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*Yaser Abbas Ateia (M.Sc, PhD) [a]
Eman Mahdi Saleh (M.Sc, PhD) [b]
Thikra N. Abdullah (CABOG) [c]
Zynab Al Musawee (M.Sc, PhD) [d]

Levels of Some Proinflammatory Cytokines in Obese Women with Polycystic Ovary Syndrome after Metformin Therapy

ARTICLE INFORMATION

Authors addresses:
[a] Department of Biochemistry
Al Kindy College of Medicine
University of Baghdad.
[b] Department of Microbiology
Al Kindy College of Medicine
University of Baghdad.
[c] Department of Gynecology
Al Kindy College of Medicine
University of Baghdad.
[d] Ministry of Health
Baghdad, Iraq

* Corresponding Author
E-mail address:
ya.1971@yahoo.com

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ABSTRACT

Background: Polycystic ovary syndrome is a heterogeneous disorder and its etiology appears to be complex and multifactorial; characterized by hyperandrogenism, chronic anovulation and infertility. It's associated with evidence of low-grade chronic inflammation, as indicated by the presence of elevated levels of high sensitive C- reactive protein levels, interleukin-6 and tumor necrosis factor-α. The source of excess circulating tumor necrosis factor-α in obese Polycystic ovary syndrome patient is likely to be the adipose tissues while in lean women increased visceral adiposity has been proposed as a source of excess tumor necrosis factor-α.

Objectives: to evaluate the levels of high sensitive C- reactive protein, tumor necrosis factor-α and interleukin-6 in patients with polycystic ovary syndrome before and after treatment with metformin; with emphasis on their relationship with the improvement in ovulation rate and body mass index in Iraqi women.

Methods: 69 Iraqi females with PCOS, with mean age of 25.8±4.4 years, body mass index 31.14±2.23 kg/m^2 and insulin resistant equal to 3.15±0.25. Additionally, 30 healthy fertile women BMI= 26.87±3.1 kg/m^2 and mean age 23.4±2.8 years), the patients were treated with metformin 1500 mg/day for 3 months. Blood samples were obtained in the morning subsequent to an overnight fasting at baseline and at the end of the 12 weeks period of treatment, the samples were analyzed for plasma glucose level estimated by enzymatic colorimetric kit, while serum insulin , TNF-α, IL-6 , hs-CRP, Progesterone and sex hormone binding globulin .

Results: BMI values were significantly increased at baseline value in patients ($P<0.05$) compared with healthy controls, then significantly decreased (12.9%) after treatment compared with baseline values, HOMA-IR index were significantly elevated in patients group at baseline compared with control, and significantly decreased by 17.4% after treatment. Regarding the influence of metformin on inflammatory markers, the present study demonstrated significant elevation of baseline levels ($P<0.05$) of TNF-α, hs-CRP and IL-6 compared with controls, and the baseline levels significantly decreased after treatment by 16%, 38% and 37% respectively. Meanwhile, sex hormone binding globulin levels were significantly decreased in PCOS patients compared with healthy controls, and significantly increased after treatment by 16.6%, also progesterone levels decline at baseline compared with control group, and it was increased significantly after treatment by 24%.

Conclusions: The study detects an increased level of inflammatory cytokines, SHBG and decrease level of progesterone in Iraqi females with PCOS, and metformin therapy improves serum levels of the inflammatory cytokines associated with increased ovulation rate.

Introduction:

polycystic ovary syndrome (PCOS) is a heterogeneous disorder and its etiology appears to be complex and multifactorial; it is one of the most frequent endocrine diseases, affecting about 5-10% of reproductive women [(1)].

PCOS is characterized by hyperandrogenism, chronic anovulation and infertility [(2)].Visceral adiposity and insulin resistance (IR) appear to play a role in the etiology of PCOS in both lean and obese women [(3,4)], and associated with evidence of low-grade chronic inflammation, as indicated by the presence of elevated levels of many markers, including high sensitive C- reactive protein levels (hs- CRP) [(5)], interleukin-6 (IL-6) [(6)] and tumor necrosis factor-α (TNF-α) [(7)]. These proinflammatory cytokines play a significant role in the pathogenesis of the PCOS. Moreover, inflammatory cytokines can induce IR by direct

actions on insulin post-receptor signaling or by inducing central obesity through activation of the hypothalamic-pituitary-adrenal axis [8]. The source of excess circulating TNF-α in obese PCOS patient is likely to be the adipose tissues while in lean women with PCOS, increased visceral adiposity has been proposed as a source of excess TNF-α [9]. Chronic elevation in these cytokines decreases the expression of glucose transporter 4 (GLUT4) in PCOS patients. Elevated level of hs-CRP, IL-6, TNF-α and oxidative stress may contribute to the dysregulation of the theca-interstitial compartment in PCOS [10-12]. IL-6 expression is well correlated with indexes of insulin resistance and decreased with weight loss, in parallel with the improvement in insulin sensitivity [13]. Insulin sensitizing agents, like metformin, have been proposed for the treatment of PCOS; they improve insulin action by increasing insulin sensitivity, and thereby decrease hyperinsulinemia [14]. Recently, metformin has been associated with a significant decrease in serum CRP levels in PCOS women, but not all authors have agreed upon this finding [15]. The present study was designed to evaluate the levels of hs-CRP, TNF-α and IL-6 in patients with PCOS before and after treatment with metformin; with emphasis on their relationship with the improvement in ovulation rate and BMI.

Methods:

This prospective study was conducted from May 2010 to October 2010 in Al-Elwiya Teaching Hospital, where 69 females with PCOS (according to the revised 2003 Rotterdam diagnostic criteria) [16], with mean age of 25.8±4.4, body mass index (BMI) 31.14±2.23 and insulin resistant (HOMA-IR) equal to 3.15±0.25. Additionally, 30 healthy fertile women (BMI= 26.87±3.1 kg/m^2 and age 23.4±2.8 yrs) without clinical evidence of PCOS, were included and served as control group. Patients with evidence of diabetes mellitus at baseline (according to the criteria of American Diabetes Association Expert Committee) [17], hyperprolactinaemia, hypertension, Cushing's syndrome, thyroid dysfunction, androgen secreting tumor, and deficiency of (21-hydroxylase enzyme), use of other medications affecting insulin sensitivity or metabolic parameters were excluded. The patients were treated with metformin 1500 mg/day for 12 weeks. The local Medical Research Ethics Committee, Al-Kindy College of Medicine, University of Baghdad approved the study protocol; all subjects were included after signing informed consent.

Blood samples were obtained in the morning subsequent to an overnight fasting at baseline and at the end of the 12 weeks period of treatment, transferred into plain tubes and left to clot. The samples were centrifuged at room temperature for 10 min. The prepared serum was stored at - 20 ^{0}C until the time of assay. The samples were analyzed for plasma glucose level estimated by enzymatic colorimetric kit obtained from (Randox) [18], serum insulin [19], TNF-α, IL-6 [20], hs-CRP [21], Progesterone [22] and SHBG [23], were determined using ready made analytical kits according to standardized methods obtained from (Sigma-Aldrich).

The data were expressed as mean ± SEM. The results were statistically analyzed using paired Student's t-test and statistical significance was set at P<0.05.

Results:

Table-1 shows that 68% of the patients appeared with hirsutism, 42% with acne; also, the percentages of infertility among the patients were 60%. Meanwhile, the percentage of amenorrhea and oligomenorrhea were 49.4% and 27.5% respectively; only 23.1% of patients have regular menstrual cycle, and the hyperandrogenemia was found in 84%. In table 2, at baseline BMI values were significantly increased in patients (P<0.05) compared with healthy controls, then significantly decreased (12.9%) after 12 weeks of treatment compared with baseline values. Additionally, HOMA-IR index were significantly elevated in patients group at baseline compared with control, and significantly decreased by 17.4% after treatment. Regarding the influence of metformin on inflammatory markers, the present study demonstrated significant elevation of baseline levels (P<0.05) of TNF-α, hs-CRP and IL-6 compared with controls, and the baseline levels significantly decreased after treatment by 16%, 38% and 37% respectively. Meanwhile, SHBG levels were significantly decreased in PCOS patients compared with healthy controls, and significantly increased after 12 weeks of treatment by 16.6% (table 2). Table-2 also shows significant decline in progesterone levels at baseline compared with control group, and it was increased significantly after treatment by 24%.

Table1: Main Clinical characteristics of PCOS patients.

Characteristics	Number of patients (%)
Hirsutism	47 (68%)
Acne	29 (42%)
Infertility	42 (60%)
Amenorrhea	34 (49.4%)
Oligomenorrhea	19 (27.5%)
Regular cycle	16 (23.1%)
Hyperandrogenemia	58 (84%)

Table1: Effects of 12 weeks metformin treatment.

Parameters	Control n=27 (Mean ± SEM)	Osteoporotic n=30 (Mean ± SEM)		Changes (%)
		Baseline	After 12 Weeks	
BMI (kg/m^2)	26.9±2.1	31.1±2.2 †	27.1±1.2 *	12.9
HOMA-IR	1.74±0.2	3.2± 0.3 †	2.6±1.3*	17.4
TNF-α (pg/ml)	14.3±1.1	32.0±2.2 †	26.6±2.1 *	16
hs-CRP (mg/L)	2.64±1.2	5.6±1.4 †	3.5±1.7*	38
IL-6 (pg/ml)	16.4±2.2	19.6±2.4 †	12.2±3.1 *	37
SHBG (nmol/L)	119.4±3.3	96.9±7.0 †	113.2±8.1*	16.6
Progesterone (nmol/L)	16.3±0.02	12.8±0.9 †	15.9±1.2 *	24

† Significant differences with controls (P<0.05)
* Significant difference compared with baseline values (P<0.05).

Discussion:

The present study demonstrated that the main features of PCOS patients presented in table 1 were in line with the characters of PCOS [24], and the significant increase in

baseline level of proinflammatory cytokines (TNF-α, hs-CRP, and IL-6) was consistent with that reported by others [25]. PCOS has already been associated with increased levels of indices of chronic low-grade inflammation [26], it is well known that obesity is linked with increased serum concentrations of TNF-α and IL-6; these cytokines are involved in the development of insulin resistance [26]. After 12 weeks of treatment with metformin, the level of IL-6 was significantly decreased by 37% from the baseline, and in tune with the reported finding of El-Mekkawi et.al [27]. Consistently, the level of TNF-α was significantly decreased after treatment, because metformin improves sensitivity of peripheral tissues to insulin, associated with improving BMI value, these findings are in tune with that reported by Goodarzi and Korenman [28], and Hotamisligil et.al [29], who reported that TNF-α expression correlates the decrease in insulin resistance and weight loss with the improvement in insulin sensitivity.

Most women with PCOS, either lean or obese, have insulin resistance and hyperinsulinemia, and insulin has a physiologic inhibitory effect on acute-phase protein synthesis in the liver [30], so the impairment of hepatic sensitivity to insulin might be the causative factor for increased synthesis of hs-CRP, production of hs-CRP was also modulated by some adipokines, including IL-6 and TNF-α [31], and serum levels of hs-CRP will be decreased after treatment due to improvement in hepatic insulin sensitivity, BMI, and also decreases serum levels of IL-6 and TNF-α. These findings were in line with the reports of Diamanti et.al [6] and Kelly et.al [32], who indicated that differences in serum hs-CRP between obese hyperandrogenic women with PCOS and obese non hyperandrogenic were no longer significant after controlling for BMI and insulin sensitivity.

Additionally, metformin attenuates the low-grade inflammatory state as judged by circulating CRP and IL-6 and reduction of central adiposity [33]. It has been shown that different adipose compartments can have varying effects on endocrine and metabolic factors in women with PCOS, and visceral fat is the main tissue in the body responsible for insulin resistance observed in obesity associated with PCOS. The present study illustrated significant increase in HOMA-IR index at baseline compared with control group, and metformin increases insulin receptor binding associated with increased glucose utilization, decreases hepatic glucose production [34], therefore, it improves HOMA-IR. In contrast, low level of SHBG was detected in selected PCOS patients, which seems consistent with other study [36], as characteristic feature of PCOS, and after treatment 16.6% increase was reported; this is in tune with other study which reported 23% increase in SHBG [36]. Serum progesterone at baseline was found significantly lower than that in controls; this finding was in line with other study [37]. The improvement in ovulation rate, as assessed by mid-luteal phase progesterone level (more than 16 nmol/L), was evaluated according to the increase in baseline progesterone level. Metformin improves menstrual cyclicity and increase the percentage of ovulatory cycles; this leads to significant increase in spontaneous ovulation rate in the first month of treatment. However, other workers found a significant enhancement in luteal progesterone level in PCOS women treated with metformin, and they suggest that insulin resistance and hyperinsulinemia may be responsible for low progesterone level during luteal phase in PCOS; therefore, the luteal progesterone level may be enhanced in PCOS by decreasing insulin levels with metformin.

Conclusions:

The study detects an increased level of inflammatory cytokines, SHBG and decrease level of progesterone in Iraqi females with PCOS, and metformin therapy improves serum levels of the inflammatory cytokines associated with increased ovulation rate.

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