

Assessment of C-reactive Protein and C3 as Inflammatory Markers of Insulin Resistance in Women with Polycystic Ovary Syndrome: A Case-Control Study

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Abstract

Background: Polycystic ovary syndrome (PCOS), a common endocrine disorder, is associated with infertility, menstrual dysfunction, hirsutism and frequent miscarriages. Insulin resistance, as a major cause of PCOS, represents a disorder with increase in inflammatory markers and risk of type 2 diabetes. We aimed to investigate whether inflammatory markers, including C-reactive protein and C3 (Complement), are related and altered in polycystic ovary syndrome.

Methods: A case-control study including forty-two women diagnosed with PCOS, according to Rotterdam criteria, and forty-two healthy controls, matched for body mass index (BMI) and age, was conducted in 2012. C-Reactive protein (CRP) and C3 were assessed as possible determinants of the homeostasis model assessment (HOMA) index. Independent-sample t-test was used to compare the means of the groups in age, BMI, C3, FBS and BS 2hpp (2 hr postprandial glucose) and for CRP, Fasting Insulin and 2 hr Plasma Insulin and HOMA index. Mann-Whitney test and Pearson correlation were used for analyzing the data. The $p < 0.05$ was considered as statistically significant.

Results: Levels of plasma CRP ($p=0.039$), 2 hr pp ($p=0.045$), Fasting Insulin ($p=0.002$), 2 hr Plasma Insulin ($p=0.002$) and HOMA index ($p=0.002$) were significantly higher in PCOS patients. But C3 was not significantly higher in cases ($p=0.885$). There was no significant correlation between C3 and CRP with HOMA index.

Conclusion: CRP increased significantly in patients with PCOS and was associated with insulin resistance, the most probable cause of PCOS. However, such an association was not found in C3.

Keywords: C3, C-reactive Protein, Insulin Resistance, Polycystic Ovary Syndrome.

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Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder, characterized by clinical and/or biochemical hyperandrogenism and chronic anovulation, polycystic ovaries in ultrasonography findings, and frequently morbid obesity, which is associated with infertility, menstrual dysfunction, hirsutism and frequent miscarriages (1, 2).

There are various reports about the prevalence of PCOS according to racial and genetic differences, it ranges from 2–20% (4).

Insulin resistance, as a major abnormality associated with PCOS, represents a disorder with increased risk of type 2 diabetes (5, 6) and is usually associated with an increase in inflammatory markers (7).

Previous studies suggest that the cytokines, arising partly from adipose tissue, could possibly be responsible for the metabolic abnormalities associated with insulin resistance.

In this respect, many markers are proven to be associated with insulin resistance, metabolic syndrome and diabetes amongst which C-reactive protein has been the most studied marker. However, the causal association has not been proven yet (7–13).

One hypothesis is that the inflammatory cytokines that stimulate the hepatic production of acute phase proteins are mainly secreted by the adipose tissue excessively and that such cytokines may result in insulin resistance by indirectly causing the phosphorylation and proteosomal degradation of insulin receptor substrates or by indirectly interfering with the insulin receptor substrate interaction (7).

Along with inflammatory markers, many other factors, such as genetic variations and infections might also play a role in the inflammation process of PCOs (1).

While the etiology of PCOS and the casual association with inflammatory markers is not clear yet, some studies have investigated the association between C3 and/or CRP with insulin resistance and PCOS. They have shown positive correlation between the increase in C3 and CRP with insulin resistance and PCOS while the association between C3 and PCOS was reported to be stronger (7, 14). The present study aimed to evaluate the serum level changes of C3 and CRP in PCOs in comparison with healthy controls matched for age and BMI. In other words, we aimed to determine the changes in CRP and C3 in patients who do not have obesity. In addition we wanted to determine the association between CRP and C3 with insulin resistance (As defined according to the homeostasis model assessment [HOMA] in patients with PCOS.)

Methods

Assessing the association of pro-inflammatory markers, CRP and C3, with PCOS was a case-control study conducted at Iran University of Medical Sciences hospitals through the year 2012. The study was conducted after achieving the approval of the university ethics board committee on medical research.

Forty-two Iranian women with established PCOS and forty-two healthy women in reproductive age were recruited in this study.

The diagnosis of PCOS was made based on the Rotterdam criteria. All patients presented with at least two of the three following criteria: 1-Oligomenorrhea or amenorrhea, 2-Clinical signs of hyperandrogenism (Hirsutism and acne) and 3-Polycystic ovaries in ultrasound study.

Androgen-secreting tumors, congenital adrenal hyperplasia, thyroid dysfunction, hyperprolactinemia, Cushing's syndrome and diabetes mellitus were ruled out in all subjects. None of the women were suffering from any other diseases, including recent infectious diseases or had been taking oral contraceptives, Metformin or Thiazolidinediones in the previous 3 months.

Forty-two BMI and age matched healthy women served as the control group. All subjects gave their informed consent to participate in the study and the study protocol conforms to the ethical guidelines of the Declaration of Helsinki.

We considered the patients with BMI \leq 40 and matched the groups due to the confounding effect of BMI on PCOS.

Blood samples were collected from all patients and healthy controls. C3, CRP, fasting plasma glucose (FPG), 2 hours postprandial glucose (2hpp), Fasting Insulin and 2 hr Plasma Insulin were measured.

C3 and CRP were measured by immunoturbidimetric methods with commercially available Latex kits (Aniston and Bionic kits, respectively). Their analytical sensitivity was 0.04 g/l for C3 and 0.01 mg/dl for CRP. Insulin was measured by an electrochemiluminescence immunoassay, with an analytical sensitivity of 0.2 mU/l.

Insulin resistance was estimated with the HOMA index: (Insulin [mU/l]×blood glucose [mmol/l])/22.5.

All statistical analyses were performed with SPSS 11.5 software. The quantitative data like BMI, C3, CRP, FBS, 2hpp, Fasting Insulin, 2 hr Plasma Insulin and HOMA Index were expressed as mean± standard deviation (SD) or median with interquartile range (IQR);

To compare means of age, BMI, C3, FBS and BS 2hpp in the groups, independent-sample t-test was used and for CRP, Fasting Insulin and 2 hr Plasma Insulin and HOMA index, Mann-Whitney U test which is a non-parametric test was used (variances of data in two groups are not equal).

For assessment of association between C3 and CRP with insulin resistance, Pearson correlation coefficient was used. P-values of less than 0.05 were considered statistically significant.

Results

Forty two Iranian women with established PCOS (case group) and mean age of 29.5 ± 5.08 and forty two healthy women in reproductive age (control group) and mean age of 28.48 ± 5.00 were recruited in this study. The mean BMI in the case and control groups were 24.40 ± 4.42 and 25.52 ± 3.76 respectively. The clinical and laboratory characteristics of the women in two groups are shown in table 1.

CRP (mean value in case group was 4.7 ± 1.5 mg/l and in control group 0 with a p-value of 0.039), 2hpp ($p=0.045$), Fasting Insulin ($p=0.002$), 2 hr Plasma Insulin ($p=0.002$) and HOMA index ($p=0.002$) were significantly higher in PCOS group than the controls. There were no significant differences between two groups with respect to C3 (median value in case group was 124(107.75-150.25) and in control group 120.5 (23.00-29.00) with a p-value of 0.885) and FBS ($p=0.498$).

In PCOS group, there were no significant associations between CRP, C3 and insulin resistance as defined according to HOMA index ($r=0.095$, $p=0.550$ and $r=0.109$, $p=0.492$) respectively (Table 2).

tween CRP or C3 levels with insulin resistance. It seems that CRP increase is involved in PCOS through other mechanisms rather than insulin resistance.

Different factors including patient's BMI have association with PCOS. There is an association between high BMI and PCOS and it was shown that losing weight in overweight women can be an effective first-line treatment in these patients (15). Moreover, body fat is associated with increased CRP in those with PCOS and hyperandrogenic state (16). In our case-control study, we tried to overcome this confounding factor by matching patients for BMI. In addition, they were matched for age to include those with almost the same condition in the study. CRP is the most studied inflammatory marker in association with insulin resistance in PCOS. Ridker et al. found CRP as a sensitive marker of mild chronic inflammation, which is produced by the liver (12).

As CRP production in the liver is modulated by some adipokines, such as interleukin-6 and tumor necrosis factor alpha, some authors have hypothesized that increased body fat may be the mechanism underlying the relation between high level of CRP and PCOS (19). Although we did not meas-

Table 1. The clinical and laboratory characteristics of PCOS cases (case group) and healthy controls (control group)

Variable	Case group	Control group	p-value
C3 (mg/dl)*	124(107.75-150.25)	120.50(23.00-29.00)	0.885
HOMA Index (mU/l)×(mmol/l)**	56.52±75.96	18.45±6.38	0.002
FBS (mg/dl)**	89.43±7.83	90.79±10.29	0.498
CRP (mg/l)**	4.7±1.5	0.00	0.039
bs2hpp (mg/dl)*	90.50(79.75-90)	98(100.50-109.50)	0.045
Fasting Insulin (mU/l)*	9(6.67-12.50)	4.25(3.70-5.20)	0.002
2 hr Plasma Insulin (mmol/l)*	23.55(7.55-38.70)	14.50(9.80-21)	0.002

* Median (IQR), ** Mean±SD

Table 2. Relationship between CRP, C3 and HOMA index (assessed by Pearson correlation coefficient)

Variable	HOMA index		
	r	R square	p-value
CRP	0.095	0.009	0.550
C ₃	0.109	0.12	0.492

Discussion

Our study revealed a significant increase of CRP in patients with PCOS in contrast to healthy controls while such a difference was not seen in C3 levels. Moreover, no association was found be-

ure the visceral fat levels in our study, probably in PCOS women the visceral fat mass is more than BMI matched controls, because the waist-to-hip ratio is greater in these women. Yet, the definite cause of CRP increase in PCOS and cardiovascular diseases stays unknown.

Like other acute-phase proteins, C3 is synthesized not only by liver, but also by activated macrophages and adipocytes. Its hepatic production is induced by cytokines like interleukin-1 and tumor necrosis factor alpha, which may interfere with insulin receptor functioning and cause insulin resistance (20).

Although serum C3 is associated with the main endogenous cardiovascular risk factors, it has been found to be strongly predictive of myocardial infarction independently of them. Evidently, and differently from CRP, the association of C3 with insulin resistance is not mainly mediated by the adipose tissue (7).

Forouhi and his colleagues showed that increased CRP levels independently predict the risk of cardiovascular disease and type 2 Diabetes Mellitus (DM) (21). However, the association and mechanism of such an effect are not clearly determined.

Today, we know that CRP is not just a marker of low-grade chronic inflammation but can be directly associated with endothelial function disorder and complement activation in atherogenesis.

According to this and previous studies it can be concluded that in PCOS women, low-grade chronic inflammation (Regarding to association of CRP with PCOS and insulin resistance) is a cooperating factor in increasing the risk of Coronary Artery Disease (CAD) and DM.

In this study, we did not find a significant relation between serum C3 level and PCOS, and also the C3 wasn't associated with insulin resistance (As defined according to HOMA index).

The strong association of C3 with insulin resistance has already been reported in young adult Pima Indians (5).

Besides production of C3 in the liver, like other acute-phase proteins, C3 also synthesizes by activated macrophages and adipocytes, therefore behaving as an inflammatory cytokine and adipokine. Its hepatic production is induced by primary wave cytokines, such as interleukin-1 and tumor necrosis factor alpha, which may interfere with insulin receptor functioning and cause insulin resistance.

However, our study had some limitations. First, the sample size was small which makes the results less generalizable. Second, we did not measure visceral fat and waist-to-hip ratio which might be a determinant of insulin resistance in cases and controls. HOMA index is a surrogate marker of insulin resistance. The latter would be more precisely measured by clamping techniques.

Conclusion

In conclusion, CRP and insulin resistance, suggesting the inflammatory processes of the disease, are the main factors in PCOS women; therefore, more attention should be paid to them in treatment

of PCOS. C3 was not associated with insulin resistance. More surveys need to be done in order to have a better look on these markers.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. Evian Annual Reproduction (EVAR) Workshop Group 2010, Fauser BC, Diedrich K, Bouchard P, Dominguez F, Matzuk M, et al. Contemporary genetic technologies and female reproduction. *Hum Reprod Update*. 2011;17(6):829-47.
2. Goodarzi MO, Korenman SG. The importance of insulin resistance in polycystic ovary syndrome. *Fertil Steril*. 2003;80(2):255-8.
3. Oktenli C, Ozgurtas T, Dede M, Sanisoglu YS, Yenen MC, Yesilova Z, et al. Metformin decreases circulating acylation-stimulating protein levels in polycystic ovary syndrome. *Gynecol Endocrinol*. 2007;23(12):710-5.
4. Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. *J Clin Endocrinol Metab*. 2004;89(6):2745-9.
5. Diamanti-Kandarakis E, Paterakis T, Alexandraki K, Piperi C, Aessopos A, Katsikis I, et al. Indices of low-grade chronic inflammation in polycystic ovary syndrome and the beneficial effect of metformin. *Hum Reprod*. 2006;21(6):1426-31.
6. Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK, Imperial J. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. *Diabetes Care*. 1999;22(1):141-6.
7. Muscari A, Antonelli S, Bianchi G, Cavrini G, Dapporto S, Ligabue A, et al. Serum C3 is a stronger inflammatory marker of insulin resistance than C-reactive protein, leukocyte count, and erythrocyte sedimentation rate: comparison study in an elderly population. *Diabetes Care*. 2007;30(9):2362-8.
8. Yudkin JS, Stehouwer CD, Emeis JJ, Coppel SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol*. 1999;19(4):972-8.
9. Florez H, Castillo-Florez S, Mendez A, Casanova-Romero P, Larreal-Urdaneta C, Lee D, et al. C reactive protein is elevated in obese patients with the

- metabolic syndrome. *Diabetes Res Clin Pract.* 2006; 71(1):92-100.
10. Ford ES. Body mass index, diabetes, and C reactive protein among U.S. adults. *Diabetes Care.* 1999;22(12):1971-7.
 11. Frohlich M, Imhof A, Berg G, Hutchinson WL, Pepys MB, Boeing H, et al. Association between C-reactive protein and features of the metabolic syndrome: a population-based study. *Diabetes Care.* 2000;23(12):1835-9.
 12. Ridker PM, Buring JE, Cook NR, Rifai N. C reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. *Circulation.* 2003;107(3):391-7.
 13. Wannamethee SG, Lowe GD, Shaper AG, Rumley A, Lennon L, Whincup PH. The metabolic syndrome and insulin resistance: relationship to haemostatic and inflammatory markers in older non-diabetic men. *Atherosclerosis.* 2005;181(1):101-8.
 14. Yang S, Li Q, Song Y, Tian B, Cheng Q, Qing H, et al. Serum complement C3 has a stronger association with insulin resistance than high-sensitivity C-reactive protein in women with polycystic ovary syndrome. *Fertil Steril.* 2011;95(5):1749-53.
 15. Ravn P, Haugen AG, Glinborg D. Overweight in polycystic ovary syndrome. An update on evidence based advice on diet, exercise and metformin use for weight loss. *Minerva Endocrinol.* 2013;38(1): 59-76.
 16. Tosi F, Dorizzi R, Castello R, Maffei C, Spiazzi G, Zoppini G, et al. Body fat and insulin resistance independently predict increased serum C-reactive protein in hyperandrogenic women with polycystic ovary syndrome. *Eur J Endocrinol.* 2009;161(5): 737-45.
 17. Adams J, Polson DW, Franks S. Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsutism. *Br Med J (Clin Res Ed).* 1986;293(6543):355-9.
 18. Robinson S, Henderson AD, Gelding SV, Kiddy D, Nithyananthan R, Bush A, et al. Dyslipidaemia is associated with insulin resistance in women with polycystic ovaries. *Clin Endocrinol (Oxf).* 1996;44 (3):277-84.
 19. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med.* 2000;342(12):836-43.
 20. Marette A. Mediators of cytokine-induced insulin resistance in obesity and other inflammatory settings. *Curr Opin Clin Nutr Metab Care.* 2002;5(4): 377-83.
 21. Forouhi NG, Sattar N, McKeigue PM. Relation of C-reactive protein to body fat distribution and features of the metabolic syndrome in Europeans and South Asians. *Int J Obes Relat Metab Disord.* 2001;25(9):1327-31.