product. Increased fasting blood glucose, fasting insulin and dyslipidemia was observed in women with PCOS when compared to control women.
Conclusions: In PCOS, alpha-1 acid glycoprotein is correlated with biomarkers of adiposity, carbohydrate metabolism.
Keywords:*Alpha-1 acid glycoprotein, c-Reactive protein, inflammation, carbohydrate metabolism, polycystic ovary syndrome.*

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I. Introduction

Polycystic ovary syndrome (PCOS)is a common infertility in reproductive age women. Many clinical consequences, such as infertility, obesity, and impaired glucose tolerance, are observed in PCOS patients. In addition, these women have an increased risk of developing cardiovascular disease and type 2 diabetes mellitus (T2DM)[1, 2]. Although insulin resistance (IR) is not included in the criteria for PCOS, this condition is a common physiological abnormality in PCOS patients with multiple metabolic dysfunctions[3]. IR plays a crucial role in the onset of PCOS[4]. Approximately 70% of PCOS women have IR[5, 6]. However, the true prevalence of IR individuals with PCOS is unclear because of limitations, such as IR assessment methods. Thus, it is important to identify reliable biomarkers for predicting IR using different features or phenotypes than those used for diagnosing PCOS.

Polycystic ovary syndrome (PCOS) is a complex endocrine disorder with a prevalence in reproductive years ranging from 3% to 26%, depending on the ethnicity, and criteria used for making the diagnosis[1-3].In general PCOS diagnosis comprises at least two of the following criteria: clinical and/or biochemical hyperandrogenism, oligo/anovulation, amenorrhea, and polycystic ovary morphology by ultrasound[4]. PCOS is frequently associated with glucose intolerance (GI), insulin resistance (IR), dyslipidemia, obesity, metabolic syndrome, non-alcoholic fatty liver disease (NAFLD), and low grade chronic inflammation[5–7]. Therefore, women with PCOS have a stimulatory effect on the development of oxidative stress, adipocyte dysfunction, endothelial dysfunction, and cardiovascular disease (CVD). In women with PCOS, atopic hypertrophic

adipocytes produce an exaggerating amount of adipocytokines, mainly those with pro-inflammatory effects,that act on the liver amino acid uptake and stimulate the synthesis of some inflammatory protein markers[8-9].

A number of adipokines are produced by tissues, such as adiponectin (ADIPOQ), zinc-α2 -glycoprotein (ZAG) and leptin. These adipokines regulate glucose and lipid metabolism and insulin action and play important roles in the pathogenesis of IR, obesity, T2DM and cardiovascular diseases[6-9].The low-grade chronic inflammation in women with PCOS has been extensively studied. Overtaking the step of measuring adipokines acting on liver fraction various simple hepatic protein biomarkers of inflammation such as C-reactive protein (CRP), albumin, and CRP/albumin ratio, are commonly used in the detection of subclinical inflammation and future risk of CVD in clinical practice[10–13]. In addition to protein biomarkers, in clinical studies enrolling many individuals, the non-specific biomarkers of low-cost, and easily measurements such as white blood cell count, neutrophil number, lymphocyte number, and HDL-C concentrations are commonly used in the evaluation of the inflammatory process in women with PCOS.

Another protein of interest with immunomodulation effects is the alpha-1 acid glycoprotein (AGP). Like CRP, AGP is an acute-phase protein synthesized by hepatocytes and, of less importance, by adipocytes, in response to the systemic reaction to inflammation. Its expression is modulated by proinflammatory cytokines, chemokines, and glucocorticoids[14]. This protein may suppress lymphocyte proliferation, and inhibits neutrophil chemotaxis response, polymorphonuclear activation, and platelet aggregation. Furthermore, it appears to integrate inflammation and metabolic signals, modulating an increased response to protect adipose tissue from excessive inflammation and metabolic dysfunction[15].To the best of our knowledge, higher levels of AGP in women with PCOS were reported in just one study and, in this condition, it appears to be correlated with free androgen index (FAI), and body mass index (BMI). Considering AGP as a biomarker of inflammation, the current study aimed to verify the role of AGP measurement in women with PCOS.

II. Material and Methods

Study design, subjects, and eligibility

This a cross sectional study, consists of 55 women with PCOS, aged 28.4 ± 4.4years old, and 55 normal controls, aged 27.9 ± 4.3years old attended at the Mahavir Institute of Medical Sciences, Vikarabad, Telangana in whom the Alpha-1 acid glycoprotein (AGP) levels were measured. Each participant signed a specific informed consent approved by the Institutional Committee for Ethics in Research.

Inclusion Criteria: PCOS patients have been included according to the Rotterdam criteria[4]and women with infertility whose final diagnosis was a male or tubal infertility.

Exclusion Criteria: Overt hypothyroidism was excluded by a thyroid-stimulating hormone (TSH) level of ≥10 μUI/ml and free thyroxin (FT4) level of ≤9.0 pmol/L. Hyperprolactinemia was excluded when prolactin (PRL) levels were ≤1.086 nmol/L. Both, controls and PCOS patients who had used sex steroids or insulin sensitizing drugs over the past 6 months or those who did not fulfill the Rotterdam criteria were excluded. Women with a history of autoimmune diseases or chronic inflammatory states were also excluded.

After exclusion of other hyperandrogenic conditions, PCOS was defined using the Rotterdam criteria[4]. The normal menstrual cycle, amenorrhea, infrequent or frequent menses were defined as recently recommended. The study included PCOS patients who attended outpatient in the department of Gynecology and Obstetrics at Mahavir Medical College and Hospital, Vikarabad, Telangana. The study was done on a total of 55 PCOS subjects. All of them were in the age group of 18 to 35 years, female subjects. Written consent is taken from all the individuals. Age and sex matched 55 healthy individuals are included in control group. Inclusion criteria: Patients who are presented with at least two of three following criteria: irregular menstrual cycle, chronic anovulation, hyperandrogenism includes hirsutism, acne and polycystic ovaries. Exclusion criteria: subjects having thyroid disorder, diabetes mellitus, congenital adrenal hyperplasia, androgen secreting tumors. Standard anthropometric data like height, weight, waist circumference (WC), were measured and noted for each subject. The BMI was calculated as the weight in kilograms divided by the square of height in meters. Hirsutism was quantified with the modified Ferriman Gallwey score. About 3 ml of blood is collected from the antecubital vein. Fasting blood samples were collected in plain, sodium fluoride and heparin tubes. Serum is separated by centrifugation. Blood samples are centrifuged at 3500 rpm for 10 min to separate serum. Serum total cholesterol, triglycerides, HDL and glucose were analyzed using commercial kits available for fully automated Humastar 600 Biochemistry analyzer. LDL and VLDL is calculated by using Fredrickson Friedwald's formula. Fasting serum insulin is estimated by Chemi Luminescent Immuno assay (CLIA) method using Beckman Coulter kit. Insulin resistance calculated by HOMA IR. Antioxidant vitamins C and E are measured by using HPLC. For adequate quality control both normal, abnormal reference control serum solutions and calibrators were run before analyzing each test sample. Other factors influencing the quality like proper functioning of instrument, glassware, cuvettes and distilled water are thoroughly checked before using.

III. Results

About 55 clinically proved and confirmed patients of Polycystic ovary syndrome in the age range of 18 to 35 years attending the outpatient department (OPD) of OBG and Endocrinology Department of Mahavir Institute of Medical College and Hospital, Vikarabad, Telangana were selected for the study. Equal number of age matched normal healthy females without any present or previous history of PCOS were selected to serve as controls. The subjects included in the study (patients as well as normal individuals) were assessed for serum Lipid profile, fasting blood sugar, Fasting insulin and inflammatory markers like C-Reactive protein and Alpha 1- Acid glycoprotein (AGP). Comparisons were made between the two groups. The following observations were made during the study. The biochemical findings of this study are presented in **Table 1**. **Table 1** shows mean, and standard deviation of all clinical and biochemical parameters measured in PCOS and control women. PCOS women had higher fasting insulin levels, lipid profile also increased levels of CRP and AGP when compared to control women.

IV. Discussion

Polycystic ovary syndrome is a common endocrinopathy characterized by chronic anovulation, hyperandrogenism and multiple small subcapsular cystic follicles in the ovary on ultrasonography, which affects 4-16% of women in reproductive age[3-5]. It is frequently associated with insulin resistance and compensatory hyperinsulinemia. In women with PCOS, mechanisms for hyperinsulinemia include functional problems in the insulin. Insulin receptors have been demonstrated in ovaries[9, 10]. Insulin can stimulate ovarian growth and steroid genesis. Insulin increases intra ovarian androgens, disrupts normal follicular genesis, causes development of multiple ovarian cysts and ovarian enlargement. When insulin resistance is increased, the uptake of FFA by the adipose tissue is decreased and release of FFA from adipose tissue is increased. This leads to increased triglyceride synthesis. Increased triglyceride synthesis in turn promotes the assembly and secretion of triglyceride containing VLDL[12]. Insulin resistance impairs VLDL particle clearance, leading to greater interchange of core triglyceride from VLDL with LDL and HDL. Triglyceride enriched LDL and HDL become substrates for hepatic lipase, resulting in smaller, denser particles. Elevated FFA levels down regulate the ABCA 1 transporter, which is involved in reverse cholesterol transport[13-15]. Obesity can be called as an underlying risk factor for atherosclerosis, cardiovascular disease because it raises the risk through the other associated risk factors that include atherogenic dyslipidemia. The marker for body fat content is the body mass index (BMI) which is determined by weight in kg/height in m^2. The best way to estimate obesity in clinical practice is to measure waist circumference. The advantage of measuring waist circumference is that an excess abdominal fat is correlated more closely with the presence of metabolic risk factors than the total body fat.

The present study includes 55 PCOS patients and 55 control women. Cutoff value of HOMA IR is taken as >2.5. In the present study serum insulin and HOMA IR in PCOS patients is increased when compared with controls and is highly significant ($p < 0.001$) and this is in accordance with the previous studies[16-19]. In the present study the mean values of cholesterol, TGL, LDL and, VLDL are increased, whereas mean value of HDL was decreased. The mean values of BMI and waist circumference are also increased. These observations show dyslipidemia and android type of obesity in PCOS subjects. Insulin resistance is associated with increased risk of hyperlipidemia, type II diabetes and coronary artery disease. Identification of IR help us to treat these unnoticed dysfunctions, give better health for people and prevent future complications. Increased free radical formation together with decreased antioxidant defense causes oxidative stress. The uncontrolled production of free radicals is considered as an important factor in tissue damage. PCOS is also associated with increased oxidative stress[17-19].

Many inflammatory mediators (cytokines, glycoproteins) are increased in PCOS, indicating an inflammatory state and an imbalance between pro-inflammatory and anti-inflammatory agents[20]. The present study evaluated and compared the association of AGP with clinical and biochemical characteristics in women with PCOS and controls. Like the classic CRP marker, baseline levels of AGP were increased in PCOS when compared with normal cycling, non-PCOS controls. Even in non PCOS, after using simple correlation, AGP was positively associated with BMI, WC, some anthropometric metabolic indexes, FAI, CRP, and CRP/albumin ratio, and negatively correlated with HDL-C and SHBG. In women with PCOS, AGP had a positive correlation with DBP, and strike correlation with anthropometric, anthropometric-metabolic indexes, biomarkers of carbohydrate metabolism, CRP, CRP/albumin ratio, ESR, and neutrophils[21, 22]. Conversely, in PCOS, AGP was negatively correlated with glucose/insulin ratio, HDL-C, SHBG, T, and B lymphocytes. In the multiple correlation model in non-PCOS women, AGP retained correlation with SHBG and LAP. In women with PCOS, the multiple regression final model retained the correlation of AGP with CRP levels and ESR. The use of simple biomarkers in evaluating on large scale the role of low-grade chronic inflammatory in women with PCOS is justified by low costs, their strong association with cytokines (IL-6, TNF-α), and the direct effect of these cytokines on the liver for the synthesis of these simple inflammatory protein markers[23]. For instance, CRP which synthesis is stimulated by IL-6, is considered the classical and most reliable marker of low-grade chronic

inflammation in PCOS[20]. Various studies have demonstrated increased levels of CRP in women with PCOS, particularly in overweight/obese women[19]. Nevertheless, increased levels of CRP have also been reported in lean women with PCOS, and other hyperandrogenic conditions[14-16]. Hyperandrogenism is associated with increased visceral adipose tissue, IR, dysfunctional adipocyte, increased release of adipokines, mainly interleukin-6, that directly stimulates the hepatic synthesis and releases of CRP[24]. Of note, CRP increases initiate an inflammatory process in vessel walls, binds LDL-C, and clears it from the site of atherosclerosis plaques by binding to cell surface receptors on macrophages, which are transformed into loan cells within the plaques. The role of androgens in CRP levels in PCOS presents inconsistencies; therefore, androgens mayor may not have a protective effect in preventing raise in CRP levels. The association between CRP levels with obesity and IR appears to be established. Many studies have correlated CRP levels with fasting insulin and markers of IR and adiposity[22-24]. Additionally, it must be considered that normal-weight women with PCOS also have higher fat accumulation in a visceral depot and higher levels of CRP.

AGP is elevated in a few inflammatory conditions[15]. Recently, it was reported to be elevated in women with PCOS in whom was correlated with BMI, and FAI. The current study endorses and expands the findings of this previous report. Of importance, in addition to be linked with chronic inflammation, PCOS is also correlated with disordered blood coagulation and endothelial cell dysfunction, and AGP appears to down-regulate the immune response, and to inhibit lymphocyte activity and platelet aggregation. Many studies have reported higher CRP levels in women with PCOS[22-24]. Most of them correlating CRP with metabolic abnormalities in this syndrome[14,21,22]. Albumin is a negative acute-phase protein as its concentrations decrease in the case of inflammation under the influence of cytokines[23]. It appears to be down-regulated in women with PCOS and type 2 diabetes mellitus (T2DM),12 perhaps through subclinical atherosclerosis changes, particularly in renal vessels[23-24].As albumin measurement is a simple test, its decrease could be an important biomarker of inflammation.

The strength of this study includes novel knowledge regarding the interplay between inflammatory markers and clinical characteristics of women with PCOS. Furthermore, all associations were matched by age and BMI. Because in non-PCOS controls the AGP has also shown to be correlated with some biomarkers of adiposity, and FAI, it should not be considered a specific marker of the inflammatory process in women with PCOS. Nevertheless, different from controls, AGP levels were extensively and strongly correlated with disturbs in carbohydrate metabolism, almost all markers of adiposity, and total testosterone[23,24].

V. Conclusion

Our study concludes that women with PCOS show high prevalence of insulin resistance. Although the CRP and Alpha 1- acid glycoprotein levels not statistically significant but showed increase in their serum levels which in turn may be responsible for the metabolic and endocrine disturbances. Hence, itissuggested thatCRP and Alpha 1- acid glycoprotein as biomarkers for PCOS prognosis.

Conflict of Interest

None declared

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Table 1: Comparison of biochemical parameters between the healthy controls and PCOS women

S.No	Parameter	Controls (n=55)	PCOS cases (n=55)	P value
1	Age (Years)	27.9 ± 4.3	28.4 ± 4.4	0.548
2	BMI (Kg/m^2)	23.5 ± 2.9	27.5 ± 4.5	0.0001*
3	Waist Circumference (cm)	72.2 ± 6.6	84.4 ± 14.6	0.0001*
4	Hip Circumference (cm)	86.25±5.84	92.26±6.1078	0.0001*
5	Waist Hip Ratio	0.74 ± 0.05	0.80 ± 0.07	0.0001*
6	FBS (mg/dl)	89.0 ± 9.1	124 ± 10.4	0.0001*
7	Total Cholesterol (mg/dl)	147.93 ± 27.02	174.03 ± 32.70	0.001*
8	Triglycerides (mg/dl)	65 ± 19.45	133.83 ± 60.16	0.001*
9	HDL-C Direct (mg/dl)	63.90 ± 7.44	32.5 ± 12.94	0.001*
10	LDL-C (mg/dl)	92.67 ± 20.2	124.83 ± 28.93	0.001*
11	VLDL-C (mg/dl)	15.73 ± 11.98	27.03 ± 12.04	0.01
12	Fasting Insulin (µlU/ml)	8.08 ± 0.33	13.93 ± 2.81	0.0001*
13	Acid Glycoprotein (µmol/L)	18.5 ± 5.1	20.1 ± 5.2	0.106
14	CRP (nmol/L)	32.4 ± 21.9	40.9 ± 36.1	0.138

*p is statistically significant

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