Effect of Metfomin Therapy on Serum Interleukin-18 Level in Patients with Polycystic Ovarian Syndrome

Thesis

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Introduction

Polycystic Ovarian Syndrome (PCOS) is one of the most common endocrine disorders of uncertain etiology, which affect 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. Elevated serum levels of interleukin-18 are associated with insulin resistance in women with polycystic ovary syndrome. ***(Tsilchoro Zidau et al., 2006).***

Infertility is the presenting complaint in 40% of women with PCOS and it is due to anovulation. The exact mechanism underlying the association between infertility and PCOS remains unknown. It was thought that the subfertility could be due to the presence of high LH and androgen levels. It was suggested that high LH level in the follicular phase of the menstrual cycle caused a premature resumption of meiosis with the consequent release of a "premature oocyte". However, it would appear that several factors interact to form part of the vicious cycle of abnormal steriodogenesis, folliculogenesis, abnormal oocyte maturation, decrease endometrial receptivity and early pregnancy loss ***(Abdulmalik et al., 2005).***

Table (2): Revised diagnostic criteria of PCOS ***(Norman et al. 2004).***

1999 criteria (both 1 and 2)

1. Chronic anovulation
2. Clinical and/or biochemical signs of hyperandrogenism, and exclusion of other aetiologies

Revised 2003 criteria (2 out of 3)

1. Oligo- and/or anovulation

2. Clinical or biochemical signs of hyperandrogenism

3. Polycystic ovaries.

Exclusion of other aetiologies (congenital adrenal hyperplasias, androgen-secreting tumors, cushing's syndrome).

Management of polycystic ovary syndrome

Traditionally, treatment of PCOS has been mainly symptom oriented as no one cause could be attributed to the condition. However, with the recent identification of the central role of insulin resistance to the etiology of PCOS, treatment is now aimed towards measures to improve insulin resistance as these have been shown to improve the regularity of menstrual cycles, ovulation, androgen levels and surrogate markers for longer term risk of diabetes and heart disease. Life-style modification and metformin which both improve insulin resistance have, therefore, been the focus of recent research ***(Sharma et al., 2005).***

Ovulation induction

Women with the polycystic ovary syndrome (PCOS) have normo-gonadotrophic and normo-estrogenic anovulation constitute the largest group of anovulatory women encountered in clinical practice**.** Current clinical protocols involve an orderly Step-by-Step (Stepwise) approach to ovulation induction in women with PCOS ***(Kim et al., 2000 and Barbiere, 2000)*** that includes;

Step I: Weight reduction if body mass index (BMI) is >27 kg/m.

Step II: Antiestorgen

Step III: Insulin sensitizer as a single agent.

Step IV: Insulin sensitizer in combination with clomiphene.

Step V: Gonadotropin therapy.

Step VI: Insulin sensitizer in combination with gonadotropin therapy.

Step VII: Laparoscopic ovarian drilling.

Step VIII: In vitro fertilization (IVF). ***(Norman et al., 2004)***

Insulin Sensitizers:

Insulin-sensitizing agents have recently been proposed as a therapy for the treatment of PCOS. These agents improve insulin action by increasing insulin sensitivity, thereby decreasing hyperinsulinemia. Since almost all, obese PCOS women and more than half of those with normal weight are insulin resistant and present with some degree of fasting or stimulated hyperinsulinemia. The use of insulin sensitizers could therefore be, suggested in most patients with PCOS ***(Pasqucli et al., 2006).***

Metformin is the oldest and still the most used insulin sensitizer worldwide in the treatment of states of glucose intolerance, particularly type 2 diabetes mellitus ***(Hundal et al., 2003).***

Metformin may also have a direct action on theca cells, reducing androgen production ***(Homburg and Lambalk, 2004).***

Interleukin -18

Interleukin-18 is a potent proinflamatory cytokine with potential atherogenic properties through effect on IL-6, TNF-α, and interferon-γ until now, there have been no reported studies that evaluated circulating IL-18 levels in human obesity but in recent studies weight loss reduces interleukin 18 levels on obese women ***(Pontillo et al., 2007).***

Interleukin -18 was originally identified as a member of interleukin -1 family and as interferon (IFN)–γ inducing factor (IGIF) ***(Zhang et al., 2006).***

Elevated serum levels of interleukin-18 are associated with insulin resistance in women with polycystic ovary syndrome ***(Escobar Morreale et al., 2006).***

Interleukin-18 may be a contributing linking inflammation and insulin resistance in PCOS women ***(Yang et al., 2006).***

It is well known that interleukin-18 acts as an important regulator of both innate and acquired immune responses in many inflammatory disease ***(Blankenberg et al., 2002).***

Serum interleukin-18 concentration are significantly increased in PCOS women and are strongly associated with insulin resistance. So IL -18, as an inflammatory cytokines, may play a role in mediating insulin resistance and inflammation ***(Héctor et al., 2006).***

The Aim of the work

The aim of the present study is to compare the level of serum interleukin-18 in patients with polycystic ovarian syndrome (PCOS) before and after treatment with metformin.

Patients and Methods

This study was conducted at the period starting from March 2007 to December 2007 the subjects recruited from the infertility clinic in Ain-Shams University Maternity Hospital. Informed consent obtained from the patients. **Aprospective study** designed on forty female patients with PCOS, diagnosed according to the international criteria of PCOS by two out of three criteria: chronic oligoovulation or anovulation after excluding secondary causes, clinical or biochemical evidence of hyperandrogenism (but not necessarily hirsutism due to inter-patient variability in hair follicle sensitivity), and radiological evidence of polycystic ovaries ***(Lanham et al., 2006).***

Inclusion criteria include:

1- Chronic oligoovulation or anovulation after excluding secondary causes, (6 or fewer menstrual periods per year).

2- Clinical evidence of hyperandrogenism.

3- Biochemical of hyperandrogenism Elevated circulating androgen levels. LH/FSH ratio 3:1

4-Ultasongraphic evidence of polycystic ovaries.

Exclusion criteria include:

1- Patients with hyperprolaetinemia.

2- Patients with androgen-secreting tumors.

3- PCOS patients with diagnosis of diabetes mellitus or who were received treatment with psychotropics, steroids, sympathomimetics or sympatholytics.

The selected cases subjected to:

1- Complete history taking.

2- General examination.

3- Investigation for serum testosterone, FSH and LH.

Single venous blood sample for measurement of serum IL-18 drawn from the PCOS patients at the start of the study then the other sample after three months of the treatment with metformin (850mg/d). The blood samples distributed to individual tubes, and each samples taken after centrifugation at 3000 rpm for 15 min. Plasma will be stored at -70°C until assay. Serum IL-18 measured by enzyme-linked immunosorbent assay (EL1SA).

Results

This study was conducted at the period starting from March 2007 to December 2007 the subjects recruited from the infertility clinic in Ain Shmas University Maternity Hospital, 40 women underwent testing for serum interleukin-18 level using ELISA before and after treatment with metformin for three months as cases of polycystic ovary syndrome.

Table (8): Description of general data among the studied cases**.**

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

Table (9): Description of anthropometric measures of the studied cases after metformin therapy**.**

Variables	Mean	±SD	Range
Weight	74	15	54-118
BMI	29	4.6	22-39

Table (10): Changes in body weight and BMI of the studied cases after metformin therapy**.**

Variables	Before	After	% of change	t	P
Weight	78.5±16	74±15	5%	**3.5**	**<0.01 HS**
BMI	31±5	29±4.6	4%	**3**	**<0.01 HS**

This table shows that body weight and hence BMI was decreased after metfomin therapy with highly statistically significant change by using paired t-test.

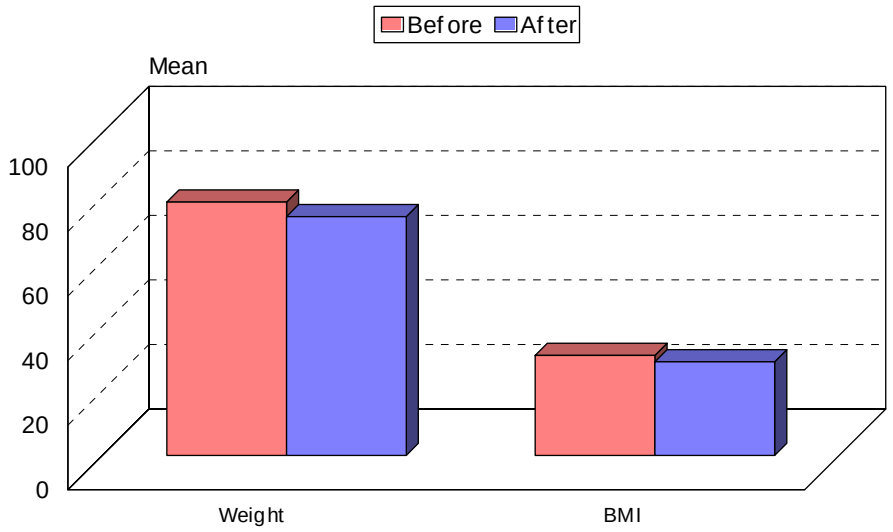

Fig. (9): Changes in body weight and BMI of the studied cases after metformin therapy.

Table (11): Description of hormonal profile among the studied cases**.**

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

Table (12): Description of parity among the studied cases

Parity	No.	%
P0	38	95%
P1	2	5%

This table shows that 95% of the studied cases are P0, while 5% of them are P1.

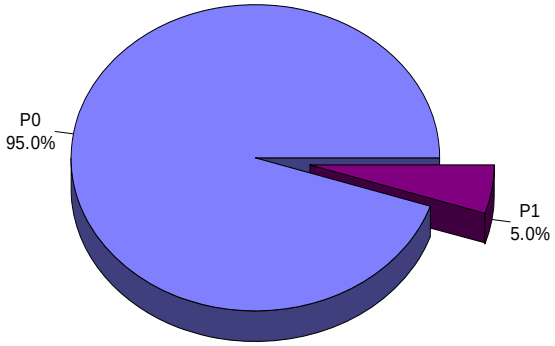

Fig. (10): Description of parity among the studied cases

Table (13): Description of infertility duration among the studied cases.

No.	Infertility duration (yrs)
3.9±2.8	**Mean ±SD**
1-12	Range

Table (14): Description of IL-18 among the studied cases before and after therapy**.**

IL-18	Before	After
Mean ±SD	451±227	125.9±103
Median	395	100
Range	160-1000	40-460

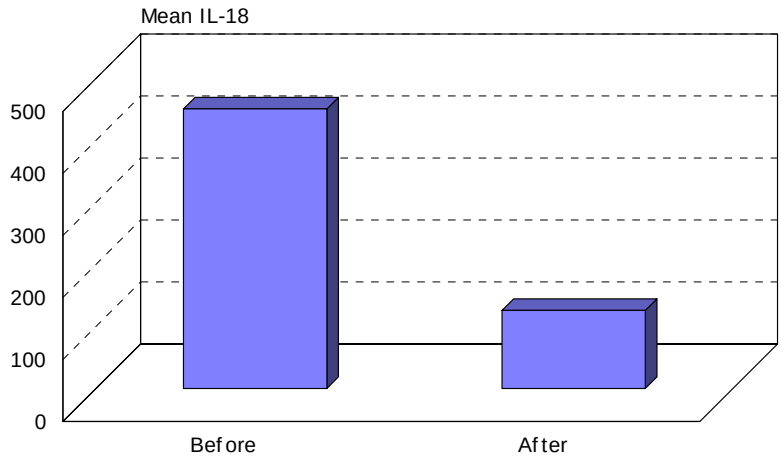

Fig. (11): Description of IL-18 among the studied cases before and after therapy

Table (15): 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	<0.01HS
Range	160-1000	40-460			

This table shows marked decrease in the level of IL-18 after therapy by metformin with highly statistically significant change by using **Willcoxon sign test**.

Table (16): Correlation between IL-18 before therapy versus general data of the studied cases**.**

Variables	IL-18		Significance
	r	P	
Maternal age	0.15	>0.05	NS
Body weight before	0.11	>0.05	NS
HT	0.10	>0.05	NS
BMI before	-0.04	>0.05	NS

There is no statistically significant correlation could be detected between IL-18 before therapy versus general and anthropometric measures of the studied cases by using **Spearman correlation test**.

Table (17) Correlation between IL-18 after therapy versus general data of the studied cases**.**

Variables	**IL-18** r	P	**Significance**
Maternal age	0.06	>0.05	NS
Body weight after	0.14	>0.05	NS
HT	0.18	>0.05	NS
BMI after	>0.09	>0.05	NS

There is no statistically significant correlation could be detected between IL-18 after therapy versus general and anthropometric measures of the studied cases by using **Spearman correlation test**.

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

Variables	**IL-18** r	P	**Significance**
LH	0.30	>0.05	**HS**
FSH	0.52	>0.01	**S**
Prolactin	0.05	>0.05	NS
Testosterone	0.10	>0.05	NS

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

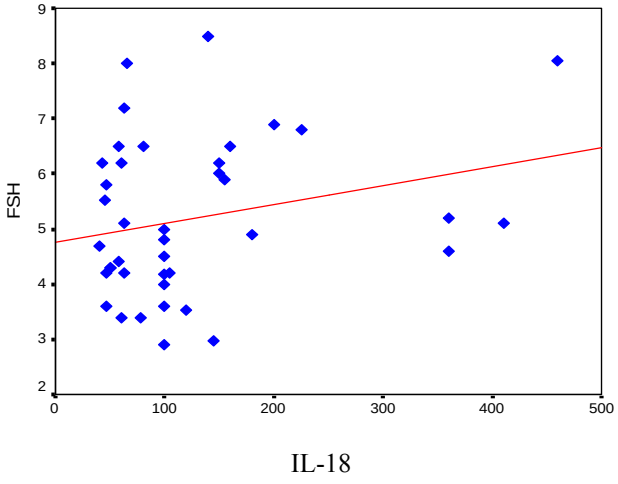

Fig. (12): Correlation between IL-18 versus FSH after therapy.

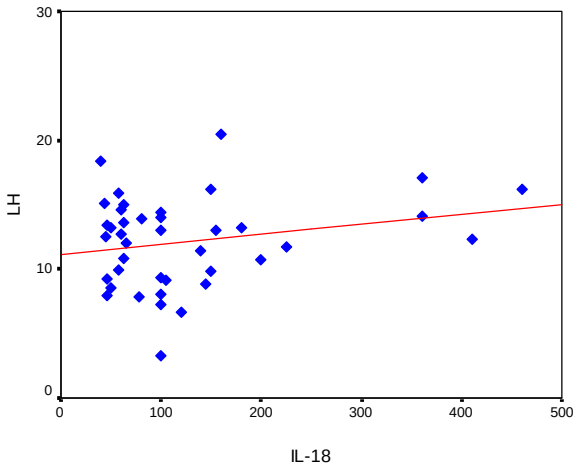

Fig. (13): Correlation between IL-18 versus LH after therapy

Table (19): Correlation between IL-18 after therapy versus hormonal profile of the studied cases.

Variables	IL-18		Significance
	r	P	
LH	0.22	>0.05	NS
FSH	0.21	>0.05	NS
Prolactin	0.11	>0.05	NS
Testosterone	0.03	>0.05	NS

This table shows no significant correlation between IL-18 versus different hormones by using **Spearman correlation test**.

Table (20): Correlation between IL-18 before therapy duration of infertility of the studied cases.

Variables	IL-18		Significance
	r	P	
Infertility duration	-0.14	>0.05	NS

This table shows no statistically significant correlation could be detected between IL-18 before therapy versus infertility duration by using **Spearman correlation test**.

Table (21): Correlation between IL-18 after therapy duration of infertility of the studied cases**.**

Significance	IL-18		Variables
	r	P	
NS	>0.05	-0.07	**Infertility duration**

This table shows no statistically significant correlation could be detected between IL-18 after therapy versus infertility duration by using **Spearman correlation test**

Discussion:

This study was conducted at the period starting from March 2007 to December 2007 the subjects recruited from the infertility clinic in Ain-Shams University Maternity Hospital. Informed consent were obtained from the patients. Aprospective study designed on forty female patients with PCOS, diagnosed according to the international criteria of PCOS by two out of three criteria: chronic oligoovulation or anovulation after excluding secondary causes, clinical or biochemical evidence of hyperandrogenism (but not necessarily hirsutism due to inter-patient variability in hair follicle sensitivity), and radiological evidence of polycystic ovaries ***(Lanham et al., 2006).***

Our aim was to compare the level of serum interleukin-18 in patients with (PCOS) before and after treatment with metformin for 3 months.

This study included 40 women who underwent testing for serum interleukin-18 before and after metformin administration for 3 months.

This study showed that the mean age for the cases group was 24.9 years. Also the mean weight 78.5kg and the mean height was 159cm. BMI was 31kg/m^2. Before treatment with metformin.

Abdominal obesity and hyper-insulinemia play a key role in the development of the polycystic ovary syndrome (PCOS). Dietary-induced weight loss and the administration of insulin-lowering drugs, such as metformin, are usually followed by improved hyperandrogenism and related clinical abnormalities. Obese women with PCOS, particularly those with the abdominal obesity phenotype, are usually more insulin resistant and more hyperinsulinemic than their normal- weight counterparts ***(Benson et al., 2008).***

The percentage of nullipara among the groups was 95% while that of Para 1 was 5%.

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 ***(Tan et al., 2007).*** Insulin resistance plays a significant role in the pathogenesis of the PCOS ***(Heutling et al., 2008).***

Also this study showed that the mean of weight among the studied group after metformin administration was 74kg. While the mean of BMI after administration of metformin was 29kg.

In this study regarding the changes in body weight and BMI of the studied groups after metformin therapy. There was a decreased in body weight and BMI with highly statistically significantly change as the p value was $p< 0.01$.

This study was consistent with a study performed by ***Pasquali and Gambineri et al., 2006,*** on 64 patient and found that there was a great reduction of body weight and abdominal fat, particularly the visceral adipose tissue.

Also there were result consistent with this study prformed by ***Pontillo et al., 2007,*** on 40 patient who declared that there was weight loss of nearly 10% of their initial body weight.

IL-18 as a member of IL-1 cytokine family is increased in obese, in diabetic, and even in polycystic ovary syndrome (PCOS) patients ***(Zhang et al., 2006).***

Regarding the level of IL-18 among the studied groups the mean level of IL-18 before metformin therapy was 451 (pg/ml) and after metformin therapy was 125.9 (pg/ml).

Also there was marked decrease in level of IL-18 after therapy by metformin with highly statistically significant change.

This result were consistent with a study performed by ***Heutling et al., 2008***, on 68 patients who where treated with metformin (850mg) daily for 6 months. Their study showed that the levels of proinflammatory cytokines (IL-6, 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. Women with PCOS have elevated levels of inflammatory markers (IL-6, IL-18) and exhibit endothelial dysfunction, that was partly reversed after a 6 month metformin treatment.

In this study regarding the correlation between IL-18 before therapy versus general data of the studied cases there was no statistically correlation could be detected between IL-18 before therapy versus general and anthropometric measures of the studied cases.

Also there was no statistically significant correlation could be detected between IL-18 after therapy versus general and anthropometric measures of the studied cases.

In this study regarding hormonal profile among the studied cases the mean of LH was (12.1pg/ml) and FSH mean was (5.2pg/ml) there was a statistically significant positive correlation between IL-18 before therapy versus FSH and highly significant versus LH.

These results were consistent with a study preformed by ***Zhang et al., 2006,*** on 30 PCO women who found that there was no statistically correlation between FSH, LH and IL-18. P value >0.179, 0.170 receptively.

Regarding hormonal profile prolactin mean level was 8.7 and testosterone mean level was 1.87 there was no statistical correlation

between IL-18 before therapy versus different hormonal profile of studied cases.

Also regarding correlation between IL-18 after therapy versus hormonal profile of studied cases there was no significant correlation between IL-18 versus different hormones.

Regarding the correlation between IL-18 before therapy duration of infertility of studied cases.

There was no statistically significant correlation could be detected between IL-18 before therapy versus infertility duration**.**

This study were not in keeping with a study performed by ***(Gambineri et al., 2007)***, who declared that the testosterone hormone were slightly reduced after metformin therapy after 6 months.

Regarding the correlation between IL-18 after therapy and the duration of infertility of studied cases. There was no statistically significant correlation could be detected between IL-18 after therapy versus infertility duration.

This study is not going with a study performed by ***Pasquali et al., 2006,*** who declared that the pregnancy rate were spontaneous improved and miscarriage rate reduced.

Summary

Polycystic Ovarian Syndrome (PCOS) is one of the most common endocrine disorders of uncertain etiology, which affect 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 ***(Tsilchoro-Zidau et al., 2006).***