Improving an Ovulation Rate in Women with Polycystic Ovary Syndrome by Using Silymarin

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Abstract

Polycystic ovary syndrome(PCOS) is a heterogeneous disorder of uncertain etiology , it is the most common endocrinopathy in women and most common cause of anovulatery infertility ,characterized by chronic anovulation and hyperandrogenemia .The present study was designed to investigate the effect of silymarin which is known to have antioxidant and insulin sensitivity effects on the levels of glucose, insulin ,testosterone ,leutinizing hormone(LH) and progesterone .Ovulation rate and Homeostasis Model Assessment of insulin Resistance (HOMA) ratio were determined .A 3-months of treatment were conducted in 60 PCOS patients in three well-matched groups .The first one (n=20),received silymarin(750mg/day) .The second group received metformin(1500mg/day) while the third group treated by combination of metformin (1500mg/day)and silymarin (750mg/day). All these groups had taken the drugs in divided doses. The results showed significant improvement in all parameters at the end of treatment .The percentage of increment in progesterone levels after completion of treatment were 12.12, 15.9, and 17.51 in groups 1, 2, and 3 respectively and the number of patients ovulated after 3 months of treatment were 4,5, and 10 in groups 1,2, and 3 respectively. However they are more better in group of patients who were treated with combination of silymarin with metformin.In conclusion the addition of silymarin to metformin in treatment of PCOS patients has improving effect on disturbed hormones and ovulation rate.

Key words: Polycystic ovary syndrome, silymarin, ovulation rate,metformin

الخلاصة

إن متلازمة المبيض متعدد الأكياس هو اضطراب متغاير لأسباب غير مؤكدة.انه اعتلال هورموني شائع عند النساء وأكثر سبب شائع للعقم المصحوب بعدم الاباضة ويتميز بعدم اباضة مزمن وارتفاع الهورمون الذكري .أن الدراسة الحالية صممت لفحص تأثير السيلمارين وهو الذي يملك خاصية ضد التأكسد و زيادة لتحسس الأنسولين وتقليل المقاومة للأنسولين على مستويات السكر , الأنسولين , الهورمون الذكري, الهورمون اللوتيني والبروجيستسيرون. تم احتساب نسبة الاباظة ودليل مقاومة للأنسولين (HOMA) أيضا.ثلاثة أشهر من العلاج أعطي ل ٦٠ مريضة بمتلازمة المبيض متعدد الأكياس في ثلاثة مجاميع . المجموعة الأولى مكونة من ٢٠ مريضة عولجن بعقار السيليمارين بجرعة ٧٥٠ ملغم يوميا والمجموعة الثانية مكونة من ٢٠ مريضة عولجن بعقار المتفورمين وبجرعة ١٥٠٠ ملغم يوميا بينما المجموعة الثالثة والمكونة من ٢٠ مريضة أيضا عولجن بمزيج الميتفورمين ١٥٠٠ ملغم يوميا والسيلمارين بجرعة ٧٥٠ ملغم يوميا .كل هذه المجاميع اخذت العلاج بجرع مجزئة .النتائج أظهرت تحسن المستوى لكل التحاليل بعد نهاية العلاج.ان زيادة نسبة مستويات البروجيستيرون ونسبة الاباضة كانت أفضل في مجموعة المريضات اللواتي عولجن بمزيج السيليمارين والميتفورمين .وفي الاستنتاج إن إضافة السيليمارين للمتفورمين لعلاج المريضات المصابات بمتلازمة المبيض متعدد الأكياس له تأثير حسن على الهومونات المضطربة ونسبة الاباضة.

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous disorder of uncertain aetiology; it is the most common endocrinopathy in women and most common cause of anovulatory infertility,affecting 5-10% of population of reproductive age.[1] It is characterized by chronic anovulation and hyperandrogenism.[2] Insulin resistance and associated hyperinsulinemia also have been recognized as important pathogenic factors in determining the majority of PCOS women particularly when obesity is present .[3] Most but not all women with PCOS have hyperinsulinemia with insulin resistance[4].The association between hyperinsulinemic insulin resistance and PCOS well recognized and play an import role in the development of PCOS[5].Hyperinsulinemia has been shown to reduce sex hormone binding globuline (SHBG) synthesis in liver[6] and insulin has a direct effect on ovarian steroidogenesis in theca cell.[7] Metformin is the oldest and still most insulin sensitizer world wide in the treatment of type2 diabetes mellitus and PCOS-associated with insulin resistance. It is a biguanide derivative and considered as an insulin sensitizer since it lowers glucose levels without increasing insulin secretion .[8]

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Silymarin is an active polyphenolic flavenoid extracted from fruits(seeds) of medicinal plant silybum marianum (milk thistle), extracts were standardized to contain 70-80% silymarin complex which comprised mainly of three major flavolignans , silybinin silychristin and silydianin of which silybinin is the most biological active. Silymarin is considered to be very safe and there are only few reports on its adverse effects,mainly a mild laxative effect has been observed in occasional instances and there are no known contraindications or side effects reported during its regular use.[9] According to the multiple pharmacological actions of silymarin, silybinin have been clinically evaluated in diabetics for their therapeutics value reduces the lipoperoxidation of cell membrane and insulin resistance significantly, decreasing endogenous insulin overproduction and the need for exogenous insulin administration.[10] So this study was designed to evaluate the efficacy of silymarin as insulin sensitizer improving an ovulation rate by treatment of PCOS and consequently its effect on hormonal and biochemical profile of the patients and comparing it with a classical one, metformin.

Materials and Methods

Patients

This study was conducted into Baghdad city ,in al-Elwia maternity teaching hospital from 12/2006-6/2007.The study groups included 80 women selected randomly, 60 patients with PCOS aged (19-39) years with a mean age (27.5) years and 20 healthy control women aged (21-32) years with mean age (24) years.The diagnosis of PCOS was made by the gynaecologists depending on ultrasound examination ,clinical features and laboratory tests according to diagnosis criteria of (Rotterdam 2003) [11]. Table-1 shows that the clinical presentations of patients in present study like those reported in other studies of polycystic ovary syndrome in that it is a heterogeneous disorder Investigations included : serum fasting glucose levels, fasting insulin levels, serum testosterone, serum progesterone and serum leutinizing hormone (LH).All patients participitated in this study were diagnosed having PCOS and were non-diabetic, not hypertensive, not pregnant , and not taking any medications that affect the reproductive or metabolic functions with 90 days of study. The patients were followed weekly regularly under gynecologist supervision during the period of treatment.The women were grouped into 4 groups as follow:

Group 1: included 20 PCOS patients ,with BMI (31.22±1.138 Kg/m2),and age (19-31) years. They received Sylimarin tablets (750mg/day) in 3 divided doses after meals for 3 months.

Group 2: included 20 patients with BMI 30.84±1.23kg/m2) and age (20-35) years. The treatment was including metformin tablets 1500mg/day in 3 divided doses (500mg after meals for 3 moths.

Group 3: included 20 patients with BMI 32.83±1.37 kg/m2), age (22-39) years. The treatment was consisting of combination of 2 drugs (sylimarin 750 mg/day) and metformin (1500 mg /day) in 3 divided doses for 3 months.

Group 4: included 20 healthy women with BMI 28.4±1.01kg/m2) ,age (21-32) years and these women were with regular cycle (21-32 days) who were taken from outside of the hospital and selected as controls.

Sample collection

Venous blood sample withdrew after overnight fasting (at least 12 hours of fasting) from PCOS women and the control group . For the subjects with regular cycles ,the samples were taken at 3-5 days after the cycle for determination of serum LH and the sample for progesterone were taken at 21 days of the cycle .The other patients with irregular cycle ,the samples were taken randomly. The samples were taken from the patients before starting and after one month of treatment .

Biochemical analysis

Determination of serum glucose and insulin levels

Fasting serum glucose and insulin levels were measured by commercial kit obtained from Randox using enzymatic method[12,13].

Determination of Homeostasis Model Assessment of insulin Resistance (HOMA-IR) ,

HOMA - IR was calculated using the following formula[14] :

HOMA-IR=Fasting glucose (mmol/L)× Fasting insulin (pmol/ml)/22.5.

Insulin resistance patients were defined as having HOMA>2.7.

Determination of serum testosterone [15] and LH levels [16] :

Serum testosterone and LH levels were determined by radioimmunoassay(RIA) method using a kit provided by Sigma-Aldrich.

Determination of serum progesterone & Ovulation Rate

Serum progesterone levels were determined using kit obtained from Sigma-Aldrich , using (RIA) method, and the ovulation rate was determined according to mid-luteal phase progesterone level that was equal to or more than 16nmol/L (5ng/ml) .[17]

Determination of body mass index(BMI)

BMI was calculated using standard formula : BMI= weight (kg)/high (m2).

Obese patients were defined as having MBI > 27kg/m2 [18].

Ultrasound study

Transvaginal ultrasound study scan is performed for each patient at about day 12 of the cycle in order to to confirm follicular changes that appear through biochemical and hormonal changes , also it was repeated for each patient who had serum progesterone levels higher than or equal to 16nmol/L in order to confirm improvement of fertility and response of patients to treatment and follow up follicular development .[19]

Diagnosis

Hyperandrogenism

Based on criteria of Androgen Excess Society (AES 2006), which recommended the following diagnostic criteria for PCOS hyperandrogenemia.[20]

1. Hyperandrogenism (hirsutism and /or hyperandrogenemia)
2. Ovarian dysfunction (oligo-anovulation and /or PCOS).
3. Exclusion of related disorders such as hyperprolactenemia and congenital adrenal hyperplasia.

Hirsutism

Based on Ferriman-Gallwey score , evaluates nine body sites including the face, chest , areolae , linea alba , upper back, lower back, buttocks, inner thighs and external genetalia .[21]

Infertility

Inability of any couple to conceive a child within a 12 months period of unprotected coitus (sexual intercourse) .[22]

Statical analysis

Student t-test was used to examine the quantitative differences in the mean parameters. The results are expressed as mean±SD and the P-values <0.05were considered statically significant.

Results

Table-1 shows that 43.3% of the patients were with hirsutism and 36.6% with acne .Most patients were obese 68.3% and 31.6% were lean.The percentage of infertility among the patients were 31% and only 7% were with regular cycle while the percentage of amenorrhea and oligomenorrhea were 19% and 34% respectively.The percentage of insulin resistance was 78.3%, morover the androgenemia feature was the highest (85%).Table 2 shows a significant elevation (P<0.05) in mean serum insulin levels (pmol/L) of base line levels in the three study groups compared with control group and it declined significantly (p<0.05) after 1^{st},2^{nd} and 3^{rd} month of treatment in all groups of patients. There is significant increment (p<0.05) in mean serum glucose levels (mmol/L) of base line levels in three groups compared with control group and it declined significantly (p<0.05) after 1^{st},2^{nd}, and 3^{rd} month of treatment in all groups of patients except in 1^{st} month of first group , it was non-significant (P>0.05).Also the same Table illustrated significant increment (P<0.05) in mean HOMA-IR of baseline level in 3 PCOS groups compared with control and it declined significantly (p<0.05) after 1^{st},2^{nd}, and 3^{rd} month of treatment in all groups of patients. There was significant increment (p<0.05) in mean serum testosterone levels (nmol/L) of base line levels in 3 groups compared with control group and it declined significantly (p<0.05)after 1^{st} ,2^{nd} and 3^{rd} month of treatment in all groups of patients. Mean serum progesterone levels (nmol/L) of baseline levels in three groups decreased significantly (p<0.05) compared with control group and elevated significantly (p<0.05) after 1^{st},2^{nd}, and 3^{rd} month of treatment in all groups of patients except 1^{st} first group , it was non-significant (p>0.05).This table also demonstrate significant increase(p<0.05) in mean serum LH levels (U/L) of base line levels compared with the control group and it declined significantly (p<0.05) after 1^{st}.2^{nd}. and 3^{rd} month of treatment in all groups of patients except in 1^{st} month of group 1 and 2 ,it was non-significant (p>0.05).Table-3 illustrated that, the percentage of increment in mean serum progesterone levels (nmol/L) was 4.28 % ,8.72 and 12.22 in group1 ,also 4.324%,8.42% and 15.9% in group2 and 4.179% ,8.79% and 17.51 in group 3 after 1^{st},2^{nd}.and 3^{rd} month of treatment for each group respectively. The numbers of women who had ovulated were 4,5 and 10 in group 1,2,3 respectively.

Table 1: Demographic data of 60 women with polycystic ovary syndrome.

Feature	No. of patients (%)
Hirsutism	26(43.3)
Acne	22(36.6)
Obesity	41(68.3)
Lean	19(31.6)
Infertility	31(51.6)
Amenorrhea	19(31.6)
Oligomenorrhea	34(56.6)
Regular cycle	7(11.6)
Insulin resistance	47(78.3)
Hyperandrogenemia	51(85)

Table 2: Effect of metformin and /or silymarin on Insulin ,glucose ,HOMA-IR ratio, total testosterone and progesterone in women with PCOS.

Groups	Analytes	Control	Base line	After 1 M	After 2M	After 3M
1	Insulin(pmol/L)	57.5±0.359	92.18±4.73	89.35±0.35*	85.65±4.28*	81.44±3.66*
	Glucose(mg/dl)	5.1±0.17	5.29± 0.29a	5.01± 0.192NS	4.88±0.128*	4.73±0.128*
	HOMA	2.13±0.015	3.11± 0.244a	2.865±0.233*	2.673±0.178*	2.02±0.178*
	Testosterone(nmol/L)	1.45±0.03	4.59± 0,223a	4.427±0.242*	4.242±0.303*	3.396±0,318
	Progesterone(nmol/L)	17.15±0,02	12.84±0,612a	13.39±0.682NS	13.96±0.804*	14.41±0.942*
	LH(u/L)	5.2±0.365	9.38± 0.317a	9.18± 0.284NS	9±0.245*	8.71±0,376*
2	Insulin(pmol/L)	57.5±0.359	83.7±4.49a	82.1±3.468	80.8±3.01*	74.5±4.73*
	Glucose(mg/dl)	5.1±0.17	5.35± 0.362a	4.49± 0.209*	4.63±0.35*	4.25±0.229*
	HOMA	2.13±0.015	2.68± 0.226a	2.59± 0.212*	2.39±0.199*	2.02±0.178*
	Testosterone(nmol/L)	1.45±0.03	4.07± 0.199a	3.938±0.213*	3.765±0.185	3.9±0.167*
	Progesterone(nmol/L)	17.15±0,02	12.95±0.967a	13.517±0.941*	14.04±1.01*	15.01±1.33*
	LH(u/L)	5.2±0.365	111.13±0.87a	10.56±0.839NS	10.10±0.644*	9.52±0.741*
3	Insulin(pmol/L)	57.5±0.359	106±6.6	94.05±4.26*	84.16±4.50*	77.22±3.09
	Glucose(mg/dl)	5.1±0.17	5.12±0.301a	4.58±0.330*	4.27±0.369*	3.87±0.22*
	HOMA	2.13±0.015	3.55±0.172a	2.75±0.144*	2.298±0.245*	1.91±0.135*
	Testosterone(nmol/L)	1.45±0.03	4.59±0.942a	4.18±0.176*	4.06±0.159*	3.9±0.167*
	Progesterone(nmol/L)	17.15±0,02	13.88±0.875a	14.46±0.792*	15.10±0.673*	16.31±0.916*
	LH(u/L)	5.2±0.365	10.08±0.510a	9.33±0.480*	8.89±4.22*	8.54±0.515*

Values are Mean±SD , a P< 0.05 for comparison with control group ,*P<0.05 for comparison with baseline , NS non-significant P>0.05

Table 3: Comparison among mean % of increament in progesterone levels (nmol/L) and number of women who had ovulated during the study in all groups of PCOS patients.

	% of 1st Month	% of 2nd Month	% of 3rd Month	No.of women ovulated
Group1 (sylimarin)	4.28	8.72	12.22	4
Group2 (Metformin)	4.324	8.42	15.9	5
Group 3 (combination)	4.179	8.79	17.51	10

Discussion

The percentage of patients with hirsutism and acne was 43.3% and 36% respectively (table-1) and this finding was consistence with other study performed in diagnosis of PCOS.Cutaneous manifestations of hyperandrogenism in PCOS include hirsutism,acne or combination , and male –pattern hair loss (androgenic alopecia); whereas acanthosis nigrigans is a cutaneous marker of hyperinsulinemia .[23] The study demonstrated that percentage of obese patients was 68.6% while it was 31.6% for lean , this is common in PCOS and it is in line with other studies which demonstrated that 40-60% of women with PCOS are obese (BMI>27 kg/m2).[24,25] The present study showed that (51.6%) of the patients were infertile , 31.6% with amenorrhea, 56% with oligomenorrhea ,11.6% with regular cycle,78.3% with insulin resistance and 85% with hyperandrogenemia ,these results are in agreement partly with other results which demonstrate the presence of infertility by (55-75%) , amenorrhea (26-15%) ,oligomenorrhea (50-90%) regular cycle (22%) and hirsutism (60-90%) in women with PCOS .[24,26] The high levels of androgens lead to chronic anovulation , menstrual disturbances and hirsutism. PCOS patients typically have elevated LH levels and LH:FSH ratios.[27] because hyperandrogenism leads to abnormal folliculogenesis and endome-

trial development .[28,29] Hyperandrogenemia is a key feature of the syndrome; but it is not always linked to hyperandrogenic symptoms such as acne or hirsutism; indeed ,ethnic groups such as Asian shown insulin resistance and associated hyperinsulinemia are also now recognized as important pathogenic factors in determining hyperandrogenism in the majority of PCOS women ,particularly when obesity is present .[30]The present study illustrated a significant ($P<0.05$) increase in serum insulin and glucose baseline levels and HOMA-IR index baseline value compared with control group, the results were compatible with those observed by Laure C.,et al.[31] , as characteristic features of women with PCOS.During three months of treatment with metformin and /or silymarin a significant ($P<0.05$) reduction in these parameters in all groups was observed except the effect of silymarin on glucose levels in the first month, was non-significant ($P>0.05$) , as shown in table (2). Metformin leads to increase glucose utilization, decrease hepatic glucose production, imcrease insulin receptor binding and insulin receptor tyrosin kinase activity, but it has adverse effect on gastrointestinal tract and liver function[32,33] , while silymarin, represents a new possibility in the treatment of PCOS, the underlying mechanistic links for this effects may be due to different possible mechanismas; silymarin increases, normalized and stimulated pancreatic activity of antioxidant and free radical quenching enzymes, (glutathion peroxidase,superoxide dismutase and catalase).[34.35] Silymarin may produce its effect on glucose and insulin levels by another mechanism through blockage of TNF-α where that serum TNF-α concentration have been high in normal-weight PCOS women and even higher levels in obese women with PCOS.[36] When combination of silymarine and metformin were used , a powerful synergism effect occurred and led to best results as illustrated in third group , because each drug act by different pharmacological mechanism and different receptor sites which means that they may not compete one with each other to get same response, so that pronounced reduction in glucose , insulin and HOMA-IR values was occurred.The present study demonstrated a significant ($P<0.05$) increase in baseline testosterone levels compared with control group ,this result was compatible with other studies which demonstrate that serum concentration of testosterone and androstenedion are elevated in women with PCOS (the mean concentration are 50%-150% higher than controls).[37] During the 3 months of treatment with metformin and /or sylimarin, a significant reduction($P<0.05$)from baseline of testosterone was observed (table -2).These results partly in agreement with Velazqwz et al. concerning metformin effect who reported that in an uncontrolled study, treatment with metformin for 8 weeks results in reduction of serum free testosterone in 29 non-diabetic women with PCOS , mostly overweight.[38] Most studies on this subject suggest that insulin lowering agents may affect the entire spectrum of endocrine, metabolic , and reproductive abnormalities in PCOS patients.However not all studies have assessed the effects of metformin in hyperandrogenic women have confirmed these findings. Interestingly, where insulin levels were reduced by treatment , serum androgens were lowered as well.[39] In an uncontrolled trial that assessed 26 obese women with PCOS before and after treatment with 1500 mg metformin /day for 8 weeks , a reduction in insulin concentrations and in serum free testosterone were reported and SHBG increased by 23% (38).The combination of silymarin and metformin resulted in a more remarkable reduction in testosterone levels than group 1 and 2 , this may be contributed to additive effect of these two drugs.It has been reported in this study significant decrease in baseline progesterone levels compared with control group, this result was compatible partly with other study.[40] Treatment with metformin and /or silymarin for 3 months , demonstrated a significant increament ($P<0.05$) in serum progesterone levels in all groups , except in first month of group 1 ,it was non-significant ($P>0.05$) (table 2). The improvement in ovulation rate (as assessed by measurement of mid-luteal phase progesterone level (>5ng/ml or >16nmol/L) was evaluated according to the percent of increment in baseline progesterone levels and number women who had ovulated , (table 3) which reflect that third group showed highest percentage of increment in progesterone levels and number of women who had ovulated. However , other researchers found a significant enhancement in luteal progesterone levels in PCOS women treated with metformin and they suggested that insulin resistance and hyperinulinemia may be responsible for low progesterone levels during luteal phase in PCOS[41] ; therefore the luteal progesterone levels may be enhanced in PCOS by decreasing insulin levels with metformin. It had been reported that an improvement in menstrual pattern or ovulation with only modest improvement in insulin resistance and hyperinsulinemia is sufficient to promote preovulatory follicular maturation.[42] Silymarin was not different entirely from metformin concerning its effect on ovulation rate and progesterone levels as a result of their effect on insulin resistance and hyperinsulinemia .There is a significant negative correlation between insulin and pro-

gesterone, and between progesterone and LH concentration.[41] Therefore it is probaple that effect of silymarin on progesterone levels were consequences of its effect on insulin resistance and hyprinsulinemia. There was remarkable response to combination treatment because each drug act by its own mechanism and higher increment in progesterone and ovulation rate exerted by each drug alone may be enhanced by their combination . The base line LH levels in this work increases significantly ($P<0.05$) compared with control group, and it was compatible with other studies which demonstrate that women with PCOS , have 55-75% of a high LH to FSH ratio due to increased levels of LH than low levels of FSH.[43] Elevated serum concentrations of LH are common in all reported series of women with PCOS.[44] Typically , PCOS associated with increased LH and androgens but with normal or low serum concentrations of FSH.Most investigations have also documented an increased LH pulse amplitude and frequency as characteristic feature of PCOS.[45] During three months of treatment with metformin /and or silymarin a significant reduction ($P<0.05$) in serum LH levels were observed in all groups except in first month of group 1 and 2 , it was non-significant ($P>0.05$), (table 2). The reduction of plasma levels of LH are not a primary event in the reduction of hyperandrogenism induced by metformin because many studies have reported a reduction in plasma androgens but not concomitant reduction in LH, indicating that in these cases the reduction of steroid synthesis cannot be secondary to reduced stimulation of LH also. It is possible that spontaneous or induced ovulation or reduction in androgens may lead to a secondary reduction in LH.Therefore androgens returned to pretreatment levels when metformin was suspended and that rise preceded the rise in LH, sustaining the hypothesis that a primary disorder of androgen hypersecretion is the cause of LH hypersecretion.[46] The effect of silymarin can be explained in the same manner, although its action on insulin levels are more pronounced when compared with metformin in current study, however there is a positive correlation between hyperinsulinemia and LH levels[41], the possible effect of silymarin on LH though its action on hyperinsulinemia and insulin resistance , indeed improvement in hyperinsulinemia may lead to decrease response of LH to GnRH. As expected from above mechanism of each drug , a highest reduction in LH levels were observed when combination used ,(table2) which indicates that each drug may improve the other.

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