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Research Article

THE RELATIONSHIP BETWEEN BODY IRON STORE AND INSULIN RESISTANCE IN PATIENTS WITH POLYCYSTIC OVARY SYNDROME TREATED BY METFORMIN

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ABSTRACT

Objective: To evaluate the effect of metformin on body mass index (BMI) and waist to hip (W/H) ratio and on insulin and ferritin serum level, and insulin resistance represented by Homeostasis Model Assessment (HOMA-IR).And to assess the relationship between serum ferritin and the above parameters in patients with polycystic ovary syndrome (PCOS), treated by metformin for more than three months. **Methodology:** This study is a case-control study, which was adopted in the Fertility and Invitro Fertilization (IVF) Center in AL. Batoll Teaching Hospital Mosul City/ Iraq, in the period between 1st of November 2013 and 1st April 2014. A group of 39 women with PCOS of reproductive age who used metformin for more than three months (metformin users) with another age-and BMI matched group of 47 women with PCOS who didn't use metformin (metformin non-users), these groups were sub-divided into subgroups according to the BMI to (obese and non-obese). A 10 ml of fasting blood sample was taken from each PCOS woman of the two groups. The serum was used to measure serum insulin and ferritin level by using commercially available kits, whereas; BMI and insulin resistance represented by Homeostasis Model Assessment (HOMA) was calculated by using especial equations. **Results**: This study revealed that about half (53 %) of the studied PCOS patients (with and without metformin therapy) found to be obese. There were significant higher mean serum insulin and ferritin level and HOMA-IR value in the obese in comparison with non obese metformin non-users PCOS women. There were a significant lower mean serum level of insulin and ferritin in obese metformin users as compared with obese metformin non-users. Insulin level of obese metformin non-users of the studied PCOS patients found to be above the normal range (<20 µIU/ ml), but there were a non significant differences in the mean FSG between the same two groups. There are no significant differences between all these parameters of the non-obese metformin users and non-users, also insulin level of all non obese studied PCOS patients (metformin users and non-users) found to be within the normal range. There were a significant positive correlation between BMI and serum insulin level, BMI and serum ferritin level, serum insulin and HOMA-IR value, and insulin and ferritin level in metformin users and non-users PCOS patients. By using linear stepwise multiple regression, this study showed a significant effect of the non-depended variables W/H ratio and HOMA-IR on insulin level in obese metformin users. This study **concluded** that obesity is an important factor that affects insulin resistance and hyperinsulinemia lead to increase ferritin level as this study found a significant correlation between serum insulin and ferritin level in metformin users which was also very highly significant in non-metformin users PCOS patients.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age, affecting (5-10%) of women worldwide[1]. It is characterized by hyperandrogenism, ovarian disorders, and polycystic ovarian morphology[2,3], and is frequently associated with insulin resistance and obesity[4].

The association between obesity and PCOS has a great deal of interest since discovery of that PCOS women are often hyperinsulinaemic and that the degree of hyperandrogenism may be positively and significantly correlated with that of hyperinsulinaemia, in which obesity lead to either over production or decreased clearance of insulin in obesity[5]. This association was confirmed by many studies[4,6,7].

The pattern of body fat distribution have significantly different effects not only on hormones and metabolism but also on clinical features of women of PCOS, these women who have greater truncal abdominal fat distribution (central obesity) as demonstrated by higher waist to hip ratio (W/R), they will be higher level of insulin concentration[8,9]. Insulin resistance and compensatory hyperinsulinemia considered the corner stone of pathogenesis of PCOS[3].

Ferritin is an intracellular protein that stores iron and releases it in a controlled fashion[10]. Mounting epidemiological evidence suggested that, in the general population, body iron stores and iron intake are positively associated with the development of disorders of glucose tolerance, including diabetes[11].The association between iron and glucose metabolism based on two directional influences, iron influence insulin secretion and sensitivity, and insulin influence iron metabolism[12,13]. Also insulin stimulates intestinal iron absorption and ferritin synthesis in parallel with the effects of insulin on glucose transport[14]. Increase ferritin level in women with PCOS was firstly noted by Escobar-morreale[15] in 2005 who noted that ferritin level increased in PCOS women in correlation with the degree of obesity and inversely with insulin sensitivity this correlation was shown also by other researchers[13,16-18].

Metformin an insulin-sensitizing agent, has been proven to be of clinical usefulness both in the short-term aiding of infertility treatments and, potentially in the prevention of the long-term sequelae for patients with PCOS[19]. The rationale for the use of metformin, in the treatment of patients with PCOS arises from the knowledge that insulin resistance with compensatory hyperinsulinemia has provided an insight into the pathogenesis of PCOS, beneficial effects of metformin on reproductive as well as metabolic aberrations of PCOS were shown by many studies[20-22].Other researchers[13,23]concluded that metformin improve PCOS symptoms through reduction in body iron stores that may break the vicious circle of reduced insulin sensitivity, increased intestinal iron absorption, and further worsening of insulin sensitivity, thus improving symptoms in these women.

The **aim** of the present study was to assess the effects of metformin therapy for more than three months on BMI, WHR, fasting serum glucose (FSG) level, serum ferritin and insulin level and insulin resistance represented by HOMA-IR value and to evaluate the relationship between serum ferritin level and these parameters, in patients with PCOS on traditional therapy with metformin for more than three months in comparison with age- and BMI-matched PCOS patients not taking metformin therapy with their traditional therapy.

METHODS

This case-control study was conducted in Fertility and Invitro Fertilization (IVF) Center in AL. Batoll Teaching Hospital that belongs to Mosul College of Medicine, both are located at the right bank of the river Tigris in Mosul City/ Iraq, from the 1st of November 2013 to 1st April 2014. This study included ninety two women at child-bearing age, who were diagnosed with PCOS according to the Rotterdam 2003 criteria[24] and were enrolled in this study. These participants were divided into two groups, the metformin users included 39 women with PCOS (age ranged from 17-38 years) on traditional therapy with metformin therapy (piophage® tablet provided by Pioneer Com. Iraqi) of doses (ranged between 1000 to 1700 mg daily) for durations ranged between3 to 18 months. Patients who had diabetes mellitus, hypertension, congenital adrenal hyperplasia, thyroid disorders, cushing's syndrome were excluded from the study. None of the patients was treated by iron containing tonics, hormonal contraceptives, antihypertensive drugs, or any other medication (except for metformin for more than three months**)**.Metformin non-users group consisted of 53 women with PCOS (age ranged from 18-42 years), who had similar criteria as the metformin users except that they did not take metformin with their traditional therapy. The participants in the two groups were subdivided according to the BMI into obese (BMI≥30), and non obese (BMI<30)[25].

Anthropometric measures (blood pressure (mmHg), body weight (Kg), height (cm), Waist and hip circumference (cm)) were taken. To determine W/H ratio the waist and hip diameters were measured while the subject was standing and breathing normally. The BMI was calculated as weight in kilograms divided by the square of height in meters.

Ten milliliters (ml) of venous blood were withdrawn from PCOS patients after 12-hour fasting, the serum was separated and kept frozen at -20 °C to be analyzed for determination of serum glucose level by enzymatic colorimetric method using Randox kit (Randox Laboratories Ltd., UK). Serum Ferritin and insulin weremeasured by chemiluminescent immunoassays (CLIA) technique, using ferritin and insulin Liaison kits supplied by Diasorin, Saluggia (VC) –(Italy.) While insulin resistance represented by HOMA value was measured using the following equation[26]: fasting serum insulin (µIU/ml) × fasting plasma glucose (mg/dl) /405.

The Descriptive and analytic statistics of the data obtained in this study was carried out by using Minitab version 16.2 software statistical program. Standard statistical methods were used to determine the mean and standard deviation (SD). Independent t-test of two means was used for continuous variables whereas catigoral variables compared by Chi-square test. Pearson`s Correlation coefficient (r) was measured between different hormonal and biochemical parameters, (simple linear correlation). Also multiple regression analysis was performed to identify the effect of some parameters on serum insulin independently. P-values ≤ 0.05 were considered statistically significant throughout data analysis.

The approval of the study protocol by an ethic committee has been obtained from the local health committee of Ministry of Health and College of Medicine -University of Mosul – Iraq.

RESULTS

A group of 39 women with PCOS who used their traditional therapy with metformin for three months were included in this study(with mean age ± SD of 24.95 ± 4.75), and considered to represent metformin- users group (case) another 47 women with PCOS (with mean age ± SD of 23.45 ± 4.58), who didn't use metformin were considered to represent metformin- non users group (control).

The general characteristic of the metformin users and non users are listed in table (1), as it appears that there were no significant difference in the mean age, BMI, WHR, SBP, DBP between the two PCOS groups. According to BMI, about half (53 %) of the studied PCOS patients (with and without metformin therapy) found to be obese.

Table 1: General characteristic of the two PCOS groups

Parameters	Mean ± SD		P-value*
	metformin users Cases(n = 39)	metformin non-users Control(n = 47)	
Age (years)	24.95 ± 4.75	23.45 ± 4.58	NS
BMI (Kg/m^2)	28.78 ± 4.45	29.70 ± 5.11	NS
W/H ratio	0.85 ± 0.056	0.88 ± 0.051	NS
Systolic BP (mmHg)	119.6 ± 7.74	116.6 ± 7.88	NS
Diastolic BP (mmHg)	80.3 ± 9.32	77.2 ± 8.00	NS

Also no significant difference in ferritin, FSG, insulin and HOMA-IR value between these groups, as it appears in table (2).

Table 2: Comparison between biochemical parameters of metformin users and non-users

Parameters	Mean ± SD		P-value*
	metformin users Cases (n = 39)	metformin non-users Control(n = 47)	
S. Ferritin (ng/mL)	38.3 ± 28.9	50.4 ± 44.0	NS
FSG (mg/dL)	91.3 ± 19.80	94.2 ± 17.32	NS
Insulin (µIU/mL)	14.38 ± 7.21	17.36 ± 9.70	NS
HOMA -IR	3.21 ± 1.64	3.80 ± 2.77	NS

This study demonstrated significant higher mean serum insulin and ferritin level and HOMA-IR value in the obese metformin non-users group in comparison to the non-obese metformin non-users, and it appears that insulin level of obese metformin non-users of the studied PCOS patients found to be above the normal range (<20 µIU/ ml)[27], but a non significant differences in the mean FSG between the same two groups(table3).

Table 3: Comparison between biochemical parameters of obese metformin non-users and non-obese metformin non-users

Parameters	Mean ± SD		P-value*
	Obese metformin non-users (n = 23)	Non-obese metformin non-users (n = 24)	
S. Ferritin (ng/mL)	69.8 ± 49.1	31.7 ± 28.6	**0.002S**
FSG (mg/dL)	97.5 ± 19.2	91.1 ± 16.5	NS
Insulin (µIU/mL)	22.0 ± 10.4	12.9 ± 6.43	**0.001S**
HOMA –IR	4.67 ± 3.32	2.96 ± 1.83	**0.032S**

While it appears that there is no significant changes between the mean serum insulin, ferritin level and FSG and HOMA-IR value of obese and non-obese metformin users, as shown in table (4).

Table 4: Comparison between biochemical parameters of obese and non-obese metformin users

Parameters	Mean ± SD		P-value*
	Obese metformin users (n = 23)	Non-obese metformin users (n = 16)	
S. Ferritin (ng/mL)	45.0 ± 31.8	28.7 ± 21.7	NS
FSG (mg/dL)	92.9 ± 23.4	89.0 ± 13.7	NS
Insulin (µIU/mL)	15.8 ± 7.46	12.4 ± 6.56	NS
HOMA –IR	3.53 ± 1.64	2.74 ± 1.59	NS

Table (5) illustrates that administration of metformin for more than three months caused a significant lowering of the mean serum level of insulin and ferritin of the obese PCOS patients in comparison with the obese metformin non-users. Insulin level of obese metformin non-users of the studied PCOS patients found to be above the normal range (<20 µIU/ ml)[27].But a non significant differences in the mean FSG between the same two groups.

Table 5: Comparison between biochemical parameters of obese metformin users and obese non-metformin users

Parameters	Mean ± SD		P-value*
	Obese metformin users (n = 23)	Obese metformin non-users (n = 23)	
S. Ferritin (ng/mL)	45.0 ± 31.8	69.8 ± 49.1	**0.048S**
FSG (mg/dL)	92.9 ± 23.4	97.5 ± 19.2	NS
Insulin (µIU/mL)	15.8 ± 7.46	22.0 ± 10.4	**0.024S**
HOMA –IR	3.53 ± 1.64	4.67 ± 3.32	NS

While in the comparison between these parameters of the non-obese metformin users and non-users, this study demonstrated that there is no significant differences, as shown in table (6), which demonstrated also that insulin level of all non obese studied PCOS patients (metformin users and non-users) found to be within the normal range (<20 µIU/ ml)[27].

Table 6: Comparison between biochemical parameters of non-obese metformin users and non-obese metformin non-users

Parameters	Mean ± SD		P-value*
	Non-Obese metformin Users (n = 16)	Non-obese metformin non-users (n = 24)	
S. Ferritin (ng/mL)	28.7 ± 21.7	31.7 ± 28.6	NS
FSG (mg/dL)	89.0 ± 13.7	91.1 ± 16.5	NS
Insulin (µIU/mL)	12.4 ± 6.56	12.9 ± 6.43	NS
HOMA –IR	2.74 ± 1.59	2.96 ± 1.83	NS

There was a highly significant positive correlation between BMI and mean serum insulin level, in the metformin users and metformin non-users PCOS patients as shown in figures (1&2) respectively.

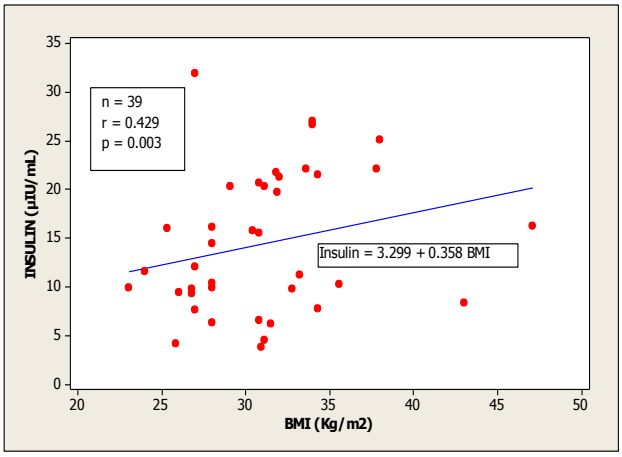

Fig. 1: Correlation between BMI and serum insulin in the metformin users

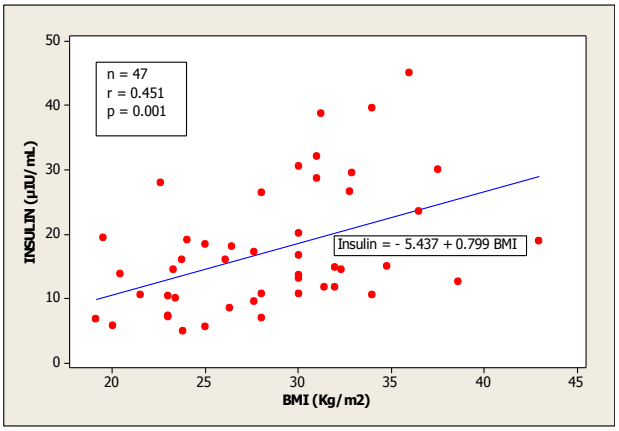

Fig. 2: Correlation between BMI and serum insulin in the metformin non-users

There was significant positive correlation between BMI and mean serum ferritin level, in the metformin users and metformin non- users PCOS patients as shown in figures(3 & 4) respectively.

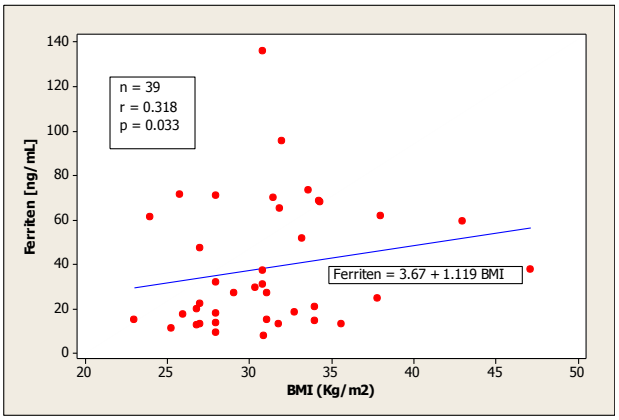

Fig. 3: Correlation between BMI and serum ferritin in the metformin users

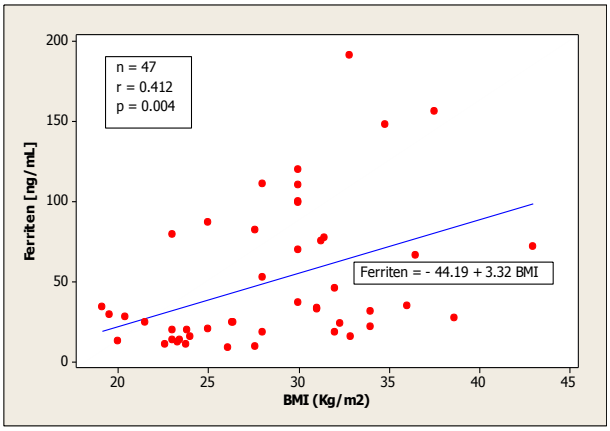

Fig. 4: Correlation between BMI and serum ferritin in the metformin non-users

There was very high significant correlation between mean serum insulin and HOMA-IR, in the metformin users and metformin non-users PCOS patients as shown in figures (5 & 6) respectively.

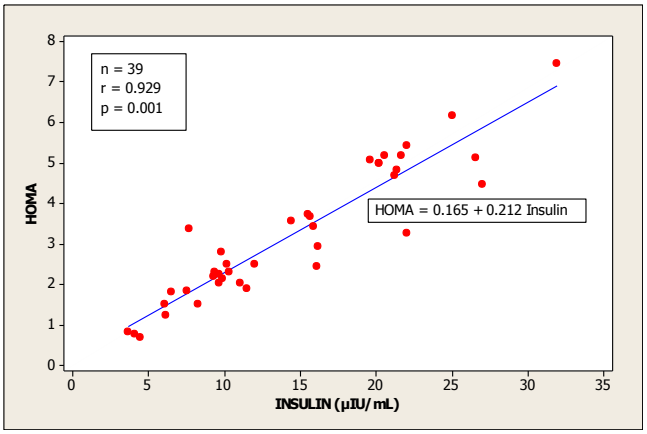

Fig. 5: Correlation between serum insulin and HOMA-IR value in the metformin users

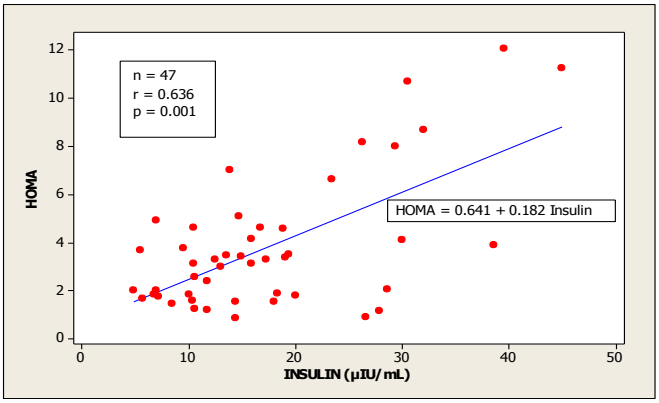

Fig. 6: Correlation between serum insulin and HOMA-IR value in the metformin non-users

There was a significant positive correlation between mean serum insulin and ferritin in the metformin users and metformin non-users PCOS patients as shown in figures(7 & 8) respectively.

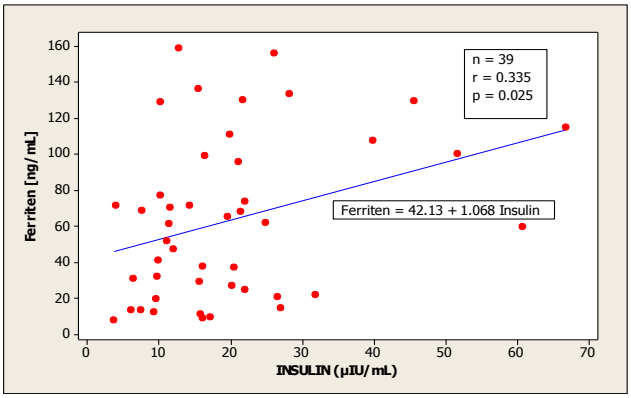

Fig. 7: Correlation between serum insulin and ferritin in the metformin users

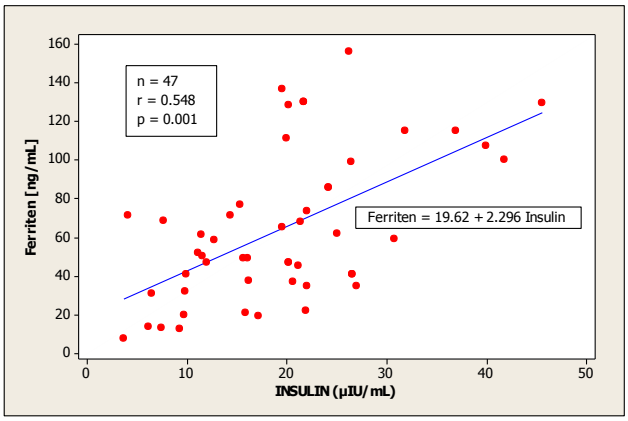

Fig. 8: Correlation between serum insulin and ferritin in the metformin non-users

By using linear stepwise multiple regression, table (7) shows a significant effect of the non-depended variables W/H ratio and HOMA-IR on insulin level in obese metformin users.

Table 7: Multiple regression analysis for the effect of some parameters on the serum insulin level in obese metformin users (n = 23)

Predictors	Regression coefficient	SE of Coefficient	P-value*
Constant	- 10.08	14.27	NS
BMI (kg/m^2)	0.182	0.180	NS
W/H ratio	5.421	0.413	0.036
S. Ferritin (ng/mL)	0.014	0.024	NS
HOMA-IR	4.116	0.459	0.001

* R^2 = 83.4%, R^2 (adjusted) = 78.5%.

DISCUSSION

In this study, all patients were carefully selected to be as matched as possible since any significant difference in the characteristics between these two groups could affect the parameters of concern either positively or negatively. Accordingly, in addition to the non-significant differences in age and BMI, there were also non-significant differences in WHR; SBP and DBP; between the two PCOS groups of patients involved in this study.

There is widespread variability in the prevalence of obese (BMI >30 kg/m^2) women in PCOS populations across different countries. The highest prevalence of obesity is reported in studies conducted in the United States and Australia, with 61% to 76% of women with PCOS considered obese[28]. While the percentage of obesity of the population of PCOS women in Mosul City in Iraq, was about 51%[29,30], which is approximately similar to that in our study (53%).Geographic location, ethnic origin, and cultural/social practices are likely contributors to the differing

manifestations of PCOS and should be recognized in routine clinical practitioners in the general population[31,32].

Obesity considered one of features of original description of syndrome by stein and leventhal[33], it plays a crucial role in the development and maintenance of PCOS and strongly influence the severity of both its clinical and endocrine features in many women with this condition by its consequences of insulin resistance and disturbance of steroid metabolism, in which upper-body obesity (android obesity) has high lipolytic activity releasing FFA into blood circulation, FFA compete with glucose uptake in muscle and fat cells, resulting in increased FFA oxidation and impaired insulin mediated glucose utilization and acceleration of glconeogenesis in the liver[34]. In consequence of this, our study found a significant higher mean serum insulin and ferritin level and HOMA-IR value in the obese in comparison with non obese metformin non-users PCOS women(table (1)). Also insulin level of obese (but not the non obese) metformin non-users of the studied PCOS patients found to be above the normal range (>20 µIU/ ml)[27]as shown in (table 3 and 5).

The significant higher mean serum insulin level and HOMA-IR value in the obese in comparison with non obese metformin non-users PCOS women, are compatible with the results of several studies [35-38]. While the significant higher mean serum ferritin level in obese in comparison with the non obese metformin non-users PCOS women, is in a agreement with[15,39], who found that ferritin level elevated in obese PCOS, but is disagreement with the study of Sharifi*et al*., (2011)[37].

The non-significant differences in FSG between the metformin users and non users PCOS women precipitated in this study, supports the idea which tells that metformin has little effect on blood glucose in non-hyperglycemic subjects[40] and is in agreement with the other studies[29,30,41,42].

Many studies indicated that elevated serum ferritin level that found in PCOS patients might be related to insulin resistance and hyperinsulinemia that frequently found in PCOS[12,13,18]. So, the use of insulin sensitizer (metformin) in PCOS reduce circulating level of insulin and shifting the endocrine balance towards ovulation[19,43], with subsequent reduction of ferritin level[14,44]. So, the amelioration of insulin resistance, and hyperinsulnemia by metformin may explain the reduction in serum ferritin levels found in the subset of PCOS patients that treated with this insulin sensitizer[14].Consequently, this study found that the mean serum insulin and ferritin level were noted to be significantly lower in obese PCOS women treated by metformin when compared with their counterpart obese PCOS women who did not take metformin(table 5). These results are consistent with other studies[13,35,45].

This study noted that serum levels of insulin and ferritin were non-significantly different in non-obese PCOS patients treated by metformin for more than three months(table, 6). These findings are disagreement with other studies[35,41,45], who found that metformin significantly improved HOMA-IR , fasting insulin and ferritin levels, in lean PCOS women after six months or three months of treatment respectively.

A significant positive correlation was observed also in this study between BMI and serum insulin level in metformin users and non-users, which is in agreement with other studies[29,46]. This is logical as hyperinsulinemia is derived from either over production or decreased clearance of insulin and in obesity insulin production was increased[47,48] with reduction of clearance rate[5,49].

A significant correlation between insulin and HOMA-IR was seen also in this study in metformin users and non-users. The insulin resistance is considered the corner stone of the pathogenesis of PCOS and the insulin resistance lead to hyperinsulinemia as a consequent effect[3,18]. However, Corkey, (2011)[48]andYe, (2013)[5]suggested that a high level of serum insulin may lead to insulin resistance in obese women.

This study found that ferritin is directly and significantly correlated with BMI in both groups of PCOS patients, this finding is in agreement with Anakal*et al*., (2013)[39]who found that ferritin levels correlated well with BMI and insulin resistance, as BMI is considered a good predictor of insulin resistance in PCOS patients. But disagreement with the study of Behradmanesh *et al.,* (2011)[41], who did not find any association between serum ferritin and BMI, WHR, HOMA-IR and serum ferritin not only before but also after metformin treatment and claimed that to the lack of normal control group or sample size differences.

Another point worth to be noted in this study that there is a significant correlation between serum insulin and ferritin level in metformin users and non-users, which was also noted by Al-Hakeim (2012)[17]. These finding gave evidence that insulin resistance and hyperinsulinemia lead to increase ferritin level by stimulate intestinal iron, absorption[13,16], in addition to that, Fernández-Real *et al.,*(1998)[50] hypothesized that serum ferritin could be a marker of insulin resistance. As well as, in epidemiological studies, serum ferritin was considered to be the second-

strongest determinant of blood glucose (after BMI) in regression models and the third-strongest determinant of serum insulin (after BMI and age)[51]. While in contrast to our study, Behradmanesh*et al.,* (2011)[41], found no significant association between serum ferritin and HOMA-IR.

Waist to hip (W/H) ratio is strongly related to the intra-abdominal fat mass, which in turn more closely correlated with glucose and insulin levels than subcutaneous fat mass[52]. Upper obesity has high lipolytic activity releasing FFA into blood circulation and compete with glucose uptake in muscle and fat cells, resulting increased in FFA oxidation and impaired insulin mediated glucose utilization with subsequent increased insulin level[53]. In the line of these facts, and the using of multiple regression analysis (table 7), this study found a significant association between insulin level and W/H ratio, and very high significant association with HOMA-IR in obese metformin users, and according to author knowledge, no study found to concentrate on such point.

CONCLUSION

The study concluded that obesity is an important factor that affect the therapeutic response to metformin treatment in PCOS patients, in that that metformin therapy for more than three months in obese but not in non-obese PCOS patients is associated with significant lower mean serum insulin and ferritin levels, and a significant correlation between BMI and serum insulin and ferritin level. Also insulin resistance and hyperinsulinemia lead to increase ferritin level as this study found a significant correlation between serum insulin and ferritin level in metformin users with very high significant in non-metformin users PCOS patients.

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