Studies of the Effects of Metformin, Diane, and Androcur in Treatment of Infertile Women with Polycystic Ovary Syndrome in Babylon Province

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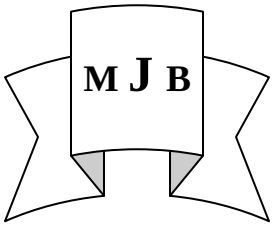

Abstract

Back ground: Infertility is one of problems that affect women and one of its cause is increase androgen level specially in women with Polycystic Ovary Syndrome (PCOS).

The aim is to assess the effect of metformin alone versus metformin with oral contraceptive and antiandrogen on PCO patients.

Patients and method: The study was conducted in Babylon province,from the infertility center of maternity and pediatrics teaching hospital. Women with PCOS were recruited from November 2008 to April 2009. The subjects study consisted of (90) married women. (n=30) received metformin only. B.(n=30)received Metformin+ ethinyloestradiol-cyproterone acetate(Diane)+cyproterone acetate (Androcur C. Thirty women were normal, and fertile as a control group.

The results: The results show that the highest incidence of PCOS among adult women occurs in age group (25-35) years and represent 58%, and 55% of patients have positive family history, and 65% of them had a history of primary infertility and high geographical distribution of PCOS occurs in urban areas .Also there is larger reduction in LH hormone in both treatment's regimes While in prolactin there is little increment after (group B) regimes, this may be due to effect of Cyproterone acetate.

Conclusion: Our study showed significant response in both groups (more in group B)that support the effect of metformin added to that of Diane together give a better response in PCO patients with moderate to sever signs of hyperandrogenism.

الخلاصة

ان العقم هو احد المشاكل التى تصيب النساء واحد أسبابه هو زيادة هرمون الاندروجين خاصة عند النساء المصابات بمتلازمة تكيس المبايض .

هدف الدراسة هو وصف التاثيرات لنوعين من الانظمة العلاجية المعطاة للنساء العقيمات لمصابات بمتلازمة تكيس المبايض في محافظة بابل. حيث تم جمع العينات للفترة من تشرين الثاني ٢٠٠٨ الى نيسان ٢٠٠٩وقسمت الى ثلاثة مجاميع ٣٠ مريضة تعالج بالمتفورمين و٣٠ مريضة بالمتفورمين والديان والانتي اندروجين و٣٠ امراة طبيعية كمجموعة سيطرة وتم قياس الهرمونات وانزيمات الكبد قبل العلاج وبعد ستة اشهر .

النتائج : بينت النتائج ان اكثر مجموعة عمرية بين ٢٥- ٣٥ (٥٨%) واغلب النساء يعانين من العقم الاولي٦٥% اكثر من العقم الثانوي ٣٥%وكانت هناك زيادة ملحوظة في الهرمون الذكري والليوتيني عن مجموعة السيطرة قبل العلاج للمجموعتين ا- ب وكان هناك انخفاض ملحوظ في هذا المستوى بعد العلاج للمجموعتين ا- ب مع بقاء الهرمون الحويصلي بالمستوى الطبيعي وبالنسبة للهرمون الحليب هناك زيادة غير ملحوظة وان معامل كتلة الجسم يظهر اختلافا كبيرا بين مريضات تكيس المبايض عن مجموعة السيطرة.

الاستنتاج: تعطى المريضات المصابات بتكيس المبايض المتفورمين مع الديان والانتياندروجين نتائج احسن خاصة للواتي يعانين من اعراض متوسطة وقوية للهرمون الذكري.

Introduction

Infertility refers to the biological inability of a person to contribute to conception [1] after 12 months of contraceptive-free intercourse if the female is under the age of 34, and after 6 months if the female is over the age of 35[2].

Polycystic ovary syndrome (PCOS) is one of the most common causes of ovulatory infertility, affects 5–10% of women [3]. It's the presence of multiple cysts in the ovaries, menstrual disturbances, hirsutism and obesity [4]. Approximately 20% of asymptomatic women who have ultrasound are found to have polycystic ovaries - but without the clinical features of the syndrome; intervention is rarely required. Diagnostic assessments done by: History-taking, Diagnostic criteria (Rotterdam criteria): Two of the three following criteria are diagnostic of the condition [5]: Polycystic ovaries (either 12 or more peripheral follicles or increased ovarian volume (greater than 10 cm3). Menstrual irregularities [6]. Clinical and/or biochemical signs of hyperandrogenism. Investigations: This may show LH elevated, LH: FSH ratio increased, with FSH normal.

Metformin is an oral anti-diabetic drug from the biguanide class. It is also used in the treatment of polycystic ovary syndrome [7]. The clinical effects of metformin may take (4-6)months.It decrease body mass index of4%, Decrease in androgen activity for around 20% .Increase in ovulatory cycles achieved within 3 months [3]. Increased rates of pregnancy and reduce risk of miscarriage [4]. Metformin may cause many side effects like: lactic acidosis, vit.B12 malabsorbtion [7], anaemia, gastrointestinal (G.I) disturbance. Metformin was recommended treatment for an ovulation[8]. Metformin can reduce hirsutism, perhaps by reducing insulin resistance.

Cyproterone acetate (Androcur): This is a progestogen with antiandrogen properties. It decreases testosterone and androstenedione levels through a decrease in circulating LH levels. It also antagonizes the effect of androgens at the peripheral level. It blocks androgen receptors and reduces androgen synthesis by inhibiting androgen-synthesizing enzymes [9] so suppresses gonadotropin secretion by maintaining the negative feedback on the pituitary . The recommended dosage for treating hirsutism is usually within the range of 50 to 100 mg daily [10]. Ethinyloestradiol -cyproterone- acetate Diane-35) The oral contraceptive pills (OCP s) is a treatment of PCOS, exerting a number of beneficial effects including regularization of menses, amelioration of hirsutism and acne by reducing ovarian androgen production [11], and protection from the development of endometrial cancer, it inhibits ovulation. Provides contraceptive protection and stabilizes cycle [12]. And because it contains the same antiandrogenic substance (cyproterone acetate), also enhances the therapeutic effect of Androcur.

Aim of the Study

To describe the beneficial and harmful effects of two types of treatment regimes given to infertile women with PCO and to evaluate some physiological parameters in relation to PCO.

Patients and Methods

The study was conducted in Babylon province. The samples were taken from the infertility center of maternity and pediatrics teaching hospital. Women with PCOS (n=60), with control group (n=30), whose chief complaints were menstrual disturbances

and infertility and /or clinical or biochemical signs of hyperandrogenism were recruited from November 2008 to April 2009, and/or ultrasound (polycystic ovaries) evidence.The diagnosis of PCOS was based the criteria of Rotterdam consensus meeting 2003,which all patients fulfilled. Also we confirm absence of cardiac,hepatic or renal disease, and un suspected pregnancy in all participants before inclusion in the study. The subjects study consisted of a total number of (90) married women. Their age ranged between 18-45(28±6) years. They were classified into three groups, according to the severity of symptoms to one of two groups: A (n=30) received metformin only. B (n=30) received Metformin (Glucophage) + ethinyloestradiol-cyproterone acetate(Diane)+cyproterone acetate (Androcur). Metformin was administered at a dose of 3×500 mg daily, except for the first week of treatment when 500mg were given only twice a day to reduce the incidence and severity of gastrointestinal side effects. [13], while cyproterone acetate was administered Per oral rout (50 mg) for first 10 days of cycle to provide contraceptive protection. Diane was administered during first 21 days of cycle [12], C. Thirty women were normal, married and fertile as a control group. Complete history and examinations were carried out.

Methods

All patients underwent clinical, and biochemical evaluations at base line and at the end of treatment period of 6 months. Clinical assessment included menstrual cycle frequency, body mass index (BMI), hirsutism score (FG), waist circumference and WHR. Hormonal studies were performed on day 2-5(early follicular phase) of cycle measure the circulating concentration of Prolactin, LH,FSH, LH/FSH ratio, total testosterone, liver function tests (ALT,AST) also measurement of Amino Transferases GOT (AST) - GPT (ALT). Statistical analysis is performed using SPSS program .Mean and standard deviation are used

Results

We classified our subjects into three equal groups: group A (those treated with metformin), group B(those treated with metformin, Diane and Androcur) and group C(those who are control).

According to the age of the patients, as shown in table (1)the most common age group was (25-35) years which represents the highest percentage of total PCOS patients, 35 out of 60(58%) followed by other groups (15-25)that represents, 19 out of 60(32%) then the lowest percent in the third group ,6 out of 60(10%).

Table 1 shows the distribution of all PCOS patients (60) according to age groups.
Distribution of PCOS patients according to age group

Name of patient's groups	No. of patient in each Age group with percentage.		
	(15-25)years	(25-35)years	(35-45)years
Group A	11(37%)	17(57%)	2(7%)
Group B	8(27%)	18(60%)	4(13%)
Total no. percentage)	19(32%)	35(58%)	6(10%)

Family History: Out of sixty patients 33 presenting to have positive family history of PCO or diabetes 53% of them were in group A and 57% were in group B.

Type of Infertility: Thirty-nine patients out of 60 (65%) had primary infertility 18 was in group A&21 in group B while the other 21 had a history of secondary infertility.

BMI (Body Mass Index):

Figure 1 shows great differences between patients and control group pre treatment, and also found highly significant differences p< 0.001 between pre and post treatment's groups, in which the mean value of BMI in group A was(30.6±5.1),and in group B was(32.05±4.4), while in group C was equal to (27.2±2.4).

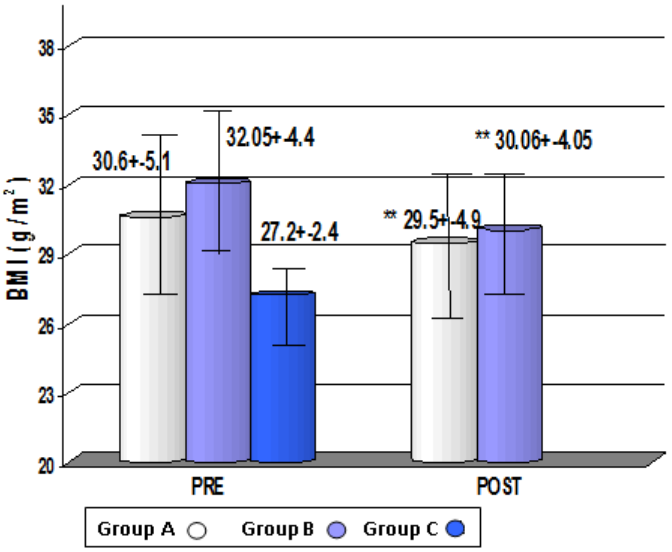

Figure 1 shows the values of BMI in the three studied groups . Relationship between BMI and Hormones in group A, B, and C

****** indicate highly significance

There is positive significant relationship between BMI , LH, FSH.in group A, p<0.05, and there is positive non significant relationship between BMI and LH,FSH hormones in group B, with negative significance to LH P<0.05, and positive non significance to FSH in group C. as shown in figure (2):

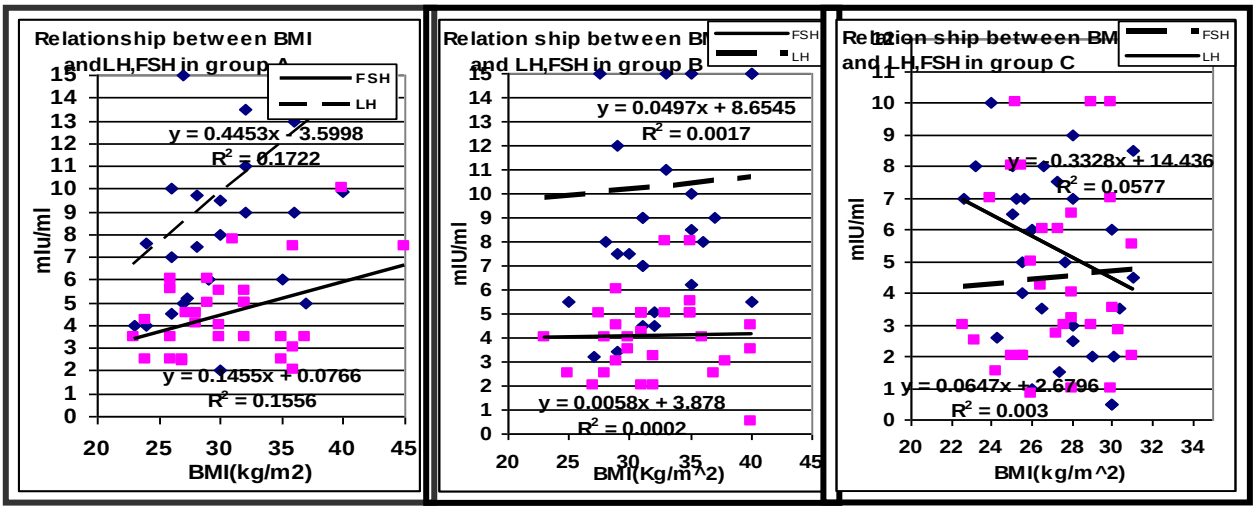

Figure 2 Relationship between BMI, LH, and FSH.in group A, B, and C

Ferriman, and Gallwey (F.G) score: Regarding degree of hirsutism there is great difference between patients and control as shown in table (2) and figure (3):

Table 2 Relationship between FG score and hormones.

Group name	S.Derocs GF±		TestosteroneTotal(ng/ml) pre± S.D	LH pre± S.D(mIU/ml)	FSH pre ±S.D (mIU/ml)
	pre	post			
Group C	6±2.3		0.67±0.26	5.29±3.3	4.44±2.8
Group A	10.8±4.3	10.2±3.8	1.25±0.75 *	10.05±5.5*	4.53±1.9
Group B	13.13±4.9	9.6 ±2.6	1.38±0.73 **	10.25±5.3*	4.06±1.7
* indicate significance p< 0.05, ** indicate highly significance p<0.001					

According to the FG score the high degree of hirsutism in the group B are more corrected with Diane and Androcur, and there is significant difference between patients and control as figure (3).

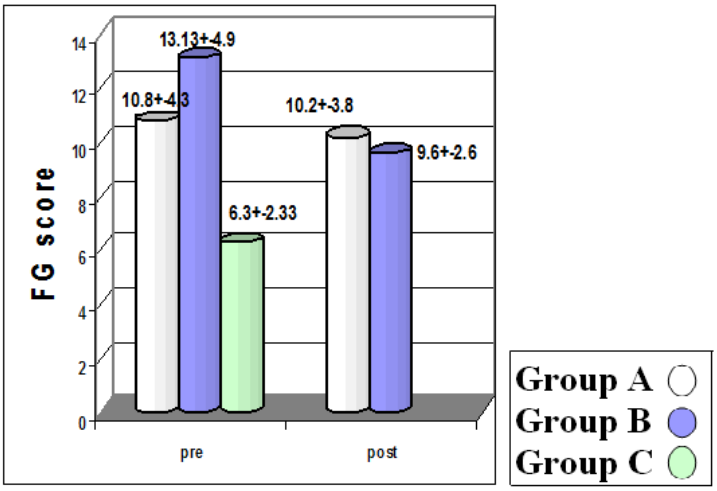

Figure 3 shows the mean and standard deviation of FG score pre and post treatment in patients and control.

Relationship between FG score and testosterone hormone in group A, B, and C.

Figure (4) shows that there is positive significant relationship between FG score and testosterone level in group A, p< 0.05, and there is highly positive significant relationship between FG score and testosterone level in group B p< 0.001, with negative non significant relationship in control group.

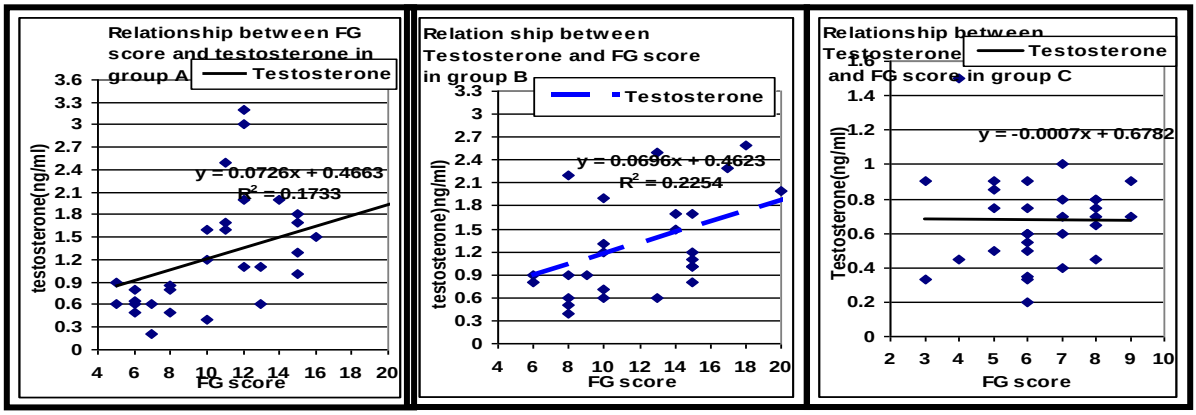

Figure 4 Relationship between FG score and testosterone in group A, B, and C. And relationship between FG score and LH, FSH hormone in group A, B, and C

There is negative significant relationship between FG and LH, P<0.05 with positive non significant relationship with FSH in group A, and there is positive significant relationship between FG score, LH. P< 0.05, with negative non significant relation ship with FSH in group B, and there is negative significant relationship between FG score and FSH with negative non significance to LH in control group.

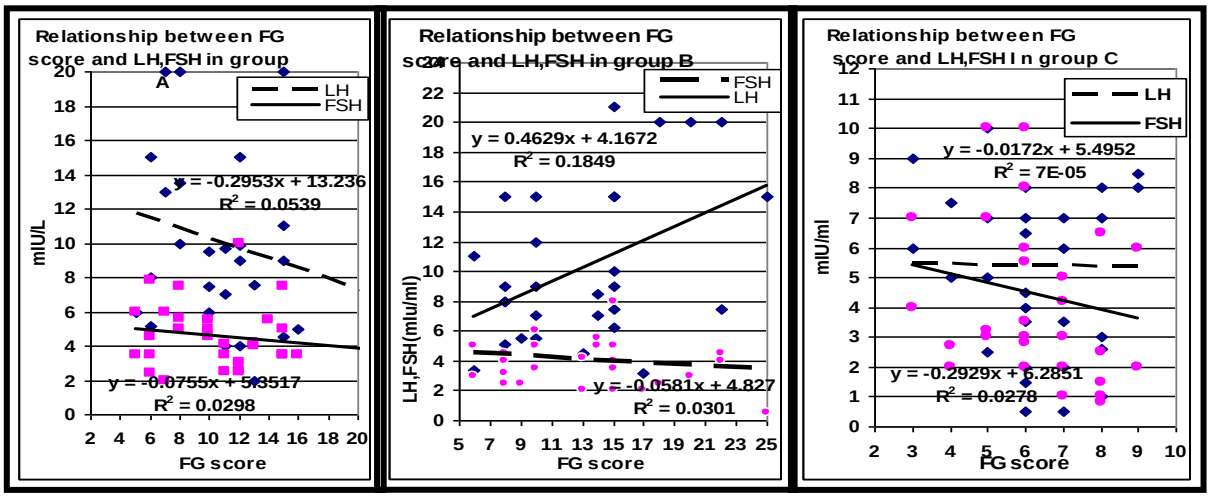

Figure 5 Relationship between FG score and LH, FSH in group A, B, C.

LH/FSH ratio in the three groups:
The mean value of LH/FSH ratio reveals highly significant increment after treatment in group A.P< 0.001. With only slight increment in group B.

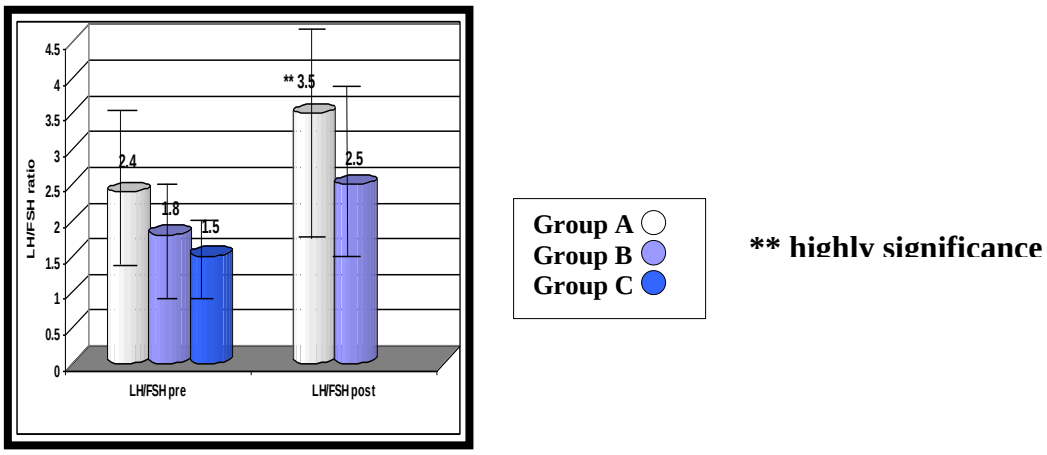

Figure 6 LH/FSH ratio in patients with control group.

FSH:

Here was decreased or normal level of FSH in patient groups pre treatment with highly significant decrement after treatment in group A p<0.001,and significant decrement in group B p< 0.05.

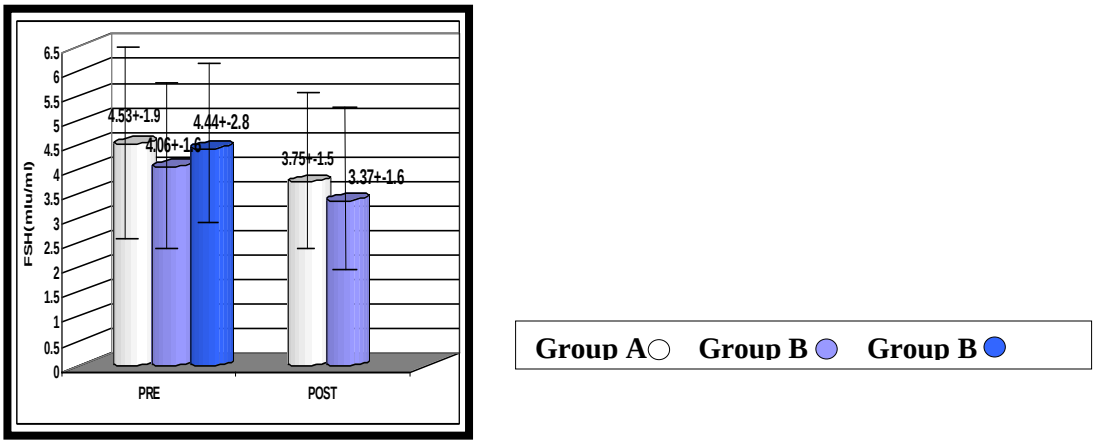

Figure 7 the FSH level pre and post treatment in patient's groups compared with control.

Testosterone:

The resulted study illustrate that the high level of testosterone in patients groups (A, B) show highly significant decrement after treatment p< 0.001.

The mean value of total testosterone pre treatment in group A and B was equal to (1.25±0.75, 1.38±0.72) while post treatment it equal to (1.05±0.6,1.05±0.56) respectively, compared with control group (0.67±0.26). figure (8):

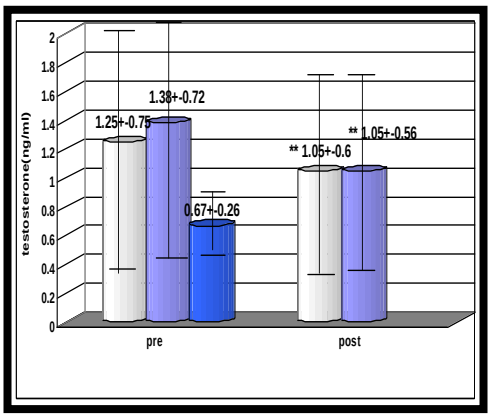

Figure 8 the testosterone hormone level pre and post treatment in patient's groups compared with control.

Prolactin:
There was normal level of prolactin pre treatment with significant increment in group B patients p< 0.05 after treatment. The mean value of prolactin pre treatment in both group A, B was equal to 13±8.3, 11.2±5.5, while post treatment was equal to 12.26±5.7, 14.14±8.8 respectively ,compared with control group 9.52±4.8.

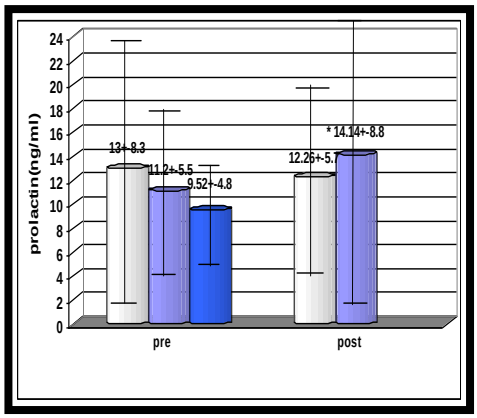

Figure 9 the prolactin level pre and post treatment in patient's groups compared with control.

Relationship between prolactin and testosterone in group A,B and C.:
There is positive non ignificant relationship between testosterone hormone and prolactin pre treatment in group A, and B, with negative non significant relationship in group C, figure (10):

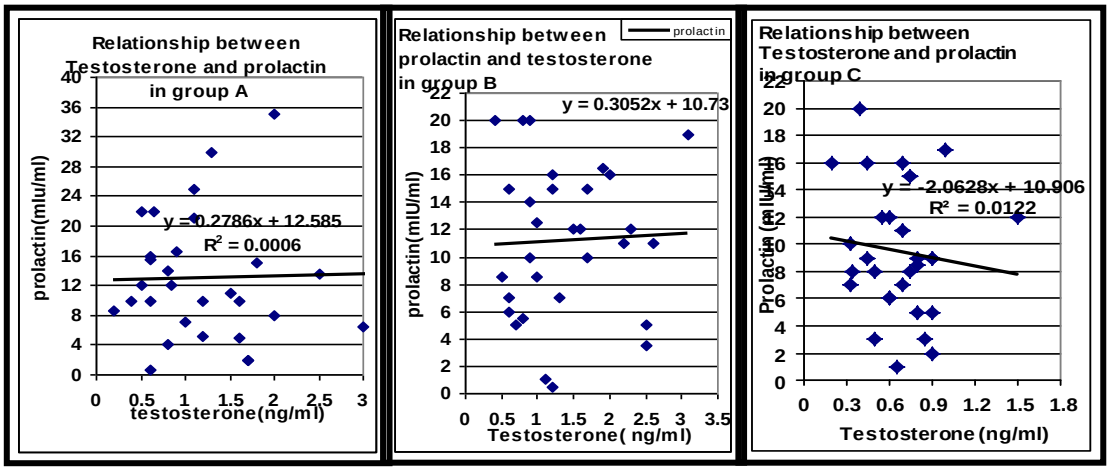

Figure 10 shows the relationship between prolactin hormone and testosterone inall groups.

Liver enzymes (ALT, AST):

There were significant increment in the level of enzymes pre-treatment especially in the second group with significant decrement after treatment p< 0.05.as shown in table (3):

Table (3) Describe the difference in the level of ALT,AST among patients and control and among patients themselves pre and post treatment. Relationship between ALT and AST in C. There was highly positive significant relationship between ALT, AST in group C. figure (11), and p <0.001.

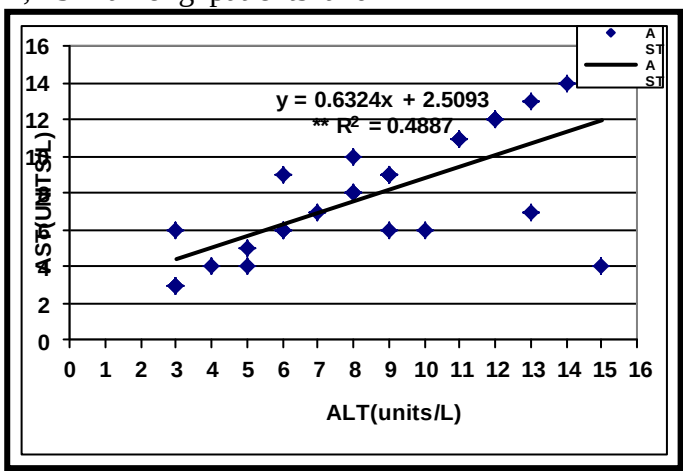

Figure 11 Relationship between liver enzymes (ALT,AST) in control group

Table 2 Relationship between FG score and hormones in the three groups

meGroup na	S.D)±Unit/LALT(		(Unit/L)± S.DAST	
	Pre	Post	Pre	Post
Group C	8.4±3.5	-	7.8±3.2	-
Group A	10.2±3.3	7.3 *±3.3	11.2±3.7	7.13 *±7.3
Group B	13±7.4	9.5 *±8.2	12.7±8.2	9.9 *±6.7

Ultrasound (U.S) findings:

Our study reveals a difference in U.S. findings among PCOS patients regarding the size of the ovary (59% of them had large ovary and the other shows normal ovarian size) and presence or absence of tinny follicles inside the ovary with ovarian thick capsule.(44% shows presence of tinny follicles)while the rest had other ovarian changes like absence of mature follicle, dense stroma ,or shows normal ultrasound findings.

Discussion

In our study we tried to cover the effects of two types of treatment regimen metformin alone and metformin +OCP(Diane) + antiandrogens (Androcur)) that had been applicable in the treatment of PCOS women according to their symptoms and it's severity ,and follow up their investigations for Clinical improvement, Biochemical changes after 6 month of treatment. The following changes were obtained from the results of the 6 month follow up study:

Distribution of Age:Table (1) shows the distribution of PCOS patients according to their age. The most common age group was ranged between 25-35(28±2) year, which represent 58% of total number .Followed by (15-25) year with mean of (21±2), 35-45(36±2) year, in a percentage of 32%, 10% respectively . This result agrees with several studies like; Carmina,[14] who mentions that PCOS is common endocrinopathy in women, occurring in about 5% of reproductive-aged women, it is rare above the age of 40 years.

The evolution of symptoms with age appears to be associated with increase of adiposity [15].

Family history, 55% of PCOS patients had positive family history and this is in agreement with most studies about family history such as Kidson[16], who states that 40% of women in families with PCOS or type 2 Diabetes have PCOS, indicating a possible inheritable tendency.

Primary infertility is more common than secondary infertility. The percentage of primary infertility was 65%(39/60)18/60 in group A, and 21/60 in group B, whereas that of secondary infertility was 35%.

Body Mass Index (BMI):As shown in figure (2) there is a great difference between patients and control pre treatment with highly significant difference after treatment in both groups $p< 0.001$.The mean value of BMI in group A pre treatment was 30.6±5.1, and post treatment was 29.5±4.9, while in group B, pre treatment was 32.05±4.4 and post treatment was 30.06±4.05, compared with control group that equal to (27.2±2.4). Like Balen [17] who states that Metformin appears to work in three ways. First, it decreases the absorption of dietary carbohydrates through the intestines. Second, it reduces the production of glucose by the liver. The liver uses the raw material in your food to create a reserve supply of blood sugar. Glucophage suppresses the roduction of this reserve fuel.Third, Metformin increases the sensitivity of muscle cells to insulin [18]. So that Metformin promotes weight loss and favorable changes in the lipid profile. In fact: weight loss can significantly improve menstrual cycles and ovulation

FG score and Degree of Hirsutism: Cyproterone acetate / ethinyloestradiol (Diane) has advantages in that more than 40% of women with facial hair find it clears within 9 months. Figure (3) shows that the high degree of hirsutism are more corrected with OCP+ antiandrogens. And there is positive significant relationship between FG score and the level of testosterone in

group A, with highly positive significant relation in group B. Also in figure (4) and (5) we see that there is positive significant relationship between FG score and testosterone level in group A ,p< 0.05.And there is highly positive significant relationship between FG score and testosterone level in group B p< 0.001, with negative non significant relationship in control group. And there is negative significant relationship between FG and LH, P<0.05 with positive non significant relationship with FSH in group A, and there is positive significant relationship between FG score, LH. P< 0.05, with negative non significant relation ship with FSH in group B, and there is negative significant relationship between FG score and FSH with negative non significance to LH in control group.

LH and FSH hormones:There is a high LH level pre treatment in both patients groups with significant decrement after treatment .

The mean value of it pre treatment was 10.05±5.5, 10.25±5.3.respectively, while post treatment was 6.76±3.5, 6.85±4.3 and compared with control mean which equal to5.39±3.3. Regarding FSH, there was a normal level pre treatment compared with control with non significant decrement after treatment in both groups as shown in figure (7) and LH/FSH ratio figure (6) shows that there is significant increment in this ratio pre treatment compared with control with highly significant increment after treatment in group A p<0.001, and significant increment in group B P<0.05.

This results were agrees with result of Banaszewska(19) who states that if you have PCOS your pituitary gland secretes higher levels of LH throughout the month instead of just before ovulation and ovulation may fail. Therefore LH/FSH ratios are increased to > 1 in women with PCOS. The ratio of LH to FSH is greater than 1:1, as tested on Day 3 of the menstrual cycle. The pattern is not very specific and was present in less than 50% in one study. Still, finding a higher LH than FSH in the early part of the menstrual cycle is a hallmark of PCOS. It would seem that confirmation of an elevation in LH is very diagnostic of PCOS.

Testosterone: In this study there is an increment in total testosterone level in some patients figure (8) ,and it remains normal in others so, the mean value of total testosterone pre treatment in group A and B was equal to (1.25±0.75, 1.38±0.72) while post treatment it equal to (1.05±0.6, 1.05±0.56) respectively, compared with control group (0.67±0.26), this result may be due to the hypothesis that says free testosterone is more specific than total testosterone as the results of Harborne [20] who report that in five of seven randomized studies ,in which insulin metabolism, SHBG, and androgens improved. This discrepancy explained by the fact that severe hyperandrogenism was not considered as a necessary inclusion criterion in our study. There are two studies byAttia[21]and Mansfied [22] that show a direct effect of metformin in reducing androgen production in theca cells.

Free testosterone provides a better diagnostic yield for ovarian hyperandrogenism because levels of SHBG are decreased thus increasing free hormone levels [23]. There is a new evidence suggests that metformin may also have a direct activity on androgen production by the ovary. Metformin use, in some women with PCOS has lowered androgen levels, increased sensitivity to clomiphene, restoration or improvement in menstruation, pregnancy and improved pregnancy outcome.The OCP (Diane) was superior to metformin in reducing serum total testosterone levels

when compared with metformin alone [24].While Diane significantly decreases serum androgen levels. The combination of OCP s with metformin thus appears to further decrease androgen levels, without any additional effect on insulin sensitivity. The estrogen component stimulates hepatic production of sex hormone-binding globulin that reduces bioavailable androgen and can reduce hirsutism and acne. The progestin component provides competitive antagonism to androgen at its receptors, reducing the action of testosterone at the target organ [25].

Prolactin: The mean level of prolactin hormone in group A pre treatment is 13±8.3, and it is 11.15±5.5 in group B, while in control it is 9.52±4.8 while post treatment it was 12.26±5.7, 14.14±8.8 respectively as in figure (9). Although it is slightly higher than control but it still within normal range. This result may goes parallel with study of Angie [26] who reveals that Some women with PCOS also have hyperprolactinemia. All women with reproductive difficulties should have a hormone screening done. Prolactin levels should be a part of that testing. More over; hyperprolactinaemia is a relatively frequent condition which affects almost half the patients suffering from poly cystic ovary (Usually higher than 200 ng/mL)[27], while Angela [28] found out that about 25% of PCOS patients exhibit elevated prolactin .Other studies differ from those results and suggest normal level of prolactin.Also there is positive relationship between prolactin level and testosterone ,figure (7)this may be contributed to the theory which says that hyperprolactinemia may associated with PCOS. After treatment with cyproterone acetate + ethinyloestradiol the prolactin level may be increased and this agrees with most studies as Ismail [29] who reports that there is a positive relationship between serum prolactin level and the duration of oral contraceptive pills intake and their steroid content, and this relationship is not related to the age and parity of the women,and with Zacur[30] who states that Prolactin levels have been rise during use of oral contraceptives.

Liver enzymes (ALTand rol group, with significant decrement after treatment in both groups, table (3). Some studies state that increased circulating concentrations of ALT are a specific marker of liver pathology and ALT has been established as a marker of liver fat accumulation in obese women 31) .Other studies have reveals a decrease in ALT level, after metformin treatment although improved liver histology has been a less consistent finding [32]. This study thus expand an evidence to support a metformin-induced reduction in liver enzymes [33].Other studies suggest that the most serious potential side effect of antiandrogens is liver toxicity, and patients should be monitored for changes in liver enzymes, especially if taking a high dose (200-300 mg/day). Toxicity is dose-dependent and the low doses used in oral contraceptive pills (2 mg) do not appear to represent a significant risk.

Ultrasound (U.S.) finding: The ultrasound findings of bilaterally enlarged polycystic ovaries. The ovary is usually greater than 9cm³ with more than 8cm³ peripherally oriented cystic structures in a sonographic plane by an increased stromal mass (>25% of the ovarian volume). Classification of polycystic suggests there are eight or more follicles present, with the follicles less than 10 mm in diameter [34].

In our study 77% of total PCOS patients had U.S findings (73%of group A and 80%of group B) 59% of them had large ovary and the other shows normal ovarian size, 44% shows presence of tinny follicles with or with out large

ovary, while the rest had other ovarian changes like absence of mature follicle, and dense stroma, and 33% of patients shows normal U.S findings . US evidence of polycystic ovaries occurs in 16% of asymptomatic women, and 66-82% have the classic ultrasound appearance expected of polycystic ovaries [49]. These ultrasound findings appear to be present in more than 90 percent of women with PCOS; they are also present in up to 25 percent of normal women.

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