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Short communication

The hallmark of pro- and anti-inflammatory cytokine ratios in women with polycystic ovary syndrome

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ABSTRACT

Polycystic Ovary Syndrome (PCOS) is a heterogeneous endocrinopathy considered to be the most common metabolic disorder in women of reproductive age. Women with PCOS present with an increased risk of non-communicable diseases (NCDs), especially low-grade chronic inflammation mediated by proinflammatory cytokines, and insulin resistance. This study aimed to investigate cytokine levels and their ratios in PCOS women compared to a healthy control group. This study evaluated 97 women with PCOS and 99 healthy women as controls. The PCOS diagnosis was performed according to ESHRE/ASRM. Plasma cytokines were evaluated by flow cytometry. We observed lower TNF levels, and decreased TNF/IL-6, TNF/IL-2, and TNF/IL-4 ratios in PCOS patients compared to the control group ($p < 0.05$). These results indicate an imbalance between pro- and anti-inflammatory cytokines, with prominent counter-regulatory cytokine production. These changes may be important in explaining the phenotypes present in PCOS and to direct better interventions for patients with this syndrome.

1. Introduction

Polycystic Ovary Syndrome (PCOS) is a multi-symptom endocrinopathy that results from androgen excess, particularly testosterone, and ovarian dysfunction [1]. The prevalence of PCOS can range from 6% to 20%, depending on the diagnostic criteria. PCOS is one of the most common metabolic/endocrine disorders in women of reproductive age [2]. The syndrome has heterogeneous clinical manifestations, and women with hyperandrogenic phenotypes are at a higher risk of developing dyslipidemia, hypertension, and type 2 diabetes mellitus (T2DM) at a younger age when compared with healthy women of the same age [3].

Adipose tissue is an active organ that secretes adipokines, hormones, and cytokines; it is associated with endocrine processes that regulate immunity and inflammatory response, glucose, and fatty metabolism, and reproductive capacity [4]. The pathophysiology of PCOS is not yet well known, but it has been suggested that abdominal obesity, hyperandrogenism, and insulin resistance are associated with its development [5]. A low-grade chronic inflammation mediated by proinflammatory cytokines may be induced by excess central fat in women with PCOS [6]. The aim of this research was to investigate plasma cytokine levels and their ratio in PCOS women compared with a healthy control group.

2. Methods

This study was approved by the local Ethics Committee (COEP) of the Federal University of Minas Gerais (UFMG) (n. CAAE 0379.0.203.000–11). Written informed consent was obtained from all participants before inclusion in the study. This case-control study included 97 women with PCOS (aged from 20 to 44 years) and 99 healthy women as a control group (18 to 45 years). The PCOS group was selected at Hospital Borges da Costa - UFMG, Brazil, from 2011 to 2013. The control group was composed of employees and students from UFMG selected in the same period. The European Society of Human Reproduction/Embryology and the American Society for Reproductive Medicine criteria (ESHRE/ASRM) was used for diagnosis of PCOS [7]. The healthy control group was characterized by ovulatory cycles with regular menses lasting 25–35 days and a luteal phase with a serum progesterone level greater than 5 ng/mL. The controls showed normal

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androgen levels, absence of skin manifestations related to androgen excess, and absence of polycystic ovaries on ultrasound. All subjects suspended physical activities 24 h prior to study participation. Individuals that presented with the following conditions were excluded: acute inflammatory disease; diabetes mellitus; thyroid, adrenal, kidney, liver, or autoimmune diseases; cancer; hypogonadism; pregnancy; or hyperprolactinemia. Women using anti-inflammatory medications, insulin, metformin, isotretinoin, antiretroviral, cyclosporine, or oral contraceptives were also excluded. Venous blood samples were collected after fasting for 12 h. The samples were centrifuged at 2500*g* for 20 min at 4 °C to obtain the plasma (EDTA) or serum. Aliquots were stored at −80 °C until use. The determination of plasma cytokines was performed by flow cytometry using the Cytometric Bead Array (CBA) (BD Biosciences), following the manufacturer's instructions. Statistical analyses were performed using the software SPSS version 21.0. Normality was assessed using the Kolmogorov-Smirnov test. Parametric variables were presented as mean and standard deviation; non-parametric were expressed as median and interquartile range. For comparison of parametric data, we used the Student *t*-test. For non-parametric variables, we used the Mann-Whitney. $P < 0.05$ value was considered significant.

3. Results and discussion

The PCOS and control groups had similar age (30.63 ± 4.99 years for PCOS and 29.53 ± 7.06 years for controls) and body mass index (BMI) [28.52 (7.20) kg/m2 for PCOS and 23.69 (5.22) kg/m2 for controls]. No difference in fasting glucose levels was observed between the groups (4.8 ± 9.1 mmol/L for PCOS, 4.7 ± 8.2 for controls, $p = 0.265$), but higher insulin levels were observed in PCOS patients [12.3 (17.4) uUI/mL] when compared to control group [7.7 (3.4) uUI/mL, $p < 0.001$]. The levels of tumor necrosis factor (TNF), transforming growth factor-beta (TGF-β), interleukins (IL) IL-10, IL-6, IL-2, IL-4 and interferon-gamma (IFN-γ) in both groups, along with statistically significant ratios of these cytokines are presented in Table 1. We observed lower TNF levels (Fig. 1**a**), as well as TNF/IL-2 (Fig. 1**b**), TNF/IL-4 (Fig. 1**c**), and TNF/IL-6 (Fig. 1**d**) ratios in women with PCOS compared to the control group.

The understanding of inflammation-related disorders in women with PCOS is an essential component for any measure of prevention of associated diseases, such as cardiovascular disease and T2DM [8].

The TGF-β and INF-γ levels did not show significant differences between the PCOS and control groups. These data corroborate the findings of a randomized clinical trial conducted with 32 volunteers (PCOS: $n = 20$; control: $n = 12$) that assessed the impact of PCOS on the levels of circulating cytokines and the effects of metformin on insulin action and cytokine levels [9]. The study suggested that the association between inflammation in skeletal muscle and insulin resistance in PCOS differed from other insulin-resistant states such as obesity and T2DM, where the circulating levels of TGF-β and INF-γ are not routinely altered by PCOS [9].

Table 1
Concentrations and ratio of biomarkers on PCOS and control groups.

Markers	PCOS group	Control group	p Value
TNF	1.71 (1.25)	2.21 (1.49)	0.011*
TGF-β	7.43 (3.23)	8.43 (6.24)	0.335
IL-10	4.00 (2.29)	3.78 (2.25)	0.779
IL-6	2.60 (3.04)	2.83 (3.38)	0.233
IFN-γ	2.31 (1.13)	2.36 (1.32)	0.111
IL-2	4.38 (0.73)	4.35 (0.64)	0.426
IL-4	5.49 (1.96)	5.12 (1.66)	0.574
TNF/IL-6	0.57 (0.19)	0.61 (0.20)	0.023*
TNF/IL-2	0.40 (0.35)	0.49 (0.39)	0.037*
TNF/IL-4	0.28 (0.41)	0.38 (0.43)	0.010*

Data are median (interquartile range). * p Value < 0.05 was considered statistically significant.

It is known that pro-inflammatory cytokines TNF and IL-6 are closely related to obesity and insulin resistance. Adipose tissue is responsible for producing both cytokines, and many PCOS women present accumulations of visceral fat and alterations in insulin sensitivity [10]. However, in the present study, higher levels of TNF were observed in the control group and no significant difference was observed for IL-6 levels. A possible explanation would be that TNF levels have an inverse correlation with endogenous hyperinsulinemia [11], which was observed in women with PCOS in our study, although this correlation was not significant ($p = 0.279$, $r = -0.043$). Inflammation and glucose are interrelated with mutual causation. Insulin, being the only glucose-lowering hormone in the body, prevents the detrimental effects of hyperglycemia through metabolic regulation. Insulin acts as an anti-inflammatory factor through suppression of proinflammatory cytokines and immune mediators [12]. Curiously, we observed a lower TNF/IL-6 ratio in PCOS women, indicating higher IL-6 production in this group, although its measurement was not sensitive enough to detect this variation between the groups.

No differences in IL-10 were observed between the groups. Although IL-10 is positively regulated in the adipose tissue to limit the systemic pro-inflammatory response commonly observed in obesity [13], a case-control study comparing lean or overweight PCOS women to overweight controls showed no significant differences in levels of IL-10, corroborating our findings in the present study [13].

We found lower TNF/IL-2 and TNF/IL-4 ratios in the PCOS group compared to controls. Although IL-2 and IL-4 are pleiotropic cytokines, their anti-inflammatory effects are commonly seen in several diseases [14,15]. Our results suggest that these cytokines are released as counter-regulators of the sub-clinical and systemic inflammatory process commonly observed in PCOS women, although individually their levels did not differ between the groups.

We assessed the ratios of pro-inflammatory to anti-inflammatory cytokines in subjects with PCOS compared to controls. The overall results indicated that PCOS is related to an imbalance between pro- and anti-inflammatory cytokines, with prominent counter-regulatory cytokine production. The interplay between PCOS, inflammation, abdominal adiposity, hyperandrogenism, and hyperinsulinism could be key in explaining the phenotypes presented by PCOS women and suggest possible interventions for the treatment of PCOS and its complications.

CRediT authorship contribution statement

Jéssica A.G. Tosatti: Writing - original draft, Data curation, Formal analysis. **Mirelle O. Sóter:** Conceptualization, Data curation. **Cláudia N. Ferreira:** Data curation, Writing - review & editing. **Ieda de F.O. Silva:** Data curation. **Ana L. Cândido:** Data curation, Writing - review & editing. **Marinez O. Sousa:** Conceptualization, Project administration. **Fernando M. Reis:** Data curation, Writing - review & editing. **Karina B. Gomes:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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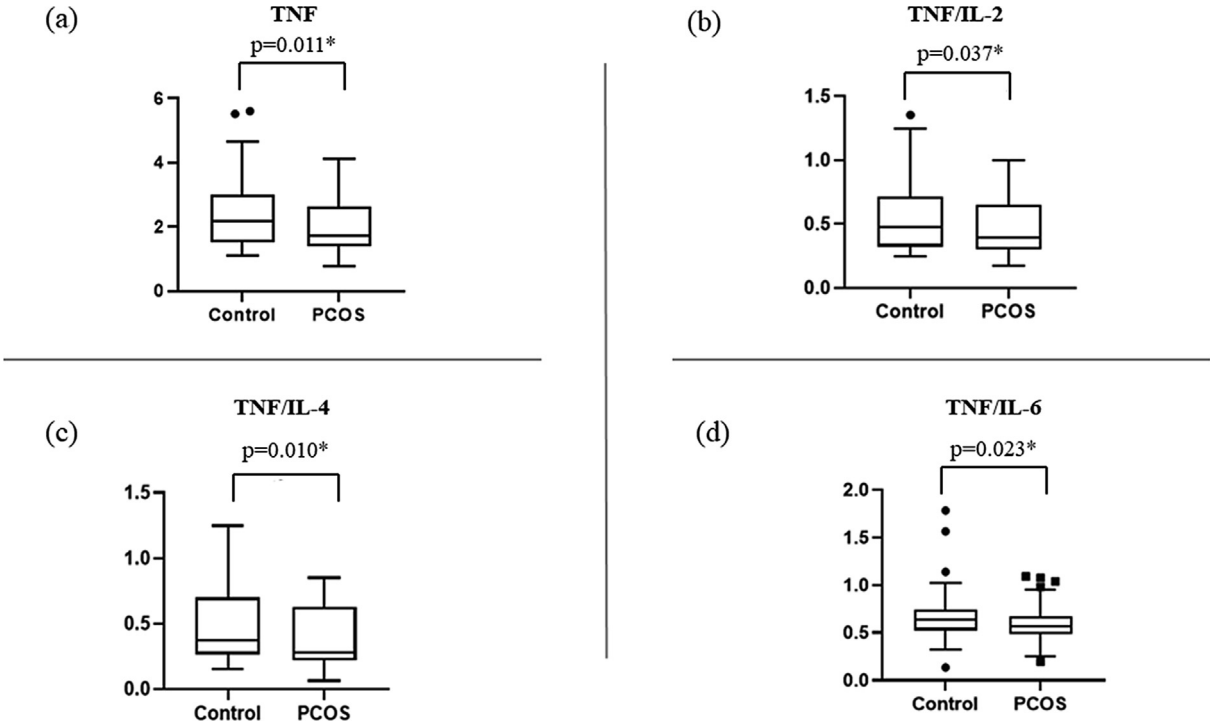

Fig. 1. Comparation of TNF concentration (a) and ratios of TNF/IL-2 (b), TNF/IL-4 (c) and TNF/IL-6 (d) on PCOS and control groups.

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