Endocrinol Nutr. 2015;62(1):31–37

ENDOCRINOLOGÍA Y NUTRICIÓN

www.elsevier.es/endo

ORIGINAL ARTICLE

Macrophage migration inhibitory factor in obese and non obese women with polycystic ovary syndrome☆

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Received 18 January 2014; accepted 28 September 2014
Available online 2 January 2015

KEYWORDS
Polycystic ovary syndrome;
Macrophage migration inhibitory factor;
Obesity

Abstract
Objective: To measure macrophage migration inhibitory factor (MIF) concentrations in obese and non-obese women diagnosed with polycystic ovary syndrome (PCOS).
Methods: Women diagnosed with PCOS and age-matched healthy controls with regular menses and normal ovaries on ultrasound examination were selected and divided into 4 groups (group A, PCOS and obese; group B, PCOS and non-obese; group C, obese controls; and group D, non-obese controls) based on body mass index (obese >30 kg/m^2 and non-obese <25 kg/m^2). Luteinizing hormone, follicle-stimulating hormone, androstenedione, testosterone, sex hormone-binding globulin, serum glucose, insulin and MIF levels were measured.
Results: Obese and non-obese women with PCOS had higher luteinizing hormone, follicle-stimulating hormone, androstenedione, testosterone, and insulin levels as compared to the obese and non-obese control groups, respectively ($P < .0001$). Women with PCOS had significantly higher MIF levels (group A, 48.6 ± 9.9 mg/ml; group B, 35.2 ± 6.0 ng/ml) as compared to controls (group C, 13.5 ± 6.0 ng/ml; group D, 12.0 ± 4.3 ng/dl; $P < .0001$). A weak, positive and significant correlation was seen between fasting blood glucose and insulin levels in women with PCOS ($P < .05$).
Conclusion: Significant differences exist in plasma MIF levels between obese and non-obese women with and without PCOS.

☆ Please cite this article as: Mejia-Montilla J, Álvarez-Mon M, Reyna-Villasmil E, Torres-Cepeda D, Santos-Bolívar J, Reyna-Villasmil N, et al. Factor inhibidor de la migración de macrófagos en mujeres obesas y no obesas con síndrome de ovarios poliquísticos. Endocrinol Nutr. 2015;62:31–37.

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2173-5093/

PALABRAS CLAVE
Síndrome de ovarios poliquísticos;
Factor inhibidor de la migración de macrófagos;
Obesidad

Factor inhibidor de la migración de macrófagos en mujeres obesas y no obesas con síndrome de ovarios poliquísticos

Resumen
Objetivo: Determinar las concentraciones del factor inhibidor de la migración de macrófagos (MIF) en mujeres obesas y no obesas con diagnóstico de síndrome de ovarios poliquísticos (SOPQ).
Método: Se seleccionaron mujeres con diagnóstico de SOPQ y controles sanas, de edades similares, con menstruaciones regulares y ovarios normales por ecografía, que fueron divididas en 4 grupos (grupo A: SOPQ obesas; grupo B: SOPQ no obesas; grupo C: controles obesas, y grupo D: controles no obesas) de acuerdo con el índice de masa corporal (obesas > 30 kg/m^2 y no obesas < 25 kg/m^2). Se analizaron las concentraciones de lutoprina, folitropina, androstendiona, testosterona, globulina fijadora de hormonas sexuales, glucosa sérica, insulina y MIF.
Resultados: Las mujeres con SOPQ obesas y no obesas presentaron concentraciones más elevadas de lutoprina, folitropina, testosterona, androstendiona e insulina comparadas con las mujeres del grupo control de obesas y no obesas, respectivamente ($p < 0,0001$). Se observó que las mujeres con SOPQ presentaron concentraciones significativamente más altas de MIF (grupo A: $48,6 \pm 9,9$ mg/ml, y grupo B: $35,2 \pm 6,0$ ng/ml) comparadas con las controles (grupo C: $13,5 \pm 6,0$ ng/ml, y grupo D: $12,0 \pm 4,3$ ng/dl; $p < 0,0001$). Se observó que las concentraciones del MIF presentaban una correlación débil, positiva y significativa con los valores de glucemia e insulina en ayunas en las mujeres con SOPQ ($p < 0,05$).
Conclusión: Existen diferencias significativas en las concentraciones plasmáticas del MIF entre las mujeres con SOPQ obesas y no obesas respecto a las controles normales.

Introduction

Polycystic ovary syndrome (PCOS), characterized by hyperandrogenism, chronic anovulation, and infertility, is one of the most common endocrine disorders in women. In addition to reproductive abnormalities, a significant proportion of women with PCOS have obesity, insulin resistance, and characteristics of metabolic syndrome.[1,2] Improvements in these metabolic abnormalities, especially those related to insulin resistance, due to lifestyle changes or drug intervention, have been shown to improve hyperandrogenism and infertility.[3]

Different studies have suggested that PCOS may be associated with increases in biochemical and physiological cardiovascular risk factors, including endothelial dysfunction. Women with PCOS have specific cardiovascular risk factors such as obesity, lipid profile abnormalities, impaired glucose tolerance, and hypertension, and therefore have a high risk of coronary heart diseasea.[4] These women have also been found to have a high risk of non-insulin-dependent diabetes mellitus.[5] It is not yet clear whether this increased risk is related to endocrine abnormalities associated with PCOS itself, such as hyperandrogenism, or wheter it is the consequence of anthropometric or metabolic abnormalities.

Macrophage migration inhibition factor (MIF) is a proinflammatory cytokine whose plasma levels are increased after endotoxin injection in experimental animals and which is also involved in the inflammatory cascade following endotoxin administration.[6,7] The endotoxin levels required to stimulate MIF are 10–100 times lower than those needed to activate the secretion of tumor necrosis factor alpha (TNF-alpha).[8] MIF is secreted by a variety of cell types which, in addition to immune cells, include macrophages/monocytes, B and T cells, and endothelial, endocrine, and epithelial cells.[9] MIF is the only cytokine that is stored in secretory cells and is rapidly released in response to a stimulus. In addition, it is also secreted by cells after de novo synthesis in response.[6,7]

MIF may play a role in atherogenesis through the stimulation of macrophages/foam cells in the atherosclerotic plaque.[8] Evidence shows that MIF also controls the metabolic and inflammatory processes underlying the development of metabolic disorders, such as glucose homeostasis during stress periods and macrophage infiltration into adipose tissue. MIF is expressed and secreted by adipose tissue, and one would therefore expect it to be elevated in obesity, similarly to TNF-alpha and interleukin-6.[10] In view of the relationship between obesity, inflammation, and atherosclerosis, on the one hand, and the role of MIF in inflammation, on the other hand, it has been suggested that MIF levels may be increased in patients with PCOS, regardless of obesity.

This research was intended to measure plasma levels of MIF in obese and non-obese women diagnosed with PCOS.

Methods

From September 2009 to July 2012, women attending the outpatient clinics of internal medicine, endocrinology, and gynecology of Hospital Central Dr. Urquinaona diagnosed with PCOS were enrolled into the study. The ethics committee of the hospital approved the study, and written consent was obtained from all women.

The diagnosis of PCOS was confirmed using the following criteria: evidence of oligoanovulation (less than six menstrual periods in the previous year), clinical or biochemical signs of hyperandrogenism (plasma testosterone levels above the upper limit of normal and an abnormal luteinizing hormone [LH]/follicle-stimulating hormone [FSH] ratio >2), and normal or enlarged ovaries (>10 mL) with the presence of subcapsular microcysts (12 or more) 2–9 mm in diameter in the abdominal ultrasound examination.[11]

Women with PCOS and obesity (body mass index [BMI] >30 kg/m^2; group A, n = 34) and non-obese women (BMI <25 kg/m^2; group B, n = 13) were selected. Hormone tests and abdominal ultrasound were performed during the early follicular phase, between the third and fifth days of the spontaneous menstrual cycle. The control group (n = 47) consisted of women of similar ages with regular menstrual periods attending the clinic for a routine gynecological check-up (between 21 and 35 days) and showing no evidence of hyperandrogenism (total testosterone <60 ng/mL, free testosterone <2 ng/mL, dehydroepiandrosterone sulphate <27 picog/mL) and with ultrasonographically normal ovaries, who were seen at the clinic for conditions other than PCOS and who were categorized based on their BMI as obese (BMI >30 kg/m^2; group C, n = 33) or non-obese (BMI <25 kg/m^2; group D, n = 13). All controls were studied on days 3–5 of their menstrual cycles.

Women with thyroid disease (TSH levels less than 0.39 or greater than 4.0 picoIU/mL), hypothalamic–pituitary dysfunction, and ovarian insufficiency (FSH less than 1.4 or greater than 20 mIU/mL and estradiol less than 20 pg/mL), androgen-secreting adrenal or ovarian tumors (total testosterone >200 ng/mL and dehydroepiandrosterone sulphate >800 picog/dL), non-classical congenital adrenal hyperplasia (17 hydroxyprogesterone >3 ng/mL), hyperprolactinemia (prolactin >26 ng/mL), secondary hypertension, active infection, Cushing's syndrome (determined by the suppression test with 1 mg of dexamethasone), vitamin B_{12} or folate deficiency, a history of liver disease, renal failure with creatinine clearance <30 mL/min by 1.73 m^2 body surface area, urinary protein excretion >1 g/day, angina pectoris, myocardial infarction, or recent cerebrovascular disease were excluded from the study. Women with secondary arterial hypertension were excluded based on clinical and laboratory tests. Women who were taking antihypertensive drugs were excluded from the study, and those taking lipid lowering drugs were asked to discontinue them four weeks before the study began. No patient was taking drugs affecting inflammation marker levels (e.g. oral contraceptives or insulin sensitizing drugs).

Ultrasound examination was performed using General Electric Logiq® Pro 3 ultrasound equipment with a convex 3.5 MHz abdominal transducer and a 5 MHz vaginal transducer. The BMI was calculated by dividing weight by squared height (kg/m^2), while the waist/hip ratio was calculated by dividing waist circumference by hip circumference. Waist circumference was measured at the midpoint between the lower costal margin and the iliac crest, and the hip was measured at the widest part of the gluteal region. Measurements were taken with a measuring tape graduated in centimeters, with the subject standing and with the arms in the anatomical position.

All venous blood samples were drawn under fasting conditions within one week of spontaneous or induced menstruation. All samples were similarly handled and were stored at −8 °C for 1–3 days. FSH, LH, estradiol, androstenedione, and testosterone levels were measured by radioimmunoassay and chemiluminescence using commercial kits (Immulite® 2000, Diagnostic Product Corp, Los Angeles, USA). Intra-assay and inter-assay coefficients of variation were 4% and 7% for FSH, 6% and 7% for LH, 7% and 9% for estradiol, 6% and 10% for androstenedione, and 4% and 7% for testosterone respectively. Sex hormone binding globulin was quantified by immunoassay (AutoDELFIA® Immunoassay analyzer, PerkinElmer, Massachusetts, USA); the inter-assay and intra-assay coefficients of variation were 3% and 4% respectively.

Serum glucose was quantified by the glucose oxidase method (Pointe Scientific Inc., Massachusetts, USA). Intra-assay and inter-assay coefficients of variation were 1.4% and 1.9% respectively. Insulin was measured by radioimmunoassay (Coat-A-Count®, Diagnostic Products Corp., Los Angeles, USA). Intra-assay and inter-assay coefficients of variation were 1.6% and 5.5% respectively. Plasma MIF levels were measured using an ELISA test (R&D Systems, Minneapolis, USA). Intra-assay and inter-assay coefficients of variation were 3.5% and 12% respectively.

Data are given as mean ± standard deviation. A Student's *t*-test for unrelated samples was used to compare the clinical and laboratory characteristics of women with PCOS and the control group. The same test was used to compare data from women in groups A and B to those from women in groups C and D. Correlation coefficients between MIF levels in women with PCOS and laboratory parameters were assessed using a Pearson's test. A linear regression analysis was conducted between the different laboratory parameters and levels of the three study markers. A value of $p < 0.05$ was considered statistically significant.

Results

Table 1 shows the clinical and endocrine characteristics of women with PCOS and controls. Groups were similar as regards age ($p = 0.2403$) and BMI ($p = 0.4445$). Findings confirmed the differences between women with PCOS and controls. LH and the FSH levels and FSH/LH ratio were significantly higher in women with PCOS as compared to the control group ($p < 0.0001$). No statistically significant differences were found in estradiol levels ($p = 0.5134$). Testosterone and androstenedione levels were significantly higher in women diagnosed with PCOS ($p < 0.0001$). SHBG levels were significantly lower in women with PCOS as compared to controls. Higher fasting insulin and blood glucose levels were also found in patients with PCOS as compared to controls ($p < 0.0001$).

MIF values are shown in Table 1. Women with PCOS showed significantly higher levels (44.9 ± 10.9 ng/dL) than the mean values seen in control women (12.9 ± 5.1 ng/dL; $p < 0.0001$).

Table 2 shows the characteristics of obese women with PCOS (group A; n = 34), non-obese women with PCOS (group B; n = 13), obese controls (group C; n = 33), and non-obese controls (group D; n = 14). No age-related statistically

Table 1 Characteristics of patients with PCOS and controls.

	Patients with PCOS (n = 47)	Controls (n = 47)	*p*
Age, years	23.2 ± 2.8	24.0 ± 3.7	0.2403
Body mass index, kg/m^2	30.6 ± 5.1	29.8 ± 5.0	0.4445
Waist/hip ratio	0.9 ± 0.1	0.9 ± 0.1	0.9999
LH, mIU/mL	9.6 ± 3.1	3.1 ± 0.8	<0.0001
FSH, mIU/mL	6.3 ± 0.9	3.8 ± 1.1	<0.0001
FSH/LH ratio	0.8 ± 0.4	1.3 ± 0.4	<0.0001
Estradiol, pg/mL	52.3 ± 5.1	53.2 ± 7.9	0.5134
Testosterone, ng/mL	5.0 ± 1.2	3.0 ± 0.8	<0.0001
Androstenedione, ng/mL	2.6 ± 0.4	1.9 ± 0.5	<0.0001
SHBG, ng/mL	1.6 ± 0.3	3.3 ± 0.4	<0.0001
Serum fasting insulin, mU/L	22.7 ± 8.2	6.2 ± 0.6	<0.0001
Serum fasting glucose, mg/dL	112.0 ± 16.8	94.4 ± 9.0	<0.0001
Macrophage migration inhibition factor, ng/mL	44.9 ± 10.9	12.9 ± 5.1	<0.0001

significant differences were found between women in the four groups (p = NS). Women in both PCOS groups (Table 2) had higher values of LH, FSH, the FSH/LH ratio, testosterone, and androstenedione as compared to women in groups C and D ($p < 0.0001$). No significant differences in estradiol levels were found between women in groups A and B and those in groups C and D ($p = 0.5360$ and $p = 0.5016$ respectively). On the other hand, SHBG levels were lower in both groups of women diagnosed with PCOS as compared to controls ($p < 0.0001$). As regards insulin levels, women in groups A and B had significantly higher values than those in the control groups C and D. Obese and non-obese women with PCOS had significantly higher serum glucose levels as compared to obese and non-obese controls respectively ($p < 0.0001$).

Obese women with PCOS had significantly higher MIF levels than obese controls (48.6 ± 9.9 versus 13.5 ± 6.0 ng/dL; $p < 0.05$). Similarly, significantly higher MIF levels were found in non-obese patients with PCOS as compared to non-obese control women (35.2 < 6.7 versus 12.0 ± 4.3. ng/dL; $p < 0.0001$).

When obese and non-obese women with PCOS were analyzed, a significant correlation was found between MIF levels and fasting blood glucose ($r = 0.285$; $p < 0.0001$) and fasting insulin levels ($r = 0.272$; $p < 0.0001$). Linear regression analysis showed that the factors affecting plasma MIF levels were insulin (beta = 0.344; $p < 0.003$) and serum glucose levels (beta = 0.665; $p < 0.001$).

Discussion

The results of the research show that obese and non-obese women with PCOS have higher MIF levels as compared to

Table 2 Characteristics of obese and non-obese patients with PCOS and controls.

	Group A, PCOS obese (n = 34)	Group B PCOS non-obese (n = 13)	Group C, obese controls (n = 33)	Group D, non-obese controls (n = 14)
Age, years	23.1 ± 2.9	23.6 ± 2.8	24.0 ± 3.6	23.7 ± 4.0
Body mass index, kg/m^2	33.5 ± 1.8	23.1 ± 2.0	32.7 ± 2.4	23.0 ± 1.2
Waist/hip ratio	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1
LH, mIU/mL	9.2 ± 3.1	10.5 ± 3.2**	3.0 ± 0.7	3.0 ± 0.8
FSH, mIU/mL	6.3 ± 0.9*	6.5 ± 0.8**	3.8 ± 1.1	3.9 ± 0.9
FSH/LH ratio	0.8 ± 0.4*	0.7 ± 0.4**	1.3 ± 0.5	1.4 ± 0.4
Estradiol, pg/mL	52.4 ± 5.3	52.0 ± 4.3	53.3 ± 8.4	52.8 ± 6.9
Testosterone, ng/mL	5.2 ± 1.1*	4.4 ± 1.2**	2.9 ± 0.8	3.2 ± 0.7
Androstenedione, ng/mL	2.6 ± 0.4*	2.5 ± 0.3**	1.9 ± 0.5	1.9 ± 0.6
SHBG, ng/mL	1.6 ± 0.4*	1.8 ± 0.3**	3.2 ± 0.4	3.6 ± 0.4
Serum fasting insulin, mU/L	27.1 ± 4.9*	11.4 ± 1.7**	6.1 ± 0.5	6.3 ± 0.6
Serum fasting glucose, mg/dL	115.7 ± 13.1*	102.3 ± 13.1**	94.7 ± 12.1	93.7 ± 9.9
Macrophage migration inhibition factor, ng/mL	48.6 ± 9.9*	35.2 ± 6.0**	13.5 ± 6.0	12.0 ± 4.3

* <0.0001 versus obese control women.
** <0.0001 versus non-obese control women.

control women. González et al.[12] first demonstrated that MIF levels were higher in women with PCOS, regardless of obesity.

Decreased insulin sensitivity is the underlying defect in most patients with PCOS, and is considered to be significant pathological mechanism for the development of cardiovascular disease.[13] Experimental and clinical studies have established the relationship, not only correlative, but also causative, between insulin resistance and chronic inflammation, especially in adipose tissue.[14,15] When macrophages/monocytes infiltrate adipose tissue, they release pro-inflammatory cytokines, and these mediators contribute, by different mechanisms, to the development of cell insensitivity and the vascular disease characteristic of atherosclerosis.[15]

The findings of this research are similar to those reported in previous studies suggesting that obesity is a pro-inflammatory state associated with increases in TNF-alpha, interleukin-6, and C-reactive protein levels, and to an increase in lipid peroxidation and oxidative damage to plasma proteins.[16,17] Increased MIF levels in plasma may contribute to accelerating the atherosclerotic process in obese subjects.[8] However, increased MIF levels in non-obese women with PCOS show accelerated atherogenesis that may occur in the syndrome, regardless of obesity.

Increased plasma MIF levels also indirectly show the increased inflammatory activity of mononuclear cells, because it is well known that, in the arterial wall, monocytes are converted into macrophages and foam cells form atherosclerotic plaques. The inflammatory mechanism may contribute to the pathogenesis of insulin resistance through insulin signaling blockade.[18]

It should be noted that in this research, both obese and non-obese women with PCOS had increased plasma levels of MIF, a key mediator in innate and adaptive immunity mechanisms especially those mediated by monocytes/macrophages. Oxidized, low density lipoprotein-laden macrophages form foam cells, which in turn collectively form fatty streaks in the arteries. Lesions with abundant foam cells and a thin fibrous layer are those which will probably break and activate thrombosis related to pro-inflammatory effects.[19] MIF is a product secreted by macrophages and stimulates them after they are secreted. This shows an autocrine and paracrine relationship and is therefore responsible for maintaining foam cell activity in the atherosclerotic plaque. It may also intensify and prolong inflammation by inhibiting foam cell apoptosis cells.[18]

TNF-alpha overexpression in adipose tissue may induce insulin resistance. Since MIF increases the expression of this cytokine, and vice versa,[20] increased levels may cause insulin resistance in adipose tissue through the action of MIF itself and/or the induction of TNF-alpha.

An additional potential role of MIF is to stimulate the secretion of pancreatic islet beta cells.[21] In this study, fasting insulin and blood glucose levels were higher in both obese and non-obese women with PCOS. High amounts of MIF in the pancreas play some role in glucose metabolism. The differentiated cell line INS-1 has the potential to express MIF, and this process may be enhanced by glucose concentration in culture medium. Moreover, in perfusion studies conducted on isolated rat islets, MIF immunoneutralization reduced the first and second phases of glucose-induced insulin secretion by 39% and 31% respectively. It has been speculated that MIF stimulates insulin secretion, which is regulated by glucose. It also acts as an enzyme that reduces and breaks sulfhydryl bonds.[8] This action may potentially decrease the biological activity of insulin and the efficiency of its receptors, which also have sulfhydryl bonds. This may contribute to insulin resistance and increase the need for insulin secretion.[18] It appears reasonable to think that MIF modulates both carbohydrate metabolism and inflammatory and immune responses, counterregulating impairment in homeostasis by the action of glucocorticoid suppression.[22]

Herder et al.[23] reported a strong association between plasma MIF levels and impaired glucose tolerance in a study of 1653 patients with noninsulin-dependent diabetes and impaired glucose tolerance, and normoglycemic control subjects. They also reported an association between high MIF allele expression and an increased risk of noninsulin-dependent diabetes. Church et al.[24] examined plasma levels in 71 obese subjects participating in a weight reduction program with diet. High MIF levels correlated to beta cell dysfunction, and decreased MIF levels were seen after the loss of more than 14 kg in 8 months. Another study in animals provided data supporting the role of MIG in the development of insulin resistance and atherosclerosis by promoting the inflammation of adipose tissue.[25]

In contrast to previous reports,[12,26] this research could not verify the correlation between MIF levels and plasma testosterone and/or androstenedione levels. A possible explanation for this finding is that a higher number of women were selected for this research as compared to the abovementioned study. Prior studies have reported a positive association between circulating levels of androgens and inflammation mediators in women with PCOS.[26,27] Experimentally induced hyperandrogenism promotes the development of atherosclerosis and appears to suppress the immune response mediated by both T and B cells.[28] It is also known that in addition to MIF, various cytokines such as TNF-alpha, interleukin-1 beta and interleukin-6, have multiple effects on hippocampal and pituitary neurons.[29] These brain structures produce in turn neuropeptides such as vasoactive intestinal peptide, somatostatin, and substance P, all of which have a significant impact on the regulation of systemic inflammation.[30]

In conclusion, these observations provide evidence showing that plasma MIF levels are increased in obese and non-obese women with PCOS as compared to healthy control women.

Conflicts of interest

The authors state that they have no conflicts of interest.

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