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Changes in IL-18 Level in Response to Metformin Treatment in Iraqi Women with Poly Cystic Ovarian Syndrome (Placebo Controlled Study)

Huda I. Al-Qadhi[1], Tharwa H. Hassan Al-Tai[2], Shoub Yassin Hussein[3]

[1]MBChB, MSc.PHD Pharmacology, Department of Pharmacology /College of medicine /University of Baghdad

[2]BSc, MSc Microbiology, Department of Biology/College of Medicine /University of Baghdad

[3]BSC, MSc, Biology, Department of Biology/College of Medicine /University of Baghdad

Abstract: ***Background: The most common endocrine disorder affect women between the ages of 18 and 44 is PCOS. ovaries stimulated by high level of luteinizing hormone (LH) from the anterior pituitary gland or by excessive insulin (hyperinsulinaemia). A majority of women with PCOS have insulin resistance. IL-18 play a role in some inflammatory conditions. [17] In women PCOS ,serum IL 18 is increased and there is a strong association with increased insulin resistance .Metformin is a drug used in type 2 diabetes. It acts by reducing glucose synthesis by the liver and increasing insulin sensitivity to enhance peripheral glucose uptake to reduce. In PCOS it is used to reduce insulin resistance and improve ovarian function. Aim of the work: To find changes in IL 18 level in response to metformine treatment. Patients and methods: Double blind placebo controlled study carried from November 2016 to February 2017 in which 50 patients blindly assigned into two groups by codes: placebo and treatment group. recieving 850mg once daily of starch and metformin successively for three months. Body mass index, hormonal profile and serum IL18 was measured at baseline and at the end of the study. Results: At the end of study the code was opened and 30 patient was in the treatment group while 20 patients in the placebo group. all measures was compared between baseline and at the end of the study. mean level of IL-18 was (312.11±99.164) before metformin treatment in the placebo group and (306.20±0.186.989) in the treatment group. At the end of the study there was marked decrease (106.66±99.281) in the treatment group with statistically significant change in the treatment group but not in the placebo group (341.33±102.996).also there was significant difference between placebo and treatment group at the end of the study. Conclusion: metformin in addition to its role as insulin sensitizer it also reduces inflammation in PCOS patients marked by significant reduction of S.IL 18.***

Keywords: Metformin, IL18, PCOS

1. Introduction

The most common endocrine disorder affect women between the ages of 18 and 44 is PCOS.[1] About 2% to 20% of this age group are affected by this condition.[2] It is one of the causes of impaired fertility.[3]

Symptoms are mainly contributed to the elevated androgens (male hormones) in women. [4] Main symptoms of PCOS include menstrual disturbances as irregular menstrual period, hypo menorrhea, menorrhagia , hirsuitism, acne, pelvic pain, poor fertility, and skin changes.[5] There might be associated conditions as type 2 diabetes, obesity, obstructive sleep apnea, heart disease, mood disorders, and endometrial cancer.[6]

Factors predisposing to PCOS are mainly genetic and environmental factors[7]. In addition many risk factors include excess body weight, poor physical activity, and a family history of PCOS. [8]

When the ovaries stimulated by high level of lutenizing hormone (LH) from the anterior pituitary gland or by excessive insulin (hyperinsulinaemia) [9] , there will be increased androgen production mainly testosterone. [10 ,11]
Diagnosis is based on two of the following three findings: oligo or un ovulation, high androgen levels, polycystic ovaries may be detectable by ultrasound[12].

PCOS has no cure but treatment may involve weight reduction.[13] Birth control pills may improving the menstrual disturbances, anti androgens may reduce excess hair growth, and acne. Metformin also help.[14] clomiphene may be added to enhance fertility and in vitro fertilization may be the final decision for patients not responding.[15]

IL-18 is an inflammatory cytokine that produced by macrophages and other cells

Following microbial infection it induces cell mediated immunity together with IL-12. It works by binding to the interleukin-18 receptor. [16]

In addition to its role in cell mediated immunity ,IL-18 play a role in some inflammatory conditions. [17] In women PCOS, serum IL 18 is increased and there is a strong association with increased insulin resistance . [18]

Metformin is a drug used in type 2 diabetes. It acts by reducing glucose synthesis by the liver and increasing insulin sensitivity to enhance peripheral glucose uptake to reduce. In PCOS it is used to reduce insulin resistance and improve ovarian function . [19]

2. Aim of the work

To find changes in IL 18 level in response to metformine treatment.

3. Patients and Methods

Study Design
Double- blind placebo controlled clinical trial consisted of a 4-week screening phase and a 12-week treatment phase.

Patient selection
65 female patients (mean age 25, rang from 22 to 29 years) selected at Kamal al- Samarrai specialist hospital(center of infertility treatment and invitro fertilization) , Baghdad- Iraq from November 2016 to February 2017. Informed consent obtained from the patients also an ethical approval obtained. They were diagnosed as PCOS patients according to international criteria of PCOS by 2 of three of : oligo or unovulation, increased androgen, radiological evidence of polycystic ovaries. Patients with diabetes mellitus , hyperprolactinemia, androgen-secreting tumors or receiving drugs as steroids, antipsychotic drugs were excluded.

All patients underwent medical screening include history, physical examination , BMI measurement and investigations include: serum testosterone, FSH and LH and prolactine.

Treatment assignment
Two sets of capsule covers labeled with a code number of (1) and (2) .(1) contain metformine 850 mg (Julfar. UAE). (2) contain 850 mg of starch. Both the prescriber and the patients were blinded to the treatment condition since the capsules given by third person. Capsules administered once daily 3successive months randomization codes were opened only after all patients had completed the whole study protocol .

Study parameter
The study parameter is IL -18 which was measured before starting the treatment and after 3 months by ELISA (enzyme linked immunosorbent assay.

Statistical analysis
Collected data were analyzed using SPSS (statistical package for social sciences, version 20 .Descriptive analysis of means and standard deviation (SD) were calculated on all demographic variables, and serum IL18 , hormones and BMI .

Multiple comparisons of paired series of data within groups were done using paired t- test. Unpaired t-test was then used to evaluate difference between placebo and treatment groups .P value <0.05 was considered the minimal for statistical significance.

4. Results

Fifteen patients dropped out of the study so only fifty patients completed the study. When the randomization list was opened at the end of the study, it was seen that 30of the patients included in the metformin treated group and 20 of the patients included in the placebo group .

Base line data of age, height, weight and BMI (body mass index) was depicated in table(1) and description of the baseline hormonal profile was shown in table (2)

Table 1: Description of general data for all patients included in the study

	N	Minimum	Maximum	Mean	Std. Deviation
Age (years)	50	22	29	25.54	2.533
Weight (kg)	50	60	105	70.22	9.792
Height (meter)	50	1.40	1.68	1.5522	.06095
BMI (kg/m^2)	50	23.88	41.02	29.1368	3.56140
Valid N	50				

Table 2: Description of baseline hormonal profile for all patients included in the study

Hormone	N	Mean	Std. Deviation
FSH	50	4.8	1.5
LH	50	13.2	3.2
Prolactin	50	9.2	3.3
Testosterone	50	2.1	o.39

There was a significant reduction in BMI among the treatment group at the end of the study.and not significant in the placebo group. Also there was a significant reduction in FSH and LH level in the treatment group at the end of the study and not significant in the placebo group. No significant changes in prolactin and testosterone was seen in all groups Description of mean BMI and hormonal profile for different groups at the end of the study was shown in table (3).

Table 3: Description of mean BMI and hormonal profile for different groups at the end of the study was shown in

Variable	Placebo group		Treatment group	
	before	after	before	after
BMI	30.2	29.3	30.4	27.2*
FSH	4.2	4.31	3.95	2.51*
LH	12.75	12.1	13.2	9.42*
prolactin	8.78	8.2	9.1	9.25
testosterone	1.87	2.1	1.56	1.58

*significant changes (unpaired t-test) p value<0.05

Description of mean serum IL18 for different groups was depicated in table (4).figure (1)

Table 4: Description of serum IL18 for all groups of patients

Groups	N	Mean	Std. Deviation
Placebo Baseline	20	312.11	99.164
Placebo After	20	341.33	102.996
Treated Baseline	30	306.20	186.989
Treated After	30	106.66	99.281

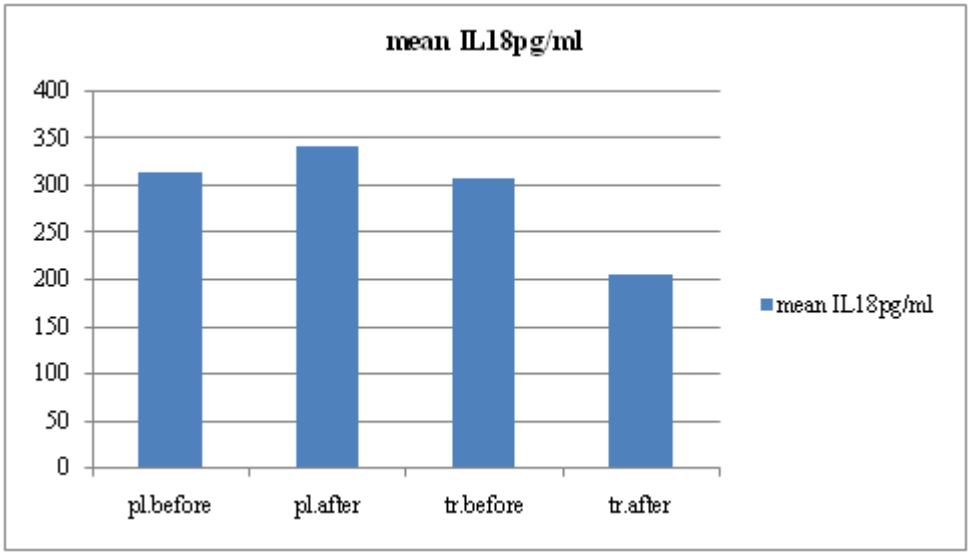

Figure 1: Mean S.IL18 for different PCOS patients groups in response to metformine treatment

Serum IL18 levels was compared at baseline with that after the study completed in the placebo group, the difference was not significant (p value >0.05).

There was a significant reduction in the mean S.IL18 level in the treatment group at the end of the study (p value<0.05). There was significant difference (p value <0.05) when serum IL18 levels was compared between placebo and treatment group at the end of the study. Table (4)

Table 4: Changes in the level of IL-18 among the studied cases before and after therapy

Groups	Mean S.IL18	(p value)	Significance
Placebo.before	312.11±99.164	0.126	NS*
Placebo. after	341.33±102.996		
Treatment .before	306.20±0.186.989	.003	S*
Treatment after	106.66±99.281		
Placebo .after	341.33±102.996	0.000	S**
Treatment after	106.66±99.281		

*Data are presented as mean+ SD. Paired t-test. ** Data are presented as mean+ SD. Independent t-test

5. Discussion

Most of PCOS patients have insulin resistance which contribute to hypothalamic-pituitary-ovarian axis disturbances that lead to PCOS. Hyperinsulinemia increases GnRH release, LH more than FSH, increased ovarian androgen release and [20] reduce follicular maturation. Insulin resistance may present among normal weight or overweight women. [21]

Chronic inflammation also present in PCOS, many studies show a correlation between inflammatory mediators , anovulation and other PCOS symptoms.[22] also a relation between PCOS and increased oxidative stress was seen.[23] Insulin-sensitizing agents was found to improve both reproductive and metabolic aspects of PCOS.[24]Metformin in addition of lowering high testosterone levels, reducing inflammatory state (Creactive protein, IL-6, and/or adiponectin), and reduces central adiposity. A potent proinflamatory cytokine is IL18 one of IL-1 cytokine family is increased in obese, diabetic, and in polycystic ovary syndrome (PCOS) patients**.**

In this study the mean level of IL-18 was (312.11±99.164) before metformin treatment in the placebo group and (306.20±0.186.989) in the treatment group. after the treatment there was marked decrease (106.66±99.281) in the treatment group with statistically significant change in the treatment group but not in the placebo group (341.33±102.996).these results were consistant with those performed by Khadiga El-Sayed Ali 2008 on 40 PCOS patients in retrospective non placebo trial and found reduction in IL18 level following metformin treatment [25]. And other study by Sherif F. ELMekkawi et al.2010 who found a decrease in IL6 and IL18 level in PCOS patients treated with metformin [26].

Another study performed by Heutling et al. 2006.showed significant increase in IL6 and IL18 in PCOS patients and metformin treatment improve metabolic and endocrine profile as well as menstruation [27].

Studies have suggested that metformin suppresses inflammatory response by inhibition of nuclear factor κB (NFκB) (which is a protein that trigger activation of the gene that codes for cytokines)via AMP-activated protein kinase (AMPK)-dependent and independent pathways. [28].

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