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A COMPARATIVE STUDY OF CLINICAL, HORMONAL, AND METABOLIC PARAMETERS AND THEIR RESPONSE TO TREATMENT WITH METFORMIN IN POLYCYSTIC OVARIAN SYNDROME

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ABSTRACT

Polycystic ovary syndrome (PCOS) showing the clinical features of an endocrine and metabolic disorder including, hyperinsulinemia and hyperandrogenism. It considered as the heterogeneous disorder and leading cause of dyslipidemia. The Purpose of this study to investigate the clinical, endocrinal and metabolic parameters, and their differential response to metformin among obese/overweight and lean body weight in polycystic syndrome women in Kerbala province. Patients and Methods: This was a prospective study for two hundred thirty-five Iraqi patients, who newly diagnosed with PCOS based on Rotterdam criteria by physician's diagnosis during their visit to Women's Hospital obstetrics education in Karbala /Iraq during the period from July, 2019 till the end of April, 2020. Patients were divided in to obese/overweight PCOS group (body mass index (BMI) ≥25 kg/m^2) and lean PCOS group (BMI <25 kg/m^2). Excluded Patients were those with thyroid dysfunction, congenital adrenal hyperplasia and Cushing's syndrome, matched age was (18-40 years). Enzymatic methods were used for blood sugar, and lipid profile measurement, LH, FSH, TSH, prolactin, total testosterone, estradiol, F. insulin were all tested by cobas e 411 analyzer, ELISA kit was used to measure SHBG. LH/FSH ratio, FAI, and HOMA-IR were calculated, all these parameters were measured for each patient and after 3-months therapy the patients had a medical checkup was repeated. Results: The total 135 PCOS women, 179 (76.17%) were in the obese/overweight group with BMI ≥25 kg/m^2 and 56 (23.83%) were in the lean PCOS group, accordingly the percentages were decrease to 162(68.94%) in obese/overweight group and increase to 73(31.02%) in lean group upon three months of metformin treatment. Prevalence of insulin resistance (IR), dyslipidemia, hirsutism and alopecia was most common in obese/overweight group. FAI, LH pulses frequency or amplitude was higher in obese/overweight, also levels of TSH and prolactin were higher, increase body weight was correlated with several abnormalities of sex hormone levels and less level of SHBG was detected in obese/overweight women, this associated with insulin resistance which caused an increase in free testosterone level. Metformin restored both LH level and ovarian function more in overweight/obese group with TSH level dropping more remarked and SHBG elevated higher with obese/overweight women. In Conclusion: Obese/overweight PCOS women have more risk of metabolic disorders like insulin resistance and dyslipidemia with more noticed signs of hyperandrogenism, Furthermore, improvement in clinical, endocrinal and metabolic parameters that noticed among this group was better than lean body weight one upon metformin therapy period.

Keywords: Fasting insulin (F.insulin), sex hormone binding globulin (SHBG), homeostasis model assessment for IR (HOMA-IR).

Introduction

Polycystic ovary syndrome (PCOS) is most prevalence endocrinological disorder seen in nearly 20% of the infertile women, it is an important cause of infertility as well as correlated with an increased risk of metabolic syndrome (MBS), diabetes mellitus type 2, cardiovascular disease and endometrial cancer (Kiałka, Milewicz *et al.*, 2016). About (80%) of women with PCOS have a high body mass index (BMI), insulin resistance (IR) and PCOS symptoms like, ovarian cysts, alopecia, acne, and hirsutism. Majority of these women are not diagnosed until fertility issues arise in adulthood (Williams, Ong *et al.,* 2013). Signs and symptoms of this syndrome including irregular or no menstrual cycle, clinical or biochemical hyperandrogenism, infertility and insulin resistance, those symptoms result in hyperinsulinemia and alteration in metabolic profile (Nahar. 2019). In 2003 Rotterdam in Netherland presented new diagnostic criteria (Dumesic, Oberfield *et al*., 2015), according to two of the following three symptoms: no ovulation, hyperandrogenism and ovarian cysts detected by ultrasound (Kabel, 2016). Insulin resistance is common finding among women with a lean weight and overweight women, furthermore adipose tissue possesses aromatase, an enzyme that converts androstenedione to estrone and testosterone to estradiol (Mauvais-Jarvis, 2016; Rothenberg, Beverley *et al.,* 2018). Clinical symptoms of PCOS are worsen in obese patients by increasing insulin resistance leading to more elevation of ovarian and adrenal androgens and of free testosterone. Treatment of obesity is one of the main goals of any therapy for PCOS, although this may be more difficult because of

insulin resistance and impaired lipolysis (Ganie, Marwaha *et al.,* 2016). Metformin as an insulin sensitizer used in PCOS treatment by suppression of endogenous glucose production, it acts by pleiotropic actions on liver, skeletal muscles, adipose tissue, endothelium and the ovaries, it also has a role in lipid metabolism by suppression of acetyl CoA carboxylase activity. It acts through AMPK activation resulting in suppression of gluconeogenesis and expression of several lipogenic genes such as fatty acid synthase-FAS, participates in improvement of insulin (Khan, Karim *et al*., 2019). Sensitivity through enhancement of peripheral glucose uptake, and regulation of lipogenesis by down regulating other genes required in lipid synthesize also by inhibiting proteolytic processing and transcriptional activity upon AMPK-mediated phosphorylation at Serine 372 (Cernea, Cahn *et al.,* 2017). The aim of presented work was to investigate the clinical, endocrinal and metabolic parameters, and comparison the differential response to metformin between the obese/overweight and lean body weight in polycystic syndrome women in Kerbala province.

Materials and Methods

It was a prospective study carried out at Women's Hospital and obstetrics education in Karbala during the period from July, 2019 till the end of April, 2020, study was conducted on two hundred thirty- five women with newly diagnosed PCOS. Participated women were recruited by consultation of an infertility according to the inclusion and exclusion criteria of the study. All women in current study were starting metformin tablet 500mg twice daily as standard adjuvant therapy for three months. Ages of women should be (18-40) years. Inclusion criteria carried out according the Rotterdam criteria when two of three of the following symptoms: hyperandrogenism, irregular menses or ultrasound PCOS morphology. Women with any insulin sensitizer drugs, lipid lowering drugs, those with endocrine abnormality or hypothalamic or pituitary dysfunction, all were excluded. Demographic parameters were taken for all the patients through history-taking specifically for: Address, age, body mass index, blood pressure, Presence of other diseases, smoking, menstrual regularity, hirsutism and alopecia. Blood samples were obtained from all patients, (5ml) of venous blood. (1ml) was placed in EDTA-tube used for measurement fasting glucose and HbA1C, (4ml) was placed in gel tube (EDTA-free) tube used for serum separation after centrifugation of blood at 3000 rpm for 10 minutes for measurement of the following parameters: follicular stimulating hormone (FSH), (LH), (TSH), Prolactin, total testosterone, (SHBG), estradiol, F.insulin and Lipid Profile including: (TG),(LDL-C), (HDL-C) and (TC). Patients were divided into two groups: one who were obese/ overweight and other who were lean body weight based on the Asian criteria (Who, 2004) with BMI $\geq$25 kg/m^2 were enrolled in the obese/overweight PCOS group and other group were those women with BMI <25 kg/m^2 (normal and underweight women) designated as lean body weight PCOS group. Various clinical, endocrinal, and metabolic parameters were compared between two groups, clinical parameters included signs of hyperandrogenism like excessive hair growth, acne, or alopecia. Menses regularity studied according to Federation of Gynecology and Obstetrics (FIGO) classification (Sen, Mandal *et al.*, 2019). Cycle length of (24–38) days was considered normal and length >38 days were considered as oligomenorrheic patients. All patients were called on Day 2 of her next cycle for measurement of bio parameters, comparison of results was reported between obese/overweight and lean PCOS groups. Homeostasis Model Assessment of Insulin Resistance HOMA-I [14] a marker of IR was used in this study. Patients with HOMA-IR >2 (DeUgarte, Bartolucci *et al.,* 2005) were classified as having IR. IR was compared between two PCOS groups. All data were analyzed using analysis system SPSS software X27, as well as represented by mean and stander deviation (M ±SD).

Results

Mean age of obese/overweight and lean PCOS groups were (26.1±5.21), (27± 3.32) respectively with a range of (18 – 40) years, 179 (76.17%) were in the obese/overweight group with BMI $\geq$25 kg/m^2 and 56 (23.83%) as lean PCOS group, accordingly the percentages were decrease to 162(68.94%) in obese/overweight group and increase to 73(31.02%) in lean group upon three-month treatment with metformin. The prevalence of IR, dyslipidemia, hirsutism and alopecia in obese/overweight and lean PCOS women were 82.34%, 3.66%, 69.76%, 21.39%, 81.93%, 33.7%, 79.88% and 19.6% respectively. Menstrual irregularity was reported among all the patients in both groups. Present studies reported that obese PCOS women recorded more improvement in menstrual cycle and clinical feature of hyperandrogenism as hirsutism and alopecia than normal weight PCOS women. Hyperandrogenism markers like (total testosterone, FAI) was higher in obese/overweight as compared to lean weight group but the results were not significant statistically [Table 2].

Insulin resistance significantly was more common in the obese/overweight group as compared to lean one PCOS patients. Fasting insulin (22.76 ± 14.43vs15.37 ± 13.3) and HOMA-IR (5.55 ± 3.7vs.3.67 ± 3.59). Fasting blood sugar was higher in obese/overweight group (97.08 ± 13.3 vs. 94.44 ± 12.46), notwithstanding more improvement in IR was recorded among obese/overweight group and the changes in those parameters between two group were significant at p value <0.05 upon metformin treatment, table [Table 2]. LH pulses frequency or amplitude was higher in over weight/obese, also levels of TSH and prolactin were higher, metformin restored both LH spontaneous episodic secretion and ovarian function more in overweight/obese group, TSH level dropping more remarked with overweight/obese.No significant differences were found in the response of two groups according to LH/FSH, TSH, Prolactin, T.testosterone and estradiol.

Increase of body weight in women was associated with several abnormalities of sex hormone balance and less level of SHBG was detected in overweight/obese women this associated with insulin resistance which caused an increase in free testosterone levels. level of lipid profile was correlated with BMI as showed in table[Table 1]. resulted from increasing release of free fatty acids (FFAs) from adipose tissue that could be improved by treatment with metformin. After three months of therapy, women compare with lean or non-obese one.

Table 1 : Correlation of BMI with Lipid profile, TG, LDL-C, TC, HDL-C.

DATA	r (Pearson's correlation coefficient test)				
	BMI	TG	LDL	TC	HDL
BMI(kg/m^2)	1				
TG(mg/dl)	0.27	1			
LDL(mg/dl)	0.15	0.2556	1		
T. cholestrole(mg/dl)	0.43	0.3667	0.3055	1	
HDL(mg/dl)	-0.01	-0.1033	-0.3044	-0.1113	1

Table 2 : Comparison of clinical, endocrinal and metabolic Parameters among lean body Wight patients (BMI<25 kg/m^2) and obese, overweight patients (BMI≥25 kg/m^2).

DATA	<25 (kg/m^2)		≥ 25 (kg/m^2)		Pre and post change in data		P value
	Pre Mean ±SD	Post Mean ± SD	Pre Mean ± SD	Post Mean ± SD	<25 (kg/m^2)	>25 (kg/m^2)	
BMI(kg/m^2)	23.13 ± 2.81	22.84 ± 3.17	32.23 ± 3.69	30.65 ± 3.67	- 1 ± 0.05	-2.05 ± 0.03	<0.05
FSH(m.Iu/ml)	5.74 ± 1.98	7.02 ± 2.57	5.74 ± 2.01	6.16 ± 2.41	2.35 ± 0.03	1.03 ± 0.04	<0.05
LH(m.Iu/ml)	9.24 ±6.47	8.48 ± 4.68	10.89 ± 5.62	8.55 ± 5.01	-0.52 ± 0.01	-2.59 ± 0.03	<0.05
LH/FSH	1.41 ± 0.89	1.33 ± 0.87	1.8± 0.12	1.5 ± 1.02	- 0.24 ± 0.04	-0.32 ± 0.05	>0.05
TSH(uI/ml)	2.01± 0.95	2.19 ± 0.92	2.8± 0.99	2.14 ± 0.85	-0.13 ± 0.02	-0.51 ± 0.05	>0.05
Prolactin(ng/ml)	23.08 ± 13.46	19.41 ± 9.96	24.55 ± 13.84	20.13 ± 11.38	-3.67 ± 0.02	-4.42± 0.03	>0.05
T.testosterone(ng/ml)	0.55 ± 0.32	0.38 ± 0.25	0.59 ± 0.37	0.51 ± 0.36	-0.24 ± 0.02	- 0.12±0.04	>0.05
SHBG(nmol/L)	48.44 ± 25.31	51.84 ± 23.82	37.96 ± 21.12	51.2 ± 20.54	3.55 ± 0.03	14.11±0.054	<0.05
FAI	8.76 ± 1.2	3.44 ± 6.07	9.64 ± 1.597	3.76 ± 8.69	-5.53 ± 0.03	-5.84 ± 0.02	>0.05
Estradiol(pg/ml)	59.97 ± 56.14	73.48 ± 49.2	56.81 ± 45.15	64.27 ± 45.8	10.26± 0.02	6.69 ± 0.05	<0.05
FBS(mg/dl)	94.44 ± 12.46	88.86 ± 13.76	97.08 ± 13.38	94.73 ± 12.11	-5.01 ± 0.07	-3.1 ± 0.01	<0.05
F.Insulin level(mIu/L)	15.37 ± 13.3	14.78 ± 7.54	22.76 ± 14.43	19.4 ± 12.47	-1.9 ± 0.05	-3.2 ± 0.02	<0.05
HOMA IR	3.67 ± 3.59	3.51 ± 1.8	5.55 ± 3.7	4.16 ± 3.17	-0.6 ± 0.05	-1.056±0.04	<0.05
HbA1C%	4.12±0.32	4.01±0.42	4.86±0.38	4.43±0.13	-0.2 ± 0.36	- 0.4± 0.28	<0.05
TG(mg/dl)	119.18 ±55.59	102.44 ± 40.53	130.5 ± 46.31	119.05 ± 37.22	-7.03 ± 0.04	-9.37 ± 0.05	<0.05
LDL(mg/dl)	92.75 ± 33.28	84.36 ± 28.1	95.74 ± 31.71	85.31 ± 23.86	-22 ± 0.05	-44 ± 0.01	<0.05
HDL(mg/dl)	45.14 ± 8.42	46.1 ± 7.75	44.22 ± 10.73	46.8 ± 9.67	1 ± 0.03	2.41 ± 0.02	<0.05
T.Cholestrol(mg/dl)	141.52+-46.61	135.96+-43.87	175.59+-42.24	153.6+-44.99	-5.77 ± 0.01	-24 ± 0.05	<0.05

Student t-test between changes of pre and post parameters of lean and obese weight .SD standard deviation. Significant at the < 0.05 level.

Discussion

Prevalence of overweight and obesity in current study was high about (76.71%), this was in agreement with the study conducted by (Essah and Nestler, 2006). Present study revealed that the prevalence of hyperandrogenism was more in the obese PCOS group, accordingly obesity results in increase in the androgens and decrease in SHBG levels, thus increasing FAI level (Yuan, Liu *et al*., 2016). Same results were reported by the study by (Kim, Lim *et al*., 2014)who reported the higher hyperandrogenism in women with increasing BMI, obesity results in hormonal imbalances resulting in hyperandrogenism and hyperinsulinemia (Blagojević, Ignjatović *et al*., 2018).

Hyperandrogenism leading to hyperinsulinemia and vice versa (Baptiste, Battista *et al*., 2010). Insulin act through a raising production of androgens and resulted in weight gain typically in the abdomen, in the ovary, insulin stimulates ovarian steroidogenesis by interacting with insulin and insulin growth factor type I receptors, accordingly in granulosa, thecal and stromal cells it decreases insulin growth factor binding protein-1(IGFBP-1) that regulates ovarian and cyst formation with adrenal steroidogenesis(Li, Chen *et al.,* 2019).

Insulin acts by increases 17-hydroxylase and 17-20 lyase activity as well as increasing the expression of 3-hydroxysteroid dehydrogenase in granulosa cells. Stimulation 17α-hydroxylase activity by PI3K, 17α-hydroxylase activity being responsible for synthesizing androgen precursors, it has synergistic action with gonadotropins in increasing LH-induced androgen synthesis in theca cells (Majumdar and Singh, 2009).

Using the HOMA-IR to investigate IR, prevalence of IR in the present study was (63.36%), these finding was consistent with the study of (DeUgarte, Bartolucci *et al.,* 2005) and (Carmina and Lobo, 2004), IR was higher in the obese PCOS group.

Metformin reduces weight through improvement of insulin sensitization and modulating the level of various peptides involved in controlling appetite like ghrelin, neuropeptide YY and adipokines, by hypothalamic adenosine 5′- monophosphate-activated kinase (AMP Kinase) (Palomba, Falbo *et al.,* 2009). Study of (De Leo, La Marca *et al.,* 2000) incompatible with current result had showed that BMI did not change significantly after metformin. At baseline measure of BMI. Obese/overweight PCOS patients were responded to treatment more than lean weight one, this result compatible with (Trolle, Flyvbjerg *et al.,* 2007) that showed metformin increased insulin sensitivity and improved

other parameters in obese women while non-obese women did not benefit from metformin.

BMI is associated with dyslipidemia in women with PCOS, this correlation due to increase BMI correlated with insulin resistance result in increased fatty acid synthesis through fatty acid oxidation because of decreasing the activity of AMPK that act through phosphorylation and inactivation of Acetyl-CoA-Carboxylase (ACC) (Palomba, Falbo *et al.,* 2009).

Elevated LH value mostly in overweight/obese compare with lean or non-obese at base line level, this compatible with finding of (Norman, Noakes *et al.,* 2004), and disagree with (Ulloa-Aguirre, Portocarrero *et al.,* 2006), that showed lean body weight and obese PCOS patients do not represent distinct pathophysiological subsets of this disorder, so did not modify mean LH level, nevertheless LH pulse frequency and amplitude and LH level improvement after therapy occurred in both lean body weight and obese but more with obese women, this result disagree with (Genazzani, Battaglia *et al.,* 2004) which showed that improvement occur within non-obese PCOS patients only.

Dropping in prolactin level upon the period of therapy was revealed in patients with BMI >25 more than lean body weight due to more improvement in insulin sensitivity. (Azziz, Carmina *et al.,* 2016) reported that weight loss within (5–7%) leads to further regular periods of menstrual cycle and improvement in infertility, moreover it prevents type 2 diabetes, circulatory diseases and endometrial cancer.

Current findings disagree with (Dunaif, Mandeli *et al.,* 1988), that showed lean body weight and obese PCOS patients do not represent distinct pathophysiological outcomes of this disorder and suggested that the impact of obesity in PCOS is not reflected in discernible changes in gonadotropin release or in the gonadal steroid feedback environment ,as well as insulin does not have a major role in the perpetuation of PCOS, since obese and non-obese PCOS women had similar reproductive hormone levels despite different degrees of insulin sensitivity.

Conclusion

Obese/overweight PCOS women have a higher risk of metabolic disorders as insulin resistance and dyslipidemia with more signs of hyperandrogenism like hirsutism and alopecia. Furthermore, this group was showed good response for metformin treatment in comparison to lean body weight one.

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Ethicalapproval

Ethical Committee in College of Pharmacy, Kerbala University was approved the study protocol.

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