Effect of Metformin Therapy on Serum Interleukin-6 and Interleukin-18 Levels in Patients with Polycystic Ovary Syndrome

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Abstract: Objective: to compare the level of plasma interleukin-6 and interleukin-18 in patients with (PCOS) before and after treatment with metformin. Subjects and method: Forty patients with PCOS were recruited. Venous blood sample was drawn for measurement of serum IL-18 and IL-6 by Elisa at the start of the study and after three months of the treatment with metformin (850mg/d). Results: Treatment with metformin for three months showed significant effect on reducing body weight,BMI, serum IL-6 and IL-18 levels in patients with PCO.Conclusion:There is a beneficial impact of metformin on the inflammatory process related to PCOS. [New York Science Journal 2010;3(8):83-86]. (ISSN: 1554-0200).

Keywords: Metformin Therapy; Serum; Interleukin-6; Interleukin-18; Patient; Polycystic Ovary Syndrome

Introduction:

Polycystic Ovarian Syndrome (PCOS) is one of the most common endocrine disorders of uncertain etiology, which affects between 6%-10% of women at reproductive age. It is characterized by menstrual abnormalities, hirsutism, acne anovulatory infertility, and elevated androgens. It has been confirmed that insulin resistance is a common feature in either obese or non obese women with PCOS. (1)

Insulin sensitizing agents have been proposed as a therapy for the treatment of polycystic ovarian syndrome. These agents improve insulin action by increasing insulin sensitivity, thereby decrease hyperinsulinemia. The use of insulin sensitizers could therefore be suggested in most patients with PCOS. Metformine is the oldest and still the most used insulin sensitizer worldwide in treatment of glucose intolerance, particularly type II diabetes Mellitus ***(Error! Reference source not found.)***

Cytokines are important mediators of inflammation and regulators of the immune response. Cytokines are divided into pro-inflammatory cytokines (TNF-α, IFN-γ, IL-1, IL-2, IL-6, IL-8, IL-12 and IL-18) and anti-inflammatory cytokines (IL-4, IL-10, and TGF-β). Recent studies indicate that inflammatory mediators are over produced in obesity, in diabetes, in metabolic syndrome in cardio vascular and even in polycystic ovarian syndrome ***(1).***

The present study aims to compare the level of plasma interleukin-6 and interleukin-18 in patients with polycystic ovary syndrome (PCOS) before and after treatment with metformin.

Subjects and Methods:

This prospective study was conducted at the period starting from March 2007 to December 2007. Forty female patients with PCOS were recruited from the infertility clinic in Ain -Shams University Maternity Hospital. The selected cases were subjected to complete history taking, general examination and investigations for serum testosterone, FSH and LH and prolactin.

Single venous blood sample was drawn from the PCOS patients and was divided into 2 different tubes for measurement of serum IL-18 and IL-6 by Elisa at the start of the study and after three months of the treatment with metformin (850mg/d).

Principle of the method

We used 2 different types of kits both are monoclonal antibody specific, the first kit specific for hIL-18 and the second kit is specific for hIL-6. The BioSource International, Inc. hIL-18 and hIL-6 ELISA kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA).

Statistical analysis:

- Unpaired t-test was used to compare quantitative parametric data (SD<50% mean).
- *Willcoxon sign test was used instead of paired t-test in non parametric data (SD>50% mean).

- *Mann Whitney Willcoxon test was used instead of unpaired t-test in non parametric data (SD>50% mean).
- *Spearman's correlation co-efficient rank test was used to rank different variables against each other in linear correlation which was positive or negative
- Chi-square test (X^2) was used to compare qualitative variables.
- R-test (correlation coefficient test) used to rank different parameters either directly or indirectly.
- Receiver- operator characteristic (ROC) curve. (Curve was used to find out the best cut off value of certain variable.)
- Stepwise regression analysis.
- P<0.05 significant

Results:

Table (1): Description of general data among the studied cases:

Variables	Mean	±SD	Range
Maternal age (yrs)	24.9	4.5	19-36
Weight (kg)	78.5	16	53-118
Height cm	159	5.5	151-173
BMI (kg/m^2)	31	5	23-42.9

Table (2): Description of hormonal profile among the studied cases at the start of the study:

Variables	Mean	±SD	Range
LH	12.1	3.5	3.3-20.5
FSH	5.2	1.4	2.9-9.5
Prolactin	8.7	3.1	3.3-13.6
Testosterone	1.87	0.48	1.3-3

Body weight and hence BMI was decreased after metformin therapy with highly statistically significant change **(table 3).**

Table (3): Changes in body weight and BMI of the studied cases after metformin therapy*:

	Before	After	% of change	T	P
Weight (kg)	78.5±16	74±15	5%	**3.5**	**<0.01HS**
BMI (kg/m^2)	31±5	29±4.6	4%	**3**	**<0.01HS**

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

There was a marked decrease in the level of IL-18 after therapy by metformin with highly statistically significant (table 4).

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

IL-18	Before	After	% of change	Z	P
Mean ±SD	451± 227	125.9±103	72.1%	8.7	<0.01 HS
Range	160-1000	40-460			

*Data are presented as mean+ SD. Wilcoxon test.

Table (5): Correlation between IL-18 before therapy versus hormonal profile of the studied cases*:

	R	P	significance
LH	0.30	<0.01	**HS**
FSH	0.52	<0.05	**S**
Prolactin	0.05	>0.05	NS
Testosterone	0.10	>0.05	NS

*Spearman correlation test.

This table shows a statistically significant positive correlation between IL-18 before therapy versus FSH and highly significant versus LH. On the other hand there is no significant correlation versus other hormones.

There were no statistically significant correlations between IL-18 before or after therapy versus general and anthropometric measures of the studied cases.

Table (6): Changes in the level of IL-6 among the studied cases before and after therapy:

IL-6	Before	After	% of change	Z	P
Mean ±SD	142.5±43	59±39	58%	**9.1**	**<0.01 HS**
Range	50-216	15.6-175			

*Data are presented as mean+ SD. Wilcoxon test.

This table shows marked decrease in the level of IL-6 after therapy by metformin with highly statistically significant change.

Table (7): Correlation between IL-6 before therapy versus hormonal profile of the studied cases

Variables	r	P	Significance
LH	0.24	>0.05	NS
FSH	**0.37**	**<0.05**	**S**
Prolactin	0.05	>0.05	NS
Testosterone	**0.52**	**<0.01**	**HS**

*Spearman's correlation.

This table shows a statistically significant positive correlation between IL-6 before therapy versus FSH and highly significant versus testosterone. On the hand there is no significant correlation versus other hormones.

There were no statistically significant correlations between IL-6 before and after therapy versus general and anthropometric measures of the studied cases.

Discussion:

Insulin resistance and obesity are common features of the PCOS. Insulin-sensitizing agents have been shown to improve both reproductive and metabolic aspects of PCOS **(7).** Insulin resistance plays a significant role in the pathogenesis of the PCOS **(8).**

When given as monotherapy to nonobese adolescents or young women with PCOS, Metformin lowers high testosterone levels, attenuates the low-grade inflammatory state (as judged by circulating C-reactive protein, IL-6, and/or adiponectin), and reduces central adiposity **(9)**.

Interleukin-18 is a potent proinflamatory cytokine with potential atherogenic properties through effect on IL-6, TNF-α, and interferon-γ. ***(10).***

In this study, there was no statistically significant correlation could be detected between IL-6 and IL-18 before therapy versus general data and anthropometric measures of the studied cases. Also, no statistically significant correlation could be found with the duration of infertility.

Regarding the changes in body weight and BMI of the studied group after metformin therapy, there was an evident decrease in body weight and BMI with highly statistically significance change.

This was consistent with a study performed by ***Qublan et al. 2007*** on 22 patients who found significant reduction in BMI after treatment with metformin (mean of 31.9kg/m²versus27.8kg/m²)**(4).**

This study showed that the mean level of IL-6 before metformin therapy was 142 pg/ml and after therapy was 59pg/ml. There was a marked decrease in level of IL-6 after treatment by metformin with highly statistically significant difference.

Regarding IL-18, the mean level of IL-18 before metformin therapy was 451 (pg/ml) and after metformin therapy was 125.9 (pg/ml). Thus there was a marked decrease in the level of IL-18 after therapy by metformin with highly statistically significant change.

Regarding hormonal profile among the studied cases, the mean LH level was 12.1mIU/ml and FSH was 5.2mIU/ml. There was a statistically significant positive correlation between IL-18 before therapy versus FSH and highly significant versus LH, while there was a statistically significant positive correlation between IL-6 before therapy versus FSH and highly significant versus testosterone.

Our results were consistent with those performed by **Heutling et al. 2006**. They showed that the levels of proinflammatory cytokines (IL-6 and IL-18) were significantly increased in women with PCOS and were found to be positively correlated with parameters of insulin sensitivity; treatment with metformin positively affected metabolic and endocrine profile as well as menstrual function and led to spontaneous pregnancies **(5)**.

However, our study was not in keeping with a study performed by **Mohlig et al. 2004** on nine obese and insulin-resistant PCOS patients who were treated with metformin. IL-6 concentrations remained largely unchanged (Mean±SD 1.72±0.30 pg/ml versus 1.91±0.31 pg/ml)**(6)**. This may be attributed to the small number of patients studied.

Conclusion:

Serum interleukin-18 and interleukin-6 levels are significantly decreased in patients with polycystic ovary syndrome after treatment with metformin for 3 months which may reflect beneficial impact of metformin on the inflammatory process related to PCOS. Further larger studies are needed to prove our results..

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