Assessments of C-Reactive Protein and its Correlation with Total Antioxidant Capacity in Woman with Poly Cystic Ovary Syndrome

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Abstract

Objective: Polycystic ovary syndrome (PCOS) is a common gynecologic endocrinopathy. The pathogenesis of PCOS is associated with both heredity and environment. PCOS has adverse impacts on female endocrine, reproduction, and metabolism. PCOS can impact women's reproductive health, leading to anovulatory infertility and higher rate of early pregnancy loss. PCOS has additional metabolic derangements, such as insulin resistance, impaired glucose tolerance, and dyslipidemia. The risks of diabetes, cardiovascular disease, hypertension, metabolic syndrome, and endometrial cancer among PCOS patients are significantly increased as well. C-reactive protein (CRP) is a member of a group of acute phase of protein which increase their concentrations during certain inflammation disorders and used as a biomarker of inflammation in the body. The aim of the presented work is to determine the level of CRP and then to evaluate its correlation with total antioxidant capacity in sera of women with PCOS.

Materials and Methods: This study was carried out on 120 women within the reproductive age (18-45 years old). Sixty patient of them were attended from gynecological and obstetric hospital in Karbala province, and they all diagnosed by their physicians as polycystic ovary syndrome, and they compared with another sixty healthy control women with regular-menstrual cycle , fertile with age range (18-45 years) from November 2015 till October

2016. Total cholesterol, triglyceride, HDL-C, LDL-C, VLDL-C, total antioxidant capacity and CRP were determined in each sample by various techniques. Then various correlations were measured by SPSS system.

Results: The PCOS patients and the control group were obese and non-obese (30 patients of each). The mean age of obese PCOS was (27.26 ± 0.83) whereas for obese control was (28.1 ± 0.713) with a high significance p < 0.01 in HDL-C, T-AOC and CRP. The comparison between non-obese PCOS, means of age (25.5 ± 0.87) with non-obese control, means of age (27.3 ± 0.68) a high significance p < 0.01 in HDL-C and T-AOC was obtained. While there is no significant correlation between T-AOC and CRP in obese and non-obese groups, respectively.

Conclusions: From the presented results, C-reactive protein levels as one of cardiac biomarkers was elevated in PCOS patients which revealed that PCOS is associated with dyslipidemia and altered oxidative status. Obesity appeared to be a major factor associated with elevated cardiac markers.

Keywords: Polycystic ovary syndrome, Total antioxidant capacity, Homocystiene , C-reactive protien

Introduction

Polycystic ovarian syndrome (PCOS) is one of the most frequent endocrine disorders in women, with an estimated prevalence of 5 – 10% of the reproductive age female population **(1)**. It is an endocrine-metabolic disturbance which has features of multiple hormonal imbalances that produce short and long term consequences on women health. PCOS is a complex disorder that may result by the susceptibility of various gene variants under the influence of environmental factors **(2,3).**

Polycystic ovary syndrome is the leading cause of anovulatory infertility in developed countries. The clinical features of the syndrome include oligomenorrhea, acne, hirsutism, obesity and insulin resistance (IR). Insulin resistance is present in the majority of women with PCOS, regardless of Body mass index (BMI), Women with PCOS have an increased risk for impaired glucose tolerance (IGT).IGT or type two diabetes mellitus (T2DM) develops in 40% of obese women with PCOS by the age of 30, decreased high-density lipoprotein cholesterol (HDL-C) levels, and increased total cholesterol, low-density lipoprotein cholesterol (LDL-C) and triglyceride levels are also associated with PCOS **(4)**. Women with PCOS manifest a wide spectrum of symptoms and clinical features, including hyperandrogenism, ovulatory disturbances and metabolic syndromes.

C-reactive protein (CRP) is not the only inflammatory biomarker that has been shown to predict myocardial infarction and stroke, more sophisticated measures of cytokine activity, cellular adhesion, and immunologic function

have all been shown to be elevated among those at increased vascular risk **(6)**. The CRP is closely linked to metabolic syndrome features, such as insulin resistance, abdominal obesity and dyslipidemia, and a large number of studies showed that serum CRP levels are significantly higher in patients with PCOS compared with healthy subjects **(7)**. Total antioxidant capacity (T-AOC) considers the cumulative action of all the antioxidants present in plasma and body fluids, thus providing an integrated parameter rather than the simple sum of measurable antioxidants **(8)**. PCOS is accompanied by oxidative stress in which increased production of free radicals is followed by decreased serum total antioxidant levels. Furthermore, it has been shown that even lean women with PCOS exhibit oxidative stress **(9)**.

Materials and Methods

The study was conducted during the period from Nov., 2015 till Oct., 2016 a period of case control study. 120 women within the reproductive age (18-45 years old) divided into two groups: 60 PCOS patient 30 obese 30 non-obese and control also 30 obese 30 non-obese. Sixty patient women out of 120 were attended from gynecological and obstetric hospital in Karbala province, and they all diagnosed by their physicians as Polycystic Ovary Syndrome, and they compared with healthy control women with comparable age. The study protocol was approved by the ethical research committee of the College of Medicine-University Karbala and Karbala Health Directorate.

Inclusion Criteria

Consisted of all patients with PCOS were already diagnosed and the diagnosis was confirmed according to European society of human reproduction and embryology and American society for reproductive medicine criteria: PCOS is diagnosed if there are any two of the following:

❖ Presence of polycystic ovary on ultrasound examination.

❖ Menstrual dysfunction with an ovulation.

❖ Clinical or biochemical hyperandrogenemia.

Exclusion Criteria

❖ All patients with hormonal therapy or any medication known to interfere with follicular development or hormonal levels under the Study for last 3 months of samples expect metformin.

❖ All patients with oligomenorrhea , amenorrhea due to other than PCOS causes.

❖ Diabetic patients.

❖ All patients suffered from heart diseases or congenital abnormality of the heart.

About 5 ml of venous blood sample was drawn from each patient and control groups for serum separation. Each serum sample was analyzed directly for total cholesterol, triglyceride, HDL-C, LDL-C, VLDL-C by using UV-VIS 1240 spectrophotometer while total antioxidant capacity was determined by using ELIZA technique. C-reactive protein was determined by Auto Analyzer procedure.

Results

The comparison results obtained between obese PCOS and obese control indicate that there is no significant difference in serum total cholesterol, TG, LDL-C,VLDL-C, age and BMI. While the results showed that there is a high significant results in serum HDL-C,T-AOC and CRP, as shown in table (1):

Table (1): Comparison between obese polycystic ovary syndrome patient group and obese control group in the measured parameter

Parameter	Control group N= 30 Mean ± SE	Patient group N= 30 Mean ± SE	P value
Age (20 – 35)	28.1 ± 0.713	27.266 ± 0.838	NS
BMI (Kg/m²)	33.09 ± 0.405	34.036 ± 0.702	NS
Total Cholesterol (mg/dl)	155.323 ± 3.662	145.277 ± 4.976	NS
TG (mg/dl)	107.107 ± 2.963	108.577 ± 6.203	NS
HDL-C (mg/dl)	53.95 ± 0.865	41.45 ± 1.843	P < 0.01
LDL-C (mg/dl)	78.003 ± 1.981	80.066 ± 2.298	NS
VLDL-C (mg/dl)	21.421 ± 0.592	21.715 ± 1.24	NS
T-AOC (U/ml)	24.347 ± 3.557	10.557 ± 1.047	P < 0.01
CRP (mg/dl)	0.579 ± 0.023	0.856 ± 0.097	P < 0.01

When we compare between non-obese polycystic ovary syndrome group and non-obese control group the results showed that there is no significant difference in serum TG, VLDL-C and CRP, high significant difference in serum total cholesterol, HDL-C, LDL-C and T-AOC.as shown in table (2):

Table (2): Comparison between non-obese polycystic ovary syndrome patient group and non-obese control group in the measured parameters

Parameter	Control group N= 30 Mean ± SE	Patient group N= 30 Mean ± SE	P value
Age (20 – 35)	27.366 ± 0.682	25.5 ± 0.875	NS
BMI (Kg/m²)	23.345 ± 0.174	23.746 ± 0.295	NS
Total Cholesterol (mg/dl)	124.57 ± 1.423	136.886 ± 3.303	P < 0.01
TG (mg/dl)	60.4 ± 4.878	62.34 ± 5.317	NS
HDL-C (mg/dl)	55.07 ± 1.203	42.044 ± 1.603	P < 0.01
LDL-C (mg/dl)	68.2 ± 1.313	76.523 ± 2.423	P < 0.01
VLDL-C (mg/dl)	12.08 ± 0.975	12.468 ± 1.063	NS
T-AOC (U/ml)	27.712 ± 3.961	11.009 ± 0.788	P < 0.01
CRP (mg/dl)	0.726 ± 0.139	0.706 ± 0.777	NS

In obese women there was a significant positive correlation between BMI and Cholesterol, TG and VLDL-C, while high significant positive correlation between BMI and LDL-C, table (3)

Table (3): r and p value between BMI and lipids profile

BMI (Kg/m²)	Cholesterol (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
	r = 0.41* p = 0.024	r = 0.399* p = 0.029	r = - 0.34 p = 0.066	r = 0.515** p = 0.004	r = 0.399* p = 0.029

In obese women there was no statistically significant correlation between T-AOC and CRP.

as shown in table (4):

Table (4): Correlation between T-AOC and CRP

T-AOC	CRP (mg/dl)
(U/ml)	r = 0.006 p = 0.977

In non-obese there was a high significant positive correlation between BMI and TG, VLDL-C, In addition to there was a high significant negative correlation between BMI and HDL-C, while there was a significant positive correlation between BMI and cholesterol.as shown in table (5)

Table (5): r and p between BMI and lipids profile in non-obese

BMI (Kg/m²)	Cholesterol (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
	r = 0.414* p = 0.023	r = 0.622** p = 0.000	r = - 0.469** p = 0.009	r = 0.015 p = 0.936	r = 0.622** p = 0.000

While, there was no statistically significant correlation between TAOC and CRP in non-obese. As shown in table (6)

Table (6): correlation between T-AOC and CRP in non-obese

	CRP (mg/dl)
T-AOC (U/ml)	**r = 0.073** **p = 0.701**

Discussion

PCOS and obesity induce an increase in serum inflammatory cardiovascular risk markers levels. The precise mechanisms underlying these associations require additional studies to clarify the state of the cardiovascular system in women with PCOS compared with controls in large numbers of patients to determine the relative contribution of different factors including insulin resistance, androgen status and BMI **(6,9)**. CRP is a sensitive, but non-specific acute-phase reactant, which (when elevated to ≥3 mg/L) is a predictor of cardiovascular events in otherwise asymptomatic individuals. Increases in CRP levels detected by assays with expanded sensitivity to very low levels of CRP, so-called high-sensitivity C-reactive protein (hs-CRP), showed a strong correlation as an independent risk factor for future cardiac events and the extent of coronary artery diseases **(10,11).**

The presented study indicated that serum C-reactive protein when compared between obese patients and obese control is statistically high significant differences than that found in PCOS (p = 0.008) while when compared non-obese groups found that no significant differences which agreement with **(12)** whose detected that the mean CRP concentrations were significantly higher in the obese PCOS group (BMI>30) compared with the control subgroups of similar BMI (P = 0.001). Sumithra et *al.* results as in agreement with presented study which indicated high BMI is yet another factor that contributes to the development of inflammation and a higher prevalence of low grade systemic inflammation was observed in overweight and obese individuals compared with normal weight persons **(13)**.

Kelly *et al.* is the first study to examine low grade chronic inflammation in women with PCOS. which have shown that CRP concentrations measured using a highly sensitive assay are significantly increased in women with PCOS relative to those in healthy women with normal menstrual rhythm and normal androgen levels after correction for BMI. It was based on only 15–17 subjects in each group and suggests that CRP may be a marker for possible prospective identification of young PCOS women prone to develop CVD in the future. Despite the fact that none of the PCOS patients had any sign of inflammation, and also noted that CRP concentrations in both PCOS and

controls correlated with the degree of obesity and inversely with insulin sensitivity, although not with total testosterone concentrations **(14).**

Mohlig *et al.* detected that CRP levels were significantly increased in both obese PCOS and obese control women as compared with their non-obese counterparts, and both parameters correlated as a markers of obesity and insulin resistance in the PCOS cohort also, found that is further supported by the fact that none of the endocrine markers of PCOS correlated with surem CRP concentrations (**15**).This study indicated that no correlation between BMI and CRP for obese and non-obese group despite while other studies found a significant positive correlation (**14-16**). Furthermore, metformin was not able to reduce the inflammation state in PCOS women **(17)**

In the presented study there was no correlation between total antioxidant capacity and C-reactive protein. Fenkci *et al*. found that C-reactive protein, which represents the increased pro inflammatory effect, increases plaque vulnerability and propensity to thrombosis. Elevated serum CRP was reported as a risk factor for the future cardiovascular events. There was an increase in CRP level in women with PCOS, and a negative correlation between TAOC and CRP. Present data strengthen the idea that there is an increased risk of cardiovascular events in women with PCOS **(18).**

 Oxidative stress and chronic inflammation are closely inter-related. A vicious cycle exists whereby inflammation induces generation of ROS, while oxidative stress promotes and aggravates inflammation **(19).** Also, PCOS alone may not be the cause for the increased oxidant status and CRP, adiposity also might play a role in this **(13)**. Several studies suggested an increased risk of PCOS for developing of cardiovascular diseases **(20).** When compared with women of similar age who have normal menstrual cycles and women with PCOS that have been found to have an increased prevalence of several cardiovascular risk factors, including obesity, insulin resistance, hyperhomocysteinaemia, dyslipidaemia, increased intima media thickness and impaired vascular elasticity **(21).**

Obesity is an additional burden in PCOS, playing a role in the pathogenesis of both hyperandrogenism and IR. High BMI (≥ 25) was found to be the main factor for endocrinology and metabolic disturbances in PCOS women **(22)**. The results of this study were in agreement with several studies showed that PCOS patients had abnormal BMI values **(23-25).** Women with PCOS are generally more obese than age matched controls, and have an elevation in BMI. The mean BMI of the investigated women with PCOS were (28.8 $\pm$ 5.95), even although patient and control groups were matched for BMI found by others **(26)**. Dyslipidemia has been reported in women with PCOS **(27).**

The results indicate that serum total cholesterol, triglyceride and very low density lipoprotein- cholesterol were higher in women with PCOS than that found in control subjects and the differences were not statistically significant (P= 0.78, p= 0.79 and p= 0.79, respectively). While serum high density lipoprotein-cholesterol (HDL-C) and low density lipoprotein-cholesterol (VLDL-C) statistically high significant differences than that found in PCOS (p = 0.000, p = 0.017) respectively. Sample size may be a critical factor in determining the statistical significance of the differences observed **(28)**. Studies evaluating lipoprotein particle concentrations in women with PCOS have been infrequent. Our results confirm that there is a higher TG levels in PCOS patients as compared with those found in normal women. Generally, dyslipidemia of PCOS is characterized by increased triglycerides and low HDL-cholesterol **(29)**, while others show that a low HDL-cholesterol is common while hypertriglyceridemia is relatively uncommon **(30).** Oxidative stress is observed in patients with polycystic ovary syndrome. PCOS women should be evaluated for status of serum lipids and oxidative stress **(31,32)**, the presented study indicates that there is a high significant difference in total antioxidant capacity (p = 0.000) between the patient and the control, either obese or non-obese, This disagree with other studies **(33,34).** While, there was no such significant difference in TAC in women with PCOS compared with controls**.** Fenkci *et al.* detected lower TAC levels in the PCOS group, but these levels did not reach a level of statistical significance. This observation suggests that the oxidative status imbalance in PCOS women might contribute to their increased risk of cardiovascular diseases. Polycystic ovary syndrome is also associated with decreased antioxidant concentrations, and is thus considered an oxidative state **(35).** On the other hand, many studies that have addressed oxidative stress and PCOS to date have not given a definitive conclusion about their possible association **(5),** while a few notable exceptions **(36,37).** These studies were suffered from lacking a sample sizes and included to less than 100 patients with PCOS. It is worth to mention that meta-analysis of Murri *et al.,* study which found that TAC unchanged when studied with other circulating markers of oxidative stress in polycystic ovary syndrome **(38).**

Conclusion

From the presented results C-reactive protein was high in PCOS patient which revealed that PCOS is associated with dyslipidemia and altered oxidative status. C-reactive protein levels as one of cardiac biomarkers was elevated in PCOS patients who revealed that PCOS is associated with dyslipidemia and altered oxidative status. Obesity appeared to be a major factor associated with elevated cardiac markers. There was an increase in

CRP level in women with PCOS, and a negative correlation between TAOC and CRP. Present data strengthen the idea that there is an increased risk of cardiovascular events in women with PCOS.

Acknowledgments

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