Gynecological Endocrinology, 2013; 29(2): 141–144

ISSN 0951-3590 print/ISSN 1473-0766 online
DOI: 10.3109/09513590.2012.730581

informa
healthcare

PCOS

Is the polycystic ovary syndrome the causative of the increase in inflammatory markers and metabolic risk?

Estela Rey-Roldan[1], Maria Belen Perez Lana[2], Liliana Galluzzo[2], Georgina Blanco[2], Claudia Onetto[2], Vanesa Straminsky[2] & Manuel Patricio Nolting[2]

[1]*Dr "R.A. Margni," Humoral Immunity Studies Institute (CONICET-UBA), Chair of Immunology, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina and* [2]*Endocrinology Section, Division of Gynecology Hospital de Clínicas José de San Martín, University of Buenos Aires, Buenos Aires, Argentina*

Aims: To investigate the relationship between the levels of C-reactive protein (CRP), interleukin-6 (IL-6) and IL-1β and the hormonal and metabolic alterations in women with polycystic ovary syndrome (PCO). _Materials and methods_: Case–control study. CRP, IL-6 and IL-1β were evaluated in combination with obesity, insulin resistance (IR) and hyperandrogenism parameters in 20 patients with PCO. Twenty healthy women were used as the control. _Results_: The average CRP values was 5.1 in the cases vs. 0.8 mg/L in the control group ($p < 0.0001$). The IL-6 average values were 2.77 in the cases vs. 2.70 pg/ml in the control group ($p = 0.254$). IL-1β levels were found to be within the normal range in all individuals. A positive correlation was found between the CRP values and the IR ($p < 0.0001$) as well as with the presence of obesity ($p < 0.02$). No correlation was found between PCR and hyperandrogenemia ($p = 0.4$) nor between IL-6 values and IR ($p = 0.3$), or between the levels of this cytokine and the presence of hyperandrogenemia ($p = 0.2$). A significant correlation was found between IL-6 levels and obesity ($p < 0.0001$). _Conclusions_: The present study demonstrates the presence of a chronic inflammation status in young women with PCO. These parameters are mainly related to obesity and, to a lesser extent, to IR.

Keywords: Polycystic ovary syndrome, c-reactive protein-cytokines

Introduction

The polycystic ovary syndrome (PCO) is one of the most frequent endocrine disorders, having an estimated prevalence of 6–10% among women in the reproductive age. PCO is featured by the presence of hyperandrogenism, chronic anovulation and infertility. Together with the latter reproductive abnormalities, some women may also present obesity, insulin resistance (IR) and the subsequent development of metabolic syndrome [1,2].

Women suffering from PCO have risk factors for coronary disease, such as IR, type 2 diabetes (DBT2), metabolic syndrome, hypertriglyceridemia and endothelial dysfunction [3,4].

Over the last years, many studies have demonstrated the high risk for the development of glucose intolerance and DBT2 in women with PCO. However, it is not clear whether the increased risk is related to the abnormalities observed in the PCO, i.e. hyperandrogenism, or whether it is associated with the anthropometrical and metabolic abnormalities featuring the PCO [5,6].

Markers of subclinical chronic inflammation such as the C-reactive protein (CRP) and interleukin-6 (IL-6) have proven to be independent predictors of both DBT2 and the increased cardiovascular disease risk [7–9]. Thus, the adipose tissue has been demonstrated to be an important source of proinflammatory mediators such as the tumor necrosis factor-α (TNFα), IL-6 and IL-1β [10,11]. Furthermore, some studies have suggested that in PCO there exists an underlying inflammatory status which, together with the endothelial dysfunction may lead to the early development of atherosclerosis. Nevertheless, it is not clear if such increased risk is related to the anthropometrical changes or to the PCO itself [12–14]. Therefore, the elucidation of the latter issue would help elaborate an efficient treatment in these patients.

Taking into account these data, the aim of this study was to investigate the co-existence of chronic inflammation markers (CRP, IL-6 and IL-1β) in women presenting PCO and their relationship with the hormonal and metabolic imbalance featuring this syndrome.

Materials and methods

Description of the cohort

A case-control study was undertaken. Twenty patients with PCO participated in the study. Diagnosis of patients was made on the basis of clinical and biochemical findings. Imaging tests were also performed and employed to diagnose the patients according to the 2003 Rotterdam classification [15]. Pathologies other than PCO such as congenital suprarenal hyperplasia, Cushing's syndrome, hyperprolactinemia, pathologies of the thyroid gland, androgen-secreting tumors and diabetes were ruled out. Patients were under no pharmacological treatment for at least three months previous to the enrollment in this study. As control group, a cohort of 20 age-matched women without PCO or other diseases were included. Each individual was offered to participate in this study. Written consent was obtained.

All blood samples were obtained during the early follicular phase (days 2–5 of the menstrual cycle). Sera were obtained and kept at −40 °C until used.

The body mass index (BMI) was calculated as the ratio between weight (kg) and size (m^2). A BMI >25 kg/m^2 and a BMI >30 kg/m^2 were taken as markers of overweight and obesity,

Correspondence: Dr María Belén Pérez Lana, Endocrinology Section, Division of Gynecology Hospital de Clínicas José de San Martín, University of Buenos Aires, Buenos Aires, Argentina. E-mail: mbelenpl@hotmail.com

respectively. Testosterone and sex hormone-binding globulin (SHBG) were both determined by radioimmunoassay (RIA). The hyperandrogenism was defined by the free androgen index (FAI), calculated as follows: FAI = (Total testosterone (ng/ml) × 3.47/ SHBG (ng/ml)) × 100. Indexes > 4.5 were considered indicators of hyperandrogenism.

IR was assessed by the HOMA (homeostasis model assessment) index, which was calculated as follows: HOMA = (glucose (mg/dl) × insulin (uU/ml))/405. HOMA values greater than 2 were considered markers of IR. Glucose and insulin levels were determined by the glucose oxidase method and chemiluminiscence, respectively (METROLAB 2300 PLUS, WIENER; ARQUITEC SR 2000). IL-1β and IL-6 levels were determined by a commercial ELISA kit (R&D Systems, Minneapolis, Minnesota, USA). Detection limits for cytokines were 2.3 and 1.9 pg/ml for IL-6 and IL-1β, respectively. Ultrasensitive CRP levels were determined by immunoturbidimetry employing a Beckman LX20 autoanalyzer. Reference values for CRP were those established by the American Heart Association [16] that is, low, intermediate or high risk for cardiovascular disease if CRP <1.0; 1.0 < CRP < 3.0 or CRP>3.0 mg/L, respectively.

Statistical analysis

The SPSS (versión 15, SPSS Inc., Chicago, IL, USA) statistical package was employed. *p* values <0.05 were considered significant. Data are presented as average, median and quartiles, according to the symmetric or asymmetric nature of the variables distribution. The Shapiro's test was employed to assess normality. Biochemical data were analyzed by the Mann–Whitney *U* test. The Pearson's and Spearman´s correlation coefficients were determined to establish the relationship between parameters.

Results

Anthropometrical and biochemical features of PCO patients

The average age of PCO patients was 25.4 vs. 24.8 in the group of PCO patients vs. control group (p = NS). BMI, HOMA, FAI, CRP, IL-6, and IL-1 values are presented in Table I.

CRP and IL-6 values

The average CRP level in the case group was 5.1 mg/L vs. 0.8 mg/L (control group; $p < 0.0001$, Figure 1, left axis). The IL-6 average value in the case group was 2.77 pg/ml vs. 2.70 pg/ml (control group; $p = 0.254$, Figure 1, right axis).

Correlation between CRP, IL-6, IL-1 with IR, obesity, and hyperandrogenism

A significant correlation was found between levels of CRP and HOMA values >2 ($r = 0.95$, $p < 0.0001$). CRP levels also correlated with obesity (r: 0.92, $p < 0.02$). On the other hand, no correlation was found between CRP values and hyperandrogenism ($r = -0.008$, $p = 0.4$). No correlation was found neither between IL-6 and HOMA values >2 ($r = -0.21$, $p = 0.3$) nor with IL-6 and hyperandrogenism ($r = -0.29$, $p = 0.2$). On the contrary, a strong correlation was found between IL-6 values and the presence of obesity ($r = 1$, $p < 0.0001$). Levels of IL-1β were found to be within the reference interval. Results are summarized in Table II.

Discussion

Currently, PCO is considered not only a reproductive, but also a metabolic disorder. This syndrome is associated with other alterations such as obesity, IR, dyslipidemia, increased PAI-1 levels and endothelial dysfunction. All these factors contribute to the atherogenic process where inflammation plays a crucial role in the pathophysiology of both atherosclerosis and cardiovascular disease [17,18]. Whether PCO is a pathological entity by itself or whether it is a group of pathologies represented by a common phenotype is subject of current debate.

A substantial group of patients with PCO is constituted by those women presenting obesity and/or IR, where the high insulin levels found in these patients play an important role in

Table I. Anthropometrical and biochemical features of PCO patients.

Variable	Number of patients	(%)
BMI <25 kg/m^2	16	80
BMI >25 kg/m^2	1	5
BMI >30 kg/m^2	3	15
HOMA <2	13	65
HOMA >2	7	35
FAI <5	10	50
FAI >5	10	50
CRP <1 mg/L	8	40
CRP >1 and <3 mg/L	5	25
CRP >3 mg/L	7	35
IL-6 >2.3 pg/ml	5	25
IL-1 detectable[a]	1	9.1

[a]Determined in 11 patients belonging to the study group.
BMI, body mass index. BMI >25 kg/m^2 and a BMI >30 kg/m^2 were taken as markers of overweight and obesity, respectively. HOMA, homeostasis model assessment index. HOMA >2 were considered markers of insulin resistance. FAI, free androgen index. FAI >4.5 were considered indicators of hyperandrogenaemia. CRP, C-reactive protein.

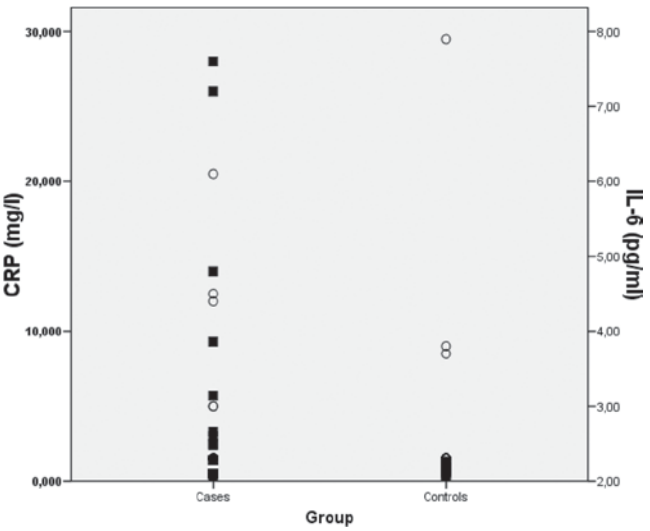

Figure 1. °C-reactive protein (CRP) (mg/ml, black square, left axis) and interleukin (IL)-6 (pg/ml, open circle, right axis) levels in the case and control group.

Table II. Correlation between CRP, IL-6, IL-1 with IR, obesity and hyperandrogenism.

	CRP	IL-6	IL-1β
HOMA >2	r: 0.95, $p < 0.001$	r: –0.21, $p = 0.3$	r: 0
BMI >25	r: 0.85, $p = 0.07$	r: –0.61, $p = 0.1$	r: 0
BMI >30	r: 0.92, $p < 0.02$	r: 1, $p < 0.001$	r: 0
FAI >4.5	r: –0.008, $p = 0.4$	r: –0.29, $p = 0.2$	r: 0

r indicates Pearson correlation coefficient. p values <0.05 were considered significant.

the pathogenesis of PCO, thus demonstrating the linkage between reproductive and metabolic alterations [5,19].

In the group of patients presented in this work, a 20% presented weight abnormalities (5% of them had overweight and a 15 % presented obesity). A 35% of patients presented abnormal HOMA values, whereas a 50% presented hyperandrogenism. Both IR and hyperinsulinemia are present in patients with PCO. Both in obese and nonobese plays a crucial role in the development of the hyperandrogenic status and anovulation.

Many studies have suggested an increased glucose intolerance and DBT2 in patients with PCO. However, it is not clear whether the risk for the development of alterations in the carbohydrate metabolism is related to the endocrine alterations observed in PCO patients such as hyperandrogenemia or whether the metabolic abnormalities are a consequence of the metabolic and anthropometric abnormalities observed in some of these patients [6,20].

IR plays an important role in the inflammatory process and many epidemiological trials have demonstrated its negative impact in the endothelial function. Over the last decade, systemic markers of inflammation have proven to be predictors of the atherosclerosis risk [4,19,21].

The CRP is a marker of inflammation and its predictive value in the incidence of acute myocardial infarction and peripheral arterial disease has been demonstrated. This marker is a more potent predictor of cardiovascular failure than the levels of low-density lipoprotein-cholesterol [22,23]. Thus, the determination of CRP levels might be a useful tool in the screening of women for PCO.

The first study demonstrating the high levels of inflammation in women with PCO were that conducted in 2001 by Kelly et al. [24]. In that study, levels of ultrasensitive CRP were significantly higher in women with PCO ($n = 17$) in comparison with healthy women ($n = 15$). These values were 2.12 and 0.67 mg/L, respectively. Two years later, Fenkci et al. [25] found higher concentrations of CRP in the PCO group (6.30 mg/L, $n = 30$) in comparison with the control group (2.35 mg/L, n:31).

In the work presented herein, a 60% of patients with PCO presented CRP values above the reference level, with a 35% of them having CRP levels greater than 3.0 mg/L, which, according to the American Heart Association, represents a high risk for cardiovascular disease [16].

The average values found in the PCO group were 5.1 mg/L vs. 0.8 mg/L (control group, $p < 0.0001$). CRP concentrations were higher in the group of women with overweight (BMI >25 kg/m^2 (r: 0.85, $p = 0.07$). A significant correlation was found between CRP values and obesity (r: 0.92, $p < 0.02$). It must be taken into account that the percentages of women with obesity and IR were low, most probably due to the small sample size, the average age of patients or to the short time of evolution of the pathology. The percentage of overweight and obesity observed in our hospital was 70% and the percentage of women having IR was 46%, over a total of 80 patients studied (unpublished data).

The spectrum of pro-inflammatory cytokines (IL-1β, IL-6, TNF α) constitutes a cascade originating in the adipose tissue [26,27]. IL-1β is a multifunctional cytokine which plays a role during ovulation, fertilization and implantation [11]. The secretion of TNFα is induced by IL-18 and the former cytokine, in turn, stimulates the synthesis of IL-6 which is responsible for the regulation of the synthesis of CRP by the liver. Both CRP and IL-6 are considered strong markers of cardiovascular disease. These markers have recently been demonstrated to be predictors for the development of DBT2 [26,27].

In our work, the group of patients with PCO, displayed an average value of IL-6 of 2.77 pg/ml and the average value for the control group was 2.70 pg/ml ($p = 0.254$). A 90% of patients did not show detectable levels of IL-1β. Moreover, no correlation was found between the cytokines analysis.

The levels of CRP and IL-6 were significantly increased in obese patients ($r = 0.92$ and $r = 1$, respectively). Besides, those patients having IR also had increased CRP levels ($r = 0.95$, $p < 0.0001$), whereas no correlation was found between the levels of IL-6 and the presence of IR. Linear regression analyses demonstrated that obesity, more than IR, is the parameter which rules CRP and IL-6 levels in the PCO patients. Hyperandrogenism parameters did not correlate neither with CRP levels nor with IL-6.

Taken together, and in line with other authors [28,29], these results suggest that the excess of adipose tissue leads to IR and inflammation and that IR promotes the increase in the CRP levels. Thus, obesity is the main cause of the chronic inflammatory status observed in PCO patients.

It is noteworthy that in our study group, hyperandrogenism was correlated with obesity.

To sum up, our results demonstrate the relationship between obesity, IR, a low degree of chronic inflammation and the levels of circulating sex hormones. The present work demonstrates the presence of a low degree of inflammation in young women with PCO and that these parameters are firstly related to obesity and, to a lesser extent, to IR. These results might be a useful tool for the treatment and follow up of patients with PCO.

Acknowledgements

The authors thank Dr Guillermo G. Nuñez for the assistance in the translation of this manuscript.

Declaration of Interest: This work was supported by grants from the University of Buenos Aires and CONICET (National Research Council of Argentina). The authors report no declarations of interest.

References

1. Ehrmann DA. Polycystic ovary syndrome. N Engl J Med 2005;352:1223–1236.
2. Carmina E, Lobo RA. Polycystic ovary syndrome (PCOS): arguably the most common endocrinopathy is associated with significant morbidity in women. J Clin Endocrinol Metab 1999;84:1897–1899.
3. Diamanti-Kandarakis E, Paterakis T, Kandarakis HA. Indices of low-grade inflammation in polycystic ovary syndrome. Ann N Y Acad Sci 2006;1092:175–186.
4. Orio F Jr, Palomba S, Cascella T, Di Biase S, Manguso F, Tauchmanovà L, Nardo LG, et al. The increase of leukocytes as a new putative marker of low-grade chronic inflammation and early cardiovascular risk in polycystic ovary syndrome. J Clin Endocrinol Metab 2005;90:2–5.
5. Legro RS. Diabetes prevalence and risk factors in polycystic ovary syndrome. Obstet Gynecol Clin North Am 2001;28:99–109.
6. Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. Endocr Rev 1997;18:774–800.
7. Duleba AJ, Dokras A. Is PCOS an inflammatory process? Fertil Steril 2012;97:7–12.
8. Lin YS, Tsai SJ, Lin MW, Yang CT, Huang MF, Wu MH. Interleukin-6 as an early chronic inflammatory marker in polycystic ovary syndrome with insulin receptor substrate-2 polymorphism. Am J Reprod Immunol 2011;66:527–533.
9. Tarkun I, Cetinarslan B, Türemen E, Cantürk Z, Biyikli M. Association between Circulating Tumor Necrosis Factor-Alpha, Interleukin-6, and Insulin Resistance in Normal-Weight Women with Polycystic Ovary Syndrome. Metab Syndr Relat Disord 2006;4:122–128.
10. Toulis KA, Goulis DG, Farmakiotis D, Georgopoulos NA, Katsikis I, Tarlatzis BC, Papadimas I, Panidis D. Adiponectin levels in women with polycystic ovary syndrome: a systematic review and a meta-analysis. Hum Reprod Update 2009;15:297–307.

11. Kolbus A, Walch K, Nagele F, Wenzl R, Unfried G, Huber JC. Interleukin-1 alpha but not interleukin-1 beta gene polymorphism is associated with polycystic ovary syndrome. J Reprod Immunol 2007;73:188–193.
12. González F, Rote NS, Minium J, Kirwan JP. Evidence of proatherogenic inflammation in polycystic ovary syndrome. Metab Clin Exp 2009;58:954–962.
13. Wu Y, Zhang J, Wen Y, Wang H, Zhang M, Cianflone K. Increased acylation-stimulating protein, C-reactive protein, and lipid levels in young women with polycystic ovary syndrome. Fertil Steril 2009;91:213–219.
14. Orio F Jr, Palomba S, Cascella T, De Simone B, Di Biase S, Russo T, Labella D, et al. Early impairment of endothelial structure and function in young normal-weight women with polycystic ovary syndrome. J Clin Endocrinol Metab 2004;89:4588–4593.
15. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 2004;81:19–25.
16. American Heart Association. Inflammation, heart disease and stroke: the role of c-reactive protein. http://www.americanheart.org/presenter.jhtml?identifier = 4648.
17. Giallauria F, Orio F, Palomba S, Lombardi G, Colao A, Vigorito C. Cardiovascular risk in women with polycystic ovary syndrome. J Cardiovasc Med (Hagerstown) 2008;9:987–992.
18. Shroff R, Kerchner A, Maifeld M, Van Beek EJ, Jagasia D, Dokras A. Young obese women with polycystic ovary syndrome have evidence of early coronary atherosclerosis. J Clin Endocrinol Metab 2007;92:4609–4614.
19. Puder JJ, Varga S, Kraenzlin M, De Geyter C, Keller U, Müller B. Central fat excess in polycystic ovary syndrome: relation to low-grade inflammation and insulin resistance. J Clin Endocrinol Metab 2005;90:6014–6021.
20. Samy N, Hashim M, Sayed M, Said M. Clinical significance of inflammatory markers in polycystic ovary syndrome: their relationship to insulin resistance and body mass index. Dis Markers 2009;26:163–170.
21. Spranger J, Kroke A, Möhlig M, Hoffmann K, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF. Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Diabetes 2003;52:812–817.
22. Ford ES. The metabolic syndrome and C-reactive protein, fibrinogen, and leukocyte count: findings from the Third National Health and Nutrition Examination Survey. Atherosclerosis 2003;168:351–358.
23. Freeman DJ, Norrie J, Caslake MJ, Gaw A, Ford I, Lowe GD, O'Reilly DS, et al.; West of Scotland Coronary Prevention Study. C-reactive protein is an independent predictor of risk for the development of diabetes in the West of Scotland Coronary Prevention Study. Diabetes 2002;51:1596–1600.
24. Kelly CC, Lyall H, Petrie JR, Gould GW, Connell JM, Sattar N. Low grade chronic inflammation in women with polycystic ovarian syndrome. J Clin Endocrinol Metab 2001;86:2453–2455.
25. Fenkci V, Fenkci S, Yilmazer M, Serteser M. Decreased total antioxidant status and increased oxidative stress in women with polycystic ovary syndrome may contribute to the risk of cardiovascular disease. Fertil Steril 2003;80:123–127.
26. Toulis KA, Goulis DG, Farmakiotis D, Georgopoulos NA, Katsikis I, Tarlatzis BC, Papadimas I et al. Adiponectin levels in women with polycystic ovary syndrome: a systematic review and a meta-analysis. Hum Reprod Update 2009;15:297–307.
27. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 2001;286:327–334.
28. Jakubowska J, Bohdanowicz-Pawlak A, Milewicz A, Szymczak J, Bednarek-Tupikowska G, Demissie M. Plasma cytokines in obese women with polycystic ovary syndrome, before and after metformin treatment. Gynecol Endocrinol 2008;24:378–384.
29. Olszanecka-Glinianowicz M, Banas M, Zahorska-Markiewicz B, Janowska J, Kocelak P, Madej P, Klimek K. Is the polycystic ovary syndrome associated with chronic inflammation per se? Eur J Obstet Gynecol Reprod Biol 2007;133:197–202.