

Assessment of chemokines MIP-1 α and MIP-1 β in Iraqi women with polycystic ovarian syndrome

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Abstract

Polycystic ovary syndrome (PCOS) is reproductive, endocrine, and metabolic disorder affecting females. The pathology of PCOS is complicated and associated to chronic low-grade inflammation, this includes a disruption in pro-inflammatory factor production, leukocytosis, and endothelial cell dysfunction, also associated with high level of pro-inflammatory cytokines, chemokines and leukocyte count. In addition, PCOS is characterized by hormonal and immunological dysfunction. Inflammation of the ovary affects ovulation and induces or aggravates systemic inflammation. Macrophage inflammatory protein-1 (MIP-1), a pro-inflammatory chemokine, is crucial in the recruitment of inflammatory and immunological cells to the place of inflammation or infection, T- and B-lymphocytes, neutrophils, macrophages, mast cells, dendritic cells and natural killer cells are all capable of producing large amounts of MIP-1. The current study aimed to investigate the role of MIP-1 α and MIP-1 β in Iraqi patients with PCOS and their correlation with obesity and other demographic parameters. This study included two groups, 60 women with PCOS and 30 control women, conducted during the period from October 2022 to January 2023. The diagnosis of PCOS women was based on two out of three of the following diagnostic criteria (hyperandrogenism - oligo or anovulation - polycystic ovaries). MIP-1 alpha and Beta levels were determined by ELISA. The outcomes revealed that the group with PCOS showed significant increase in the level of MIP-1 α (635.28 ± 20.58) than in the control women, (571.20 ± 25.92), ($p < 0.05$). Although there was an increase the level of MIP-1 β in women with PCOS (191.85 ± 17.54) than in the control group (165.31 ± 11.01), the difference did not reach statistical significance. In conclusion, based on our findings, that MIP-1 α and MIP-1 β increased in PCOS cases, this may indicate that PCOS is low grade chronic inflammation.

Keywords: polycystic ovarian syndrome, MIP-1- α and MIP-1- β .

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Introduction

Polycystic ovary syndrome (PCOS) is a hyperandrogenic heterogeneous endocrine condition that affects 15-20% of women of

reproductive age according to Rotterdam criteria¹ which states that PCOS is defined as the presence of at least two symptoms: hyperandrogenism, polycystic ovaries and

oligo/anovulation.^{1,2} It is distinguished by ovulatory failure, androgen excess and/or polycystic ovaries. Moreover, it is a chronic disorder linked with a number of clinical sequelae that may have an influence on PCOS patients' lifetime. Such features include reproductive issues (androgenic alopecia, hirsutism, obstetric troubles, and infertility), metabolic symptoms (insulin resistance and obesity).³ It is essential to remember that PCOS is considered a low-grade inflammatory condition regardless of obesity.⁴

The rise in several inflammatory markers, as well as increased oxidative stress and endothelial dysfunction, provides evidence that PCOS is frequently associated with low-grade systemic inflammation.⁵ Ovarian inflammation impacts ovulation and causes or exacerbates systemic inflammation. Obesity, insulin resistance, and hyperandrogenism are considered to react with systemic inflammation, resulting in PCOS.^{6, 7} Chemokines are crucial in the recruitment of inflammatory and immunological cells to the site of inflammation or infection. They are sometimes referred to as chemoattractant cytokines. A chemokine's usual structure is made up of four cysteine residues. Chemokines are divided into two subfamilies depending on the absence or presence of an amino acid between the first two cysteine residues: CXC (alpha) and CC (beta), it have a variety of biological processes, such as leukocyte recruitment, homeostasis, tumor metastasis, wound healing, angiogenesis, and the induction of innate and acquired immune responses.⁷

The macrophage inflammatory protein 1 α (MIP-1 α), also known as chemokine (C-C motif) ligand 3 (CCL3), is a pro-inflammatory chemokine generated by different cells including dendritic cells, macrophage, neutrophils, lymphocytes, and natural killer cells. It is encoded by CCL3 genes that are located on chromosomes 11 and 17.⁸ The biological role of macrophage inflammatory protein-1 (MIP-1) is to bind to a specific receptor, triggering a series of intracellular events that result in several target cell responses such as degranulation, chemotaxis, mediator production, and phagocytosis.

It is interesting to note that CCL3 can bind to several chemokine receptors including CCR1, CCR4, and CCR5, and have different effects on different immune cell subtypes.⁹ Analysis of upregulation genes for the chemokine CCL3 in cells isolated from obese adipose tissue indicated that adipose tissue of obese is a key source of inflammatory cytokines. Furthermore, macrophage increase in adipose tissue has been linked to the development of insulin resistance and the advancement of type II diabetes. However, not all women with a high body mass index (BMI) suffers insulin resistance.¹⁰ Increased chemokine transfer from dysregulated adipose tissue into the bloodstream causes persistent low-grade inflammation, atherosclerosis, and insulin resistance.¹¹

MIP-1 β , also known as chemokine cysteine–cysteine (C–C) motif ligand (CCL) 4, is a chemokine from the CC family.¹² CCL4 is involved in immune cell chemotactic activity.¹³ Lymphocytes and macrophages are the primary producers of CCL4.¹⁴ It is linked to atherosclerosis and cardiovascular disease.¹⁵ MIP-1 β is a strong chemoattractant for monocytes/macrophages, T cells and dendritic cells through its associated receptor CCR5.¹⁶ It is a particularly potent chemoattractant for Th1 cells, which have anticancer properties.¹⁷ MIP-1 β has been found to trigger the human acute inflammatory response by increasing the levels of pro-inflammatory cytokines tumor necrosis factor alpha (TNF- α), interleukin 6 (IL-6), and IL-18.¹⁸ Furthermore, high circulating MIP-1 β levels have been seen in people who have cardiovascular disease and/or type 2 diabetes.¹³

The current study aimed to determine the role of two inflammatory mediators, MIP-1 α and MIP-1 β , in Iraqi patients with PCOS and their correlation with obesity and other demographic parameters.

Subjects and Methods

The study was conducted on a sample of PCOS patients on infertility consultation, Medical City of Baghdad. A total of 90 women were enrolled in the study, 60 PCOS women, along with 30 non-PCOS (control) women. The average age of

the patients and control groups was 20-40 years.

The study protocol was reviewed and approved by the Ethics Committee, College of Science, University of Baghdad (Ref. No.: CSEC/1022/0120, dated October 2022). A written informed consent was obtained from each patient and control before participating in this study.

All parameters were determined using serum samples that were collected from PCOS and non-PCOS women at the early follicular phase. Diagnosis of PCOS was based on the 2003 Rotterdam criteria during the period from October 2022 to January 2023.^{2, 19} Laboratory tests included chemokine (MIP-1 α and MIP-1 β). Venous blood samples (5 ml) were obtained from all participants, and serum was separated and stored at (-20°C) until used.

The chemokines (MIP-1 α and MIP-1 β) values were measured by an ELISA technique using commercial immunological kits (Cat NO: ELK1115, ELK Biotechnology company, China,

and Cat NO: ELK1117, ELK Biotechnology company, China, respectively), according to the manufacturer's instructions. The optical density of the obtained final ELISA products was measured by an ELISA reader apparatus (Universal microplate reader, ELX 800, UK). BMI was measured according to the equation of weight divided by square height in meters, also waist hips ratio was calculated.

Statistical analysis

The Statistical Analysis System (SAS, 2018) program was used to perform different statistical analysis and to determine the effect of different factors on study parameters.

Results

The present study revealed that the MIP-1 α value was significantly increased in PCOS patients (635.28 \pm 20.58) compared to controls (571.20 \pm 25.92, $p < 0.05$). MIP-1 β was higher in PCOS patients (191.85 \pm 17.54) but the difference than controls (165.31 \pm 11.01) was not significant ($p > 0.05$), as shown in Table 1.

Table 1. Comparison between MIP-1 alpha and MIP-1 Beta levels in patients and control groups.

Studied Group	Mean \pm SE	
	MIP α	MIP 1 β
Patients	635.28 \pm 20.58	191.85 \pm 17.54
Controls	571.20 \pm 25.92	165.31 \pm 11.01
<i>p</i> -value	0.0492	NS

$P > 0.05$ is not significant (NS).

In addition, there was a significant difference in age between PCOS patients (27.05 \pm 0.71 years) and controls (29.73 \pm 0.87 years), and a significant increase in the mean of BMI between patients (28.96 \pm 0.75) and controls (24.93 \pm 0.44, $p < 0.05$). While there was no significant difference in waist to hips ratio, they were (0.825 \pm 0.008), (0.824 \pm 0.007) in the PCOS

patients and controls, respectively. The number of borne children in the control group (1.433 \pm 0.23) was significantly higher than those of patients (0.631 \pm 0.12) (Table 2). Data in Table 3 show that there was significant increase in the percentage of infertility in PCOS patients (45.33 %) when compared to controls (8 %, $p < 0.05$).

Table 2. Comparison between Age, BMI, Waist hips Ratio in patients and control groups.

Group	Mean \pm SE		
	Age (year)	BMI (kg/m ²)	Waist/hips Ratio
Patients	27.05 \pm 0.71	28.96 \pm 0.75	0.825 \pm 0.008
Controls	29.73 \pm 0.87	24.93 \pm 0.44	0.824 \pm 0.007
<i>p</i> -value	0.0242	0.0004	NS

$P > 0.05$ is not significant (NS).

Table 3. Comparison between percentage of infertility in the patients and control group.

Studied group	Have Children	No children	p-value
Patients	54.67 %	45.33 %	0.0008
Control	92 %	8 %	

* $P \leq 0.05$ is significant.

The correlation between MIP-1 α and MIP-1 β and demographic data is illustrated in (Table 4). There was no significant correlation between age, BMI, waist to hips ratio, and MIP-1 α and MIP-1 β . There was a significant correlation

between MIP-1 β and the duration of disease while MIP-1 α exhibited no significant correlation. Finally, the MIP-1 subtypes showed no correlation with the number of born children.

Table 4. Correlation coefficient between MIP-1 α and MIP-1 β and other studied parameters.

Parameters	Correlation coefficient-r	
	MIP α	MIP 1 β
Age	0.22	0.06
BMI	0.03	0.08
Waist hips ratio	0.13	0.14
Duration of PCOS	0.12	0.34
Fertility	0.11	0.06

Discussion

PCOS is a prevalent reproductive disorder that can result in infertility and has substantial social, medical, and economic ramifications for individuals, however, its causes remain unknown.²⁰ A previous published research studied the levels of MCP -1 and MIP-1 α in PCOS women.²¹

Ovarian inflammation impacts ovulation and causes or exacerbates systemic inflammation. Earlier investigations indicated that significantly greater levels of inflammatory cells were discovered in the peripheral blood of PCOS patients including neutrophils and lymphocytes.²² The purpose of this study was to find the role of C-C chemokines like MIP-1 α (CCL3) and MIP-1 β (CCL4) in women with PCOS, and their correlation with obesity and other demographic parameters.

The current study is similar to some previous studies that concluded an increase in the levels of MIP-1 α , according to available data, MIP-1 α levels were increased in PCOS patients more than women without PCOS. Continuous MIP-1 α production is linked to long-term metabolic problems and an increased risk of cardiovascular disease.²²⁻²⁴ Although the specific

mechanisms are not entirely known, multiple studies have shown that obesity and insulin resistance have a reciprocal impact on elevated inflammation, implying a probability of initiating or modifying the role of both conditions on PCOS pathogenesis.²⁴ In the presence of abdominal obesity (visceral adiposity) which is associated with insulin resistance, these adipocyte exerts paracrine and endocrine effect by secretion of inflammatory markers.²⁵ This was approved by an increase of C-Reactive protein level (CRP) which is produced by hepatocytes under stimulation of pro-inflammatory cytokines like IL-6, TNF α , and IL-18. CRP act as a mediator in the inflammatory cascade may lead to endothelial dysfunction and promote monocyte chemoattractant protein -1 chemotaxis.²⁶ CRP acts as a mediator in inflammatory cascade and may lead to endothelial dysfunction and promote monocyte chemoattractant protein -1, MIP-1 α chemotaxis in addition to increased white blood cells.²⁷

Our main observation was that the level of MIP-1 β was increased in women with PCOS, but the increase did not reach statistical significance ($p=0.301$). Previous studies indicated that apoptosis was attributed to ovarian

anovulation. In PCOS ovaries, they observed a significantly high number of macrophages and a relatively high number of lymphocytes. Activated macrophages and lymphocytes can produce pro-apoptotic cytokines. Macrophages are able to phagocytose apoptotic follicular cells. Lymphocytes can facilitate cytotoxicity, mediate follicular cell apoptosis, and cause follicular shrinkage. As a result, the researchers noted that increased macrophage, lymphocyte numbers especially attract cytotoxic CD8+ T cell, may trigger granular and thecal cell apoptosis via these cytokines.¹⁶ MIP-1 β signaling through CCR5 has been shown to decrease inflammation by attracting regulatory T-cells.²⁸ We postulate that lymphocytes and monocytes production of MIP-1 β is changed. As a result, the link between CCL4 levels in plasma and PCOS condition may be obscured. MIP-1 β was negatively associated with diabetes mellitus, it has been demonstrated to be negatively proportionate to the cell stress marker proinsulin.²⁸

The non-significant difference in mean age between PCOS patients and controls in this study might be attributed to participant selection. Females with PCOS had a greater BMI than controls, the results of this study are in agreement with previous reports^{29,30} who found a high frequency of BMI in women with PCOS. Obesity and weight increase are typical clinical and biochemical symptoms of PCOS, in women who are genetically susceptible to it. As a result, there are strong correlations between obesity and PCOS, most PCOS women (38%-88%) are either obese or overweight which leads to an increase in waist-to hips ratio related to abdominal obesity.³¹

The results of the present study demonstrated that the number of children born for women in the control group was significantly higher than those born for patients ($p < 0.05$). This finding of our study is compatible with a previous work,¹ which elicited a direct association between PCOS and irregular or delayed menstruation. Thus, having no children can be considered a symptom for PCOS diagnosis,^{32,33,34} this agreed with the results of the current study, correlated MIP-1 β with duration of PCOS and pregnancy delay. Mild

inflammation is physiologically normal during the follicle formation and ovulation processes. Nevertheless, abnormal inflammation can lead to oocyte quality problems, oligo-anovulation, and infertility.^{35,36}

There are certain limitations to our study that should be noted. The relatively small sample size of the patients and controls is a restriction. Furthermore, they were chosen from a single center/hospital and therefore, may not be representative of the whole community. Further multicenter studies with larger sample size would be helpful to verify the current study findings.

Based on our study findings, we may conclude that MIP-1 α and MIP-1 β increased in PCOS. This may prove their role in PCOS pathophysiology, and that the disease is of low-grade chronic inflammation.

Author Contributions

SFA designed the research, performed the statistical analysis and revised the manuscript. SKI collected the data and wrote the manuscript. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Ethics Committee, College of Science, University of Baghdad (Ref. No.: CSEC/1022/0120, dated October 2022).

Informed consent

A written informed consent was obtained from each patient and control before participating in this study.

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