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ASSESSMENT OF HOMOCYSTEINE LEVEL IN PLASMA OF IRAQI WOMEN WITH POLYCYSTIC OVARY SYNDROME AND INSULIN RESISTANCE

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ABSTRACT
Polycystic ovary syndrome (PCOS) is a common endocrine disorder which affects women at reproductive age. This study was aimed to detect homocysteine level in women with polycystic ovary syndrome. The present study was carried out for (51) PCOS women and (10) healthy women as controls at High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/AL-Nahrain University, from February to August, 2014. Homocysteine test and OGTT were performed for all women. Age and BMI were recorded for all subjects and duration of infertility recorded for infertile PCOS women. Significant increment (P<0.05) in homocysteine level was observed in obese PCOS group with duration of infertility >9 years, but the differences in homocysteine was not significant (P>0.05) between PCOS women and controls, and among PCOS subgroups. Significant increment (P<0.05) in OGTT was recorded in obese patients when compared with controls. The present study concluded that homocysteine elevation in PCOS women related to obesity and insulin resistance. Also, it has a relation with duration of infertility.

KEYWORDS: Polycystic ovary syndrome, Homocysteine, insulin resistance.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common endocrinopathy affecting 6-10% women of reproductive age[1]. PCOS had been initially described in 1844 by Chereau and Rokitansky[2]. PCOS may occur as high as 30% in women suffering from secondary amenorrhea, 75% in women suffering from oligomenorrhea, and 90% in women with hirsutism[3]. About 50% of PCOS patients are obese or they have a greater risk of overweight, obesity, and central obesity[4]. Also, many PCOS women may have high increased risk of cardiovascular disease (CVD) and endometrial cancer[5]. Additionally, the late complications of PCOS, such as primarily diabetes, dyslipidemia, and CVD seem to be connected to insulin resistance[6], and the multiple risk factors for CVD in young women with PCOS including; type 2 *diabetes mellitus* (T2DM), metabolic syndrome (MS), abdominal obesity, dyslipidemia, and hypertension[7]. There are different risk factors for cardiovascular disease (CVD) have been studied in women with PCOS, one of them is homocysteine[8]. Homocysteine (HCY) is an amino acid containing thiol, which is an independent risk factor for CVD[9]. It is an intermediate product of methionine metabolism, and homocysteine itself metabolized by two pathways: the re-methylation pathway, which regenerates methionine, and the transsulfuration pathway, which is degrades homocysteine into cysteine and then taurine[10]. In women with PCOS, the mean serum homocysteine concentrations are increased, which the most variables that influence the homocysteine concentration were taken into account and the higher homocysteine levels may associate with hyperinsulinemia in these patients. Additionally, in women with PCOS, together with other risk factors like hyperinsulinemia or dyslipidemia, homocysteine elevation may contribute to the risk of cardiovascular disease[11]. PCOS diagnose by the presence of two out of three which include[12]: 1.Oligomenorrhea or anovulation; 2.Clinical signs which include hirsutism and acne, biochemical signs of hyperandrogenism which include the elevation of free testosterone or testosterone; 3.Polycystic ovaries confirms using ultrasound scan which is clear the presence of 12 or more follicles in each ovary measuring 2-9mm in diameter. Therefore, the aim of this study is to detect homocysteine level in PCOS women with insulin resistance which was detected by using OGTT.

MATERIALS & METHODS

This study was carried out at the laboratory of Hormonal and Biochemical assays of High Institute for Infertility Diagnosis and Assisted Reproductive Technologies / AL-Nahrain University from 1 February to 1 August, 2014. From the total of 61 women with age range 17-49 was studied, 51 women were suffering from PCOS, and 10 healthy women were considered as controls to compare with PCOS's women. Blood samples (about 5mL) were collected from each fasting woman in the morning and 75 gm of glucose loaded in 400-500 ml of water and given to the subjects. From the collected blood in previous step, 4 mL of it was transferred to gel and clot activator tube and incubated for 10 minute in incubator at 37ºC to coagulate, and serum separated by centrifuge and freezing in -4 ºC, but the remaining blood (1mL) transferred to tube with EDTA as an anticoagulant to separate plasma. Then the samples centrifuged and the plasma stored at -20°C which prepared for homocysteine test. OGTT test was performed after 30, 60, and 90 minute after glucose loading. The principle assay of homocysteine test employs the quantitative sandwich enzyme immunoassay technique by

using ELISA kit of CUSABIO (China). The normal value of laboratory homocysteine is range from (0.4-3 nmol/ml). Patients considered with hyperhomocysteinemia with homocysteine level >3 nmol/ml. GTT was performed using glucose MR kit of Linear (Spain). The normal value of OGTT is <140 mg/dl, OGTT between 140-199mg/dl refer to impaired glucose tolerance, and its considered diabetic if OGTT ≥ 200 mg/dl[13]. Body mass index (BMI) was measured for all subjects by dividing weight into kilogram on height in meter square. BMI value indicates for body weight, subjects with BMI 18.5-24.9 Kg/m^2 considered normal weight, with BMI 25-29.9 Kg/m^2 considered overweight, and with BMI ≥30 Kg/m^2 considered obese. The duration of infertility recorded for all infertile PCOS patients.

Statistical analysis was performed using Statistical Package for Social Science (SPSS); version 20. The differences among more than two groups were assessed by using ANOVA table- Duncan test with means and standard error of means while the comparison between two groups were assessed by using F test. The confidence level has been chosen as 95% and P value <0.05 was considered as significant.

RESULTS

There was a comparison of parameters including; age, BMI, GTT (FBS and OGTT), and homocysteine levels among PCOS women and control group. As shown in table (1), there were no significant differences (*P*>0.05) in the age, BMI and in GTT (FBS and OGTT) between the two groups. Also, in comparison to homocysteine level between PCOS group and control subjects. No significant differences (*P*>0.05) in homocysteine level were observed between the two groups.

TABLE 1: Comparison in parameters between

Parameters	Control (n=10) 16.40%	PCOS (n=51) 83.60%	P_{value}
	Mean ± S.E	Mean ± S.E	
Age (Years)	33.10±3.139	28.53±1.060	NS
BMI (Kg/m^2)	26.99±1.746	30.0±0.902	NS
FBS (0 time) (mg/dl)	82.48±1.768	95.77±3.614	NS
R1BS(30min)(mg/dl)	136.2±2.928	155.62±6.136	NS
R2BS(60 min)(mg/dl)	125.38±5.438	155.39±8.389	NS
R3BS(90 min)(mg/dl)	115.68±5.407	139.49±7.671	NS
HCY (nmol/ml)	3.69±1.076	5.04±1.620	NS

PCOS women and control group

PCOS=polycystic ovary syndrome, BMI=Body mass index, FBS = fasting blood sugar, R1BS=Reading 1 blood sugar, R2BS= Reading 2 blood sugar, R3BS= Reading 3 blood sugar HCY=homocysteine, NS= non significant

As observed in the table (2), a comparison of parameters among PCOS subgroups according to BMI included; women with normal Weight (BMI 23.38 kg/m^2), as contrast PCOS women with overweight (BMI 27.30 kg/m^2), PCOS obese groups (BMI 35.92kg/m^2), and control group. However, no significant differences (*P*>0.05) were assessed at the age among the four groups. Highly significant differences (*P*<0.05) were observed in BMI of obese PCOS than normal weight PCOS, overweight PCOS and control group. Also, there was higher significant differences (*P*<0.05) in overweight PCOS than normal weight PCOS group. Normal weight PCOS were had lower value of BMI than control group, but there were no significant difference (*P*>0.05) in BMI between overweight PCOS and control group.

TABLE 2: Comparison in parameters among PCOS group (normal weight, overweight, and obese) and control group

Parameters	control (n=10) 16.40%	PCOS Normal weight(n=13) 21.31%	PCOS Overweight (n=16) 26.23%	PCOS Obese (n=22) 36.06%
	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E
Age (Years)	33.10±3.139a	28.46±2.712 a	30.06±1.764 a	27.45±1.403 a
BMI (Kg/m^2)	26.99±1.746 b	23.38±0.275 A	27.30±0.304 b	35.92±1.126 a
FBS (0 time) (mg/dl)	82.48±1.768 b	90.57±4.174 ab	87.71±2.529 ab	104.70±7.487 a
R1BS(30min)(mg/dl)	136.20±2.98 b	140.69±9.22 ab	147.04±7.367 ab	170.67±11.43 a
R2BS(60min) (mg/dl)	125.38±5.48 a	125.29±10.78 a	151.89±11.73 ab	175.73±15.73 b
R3BS(90min)(mg/dl)	115.68±5.47 b	119.27±9.16 ab	131.22±10.23 ab	157.45±14.67 a
HCY(nmol/ml)	3.69±1.076a	3.21±0.815a	7.90±4.866 a	4.04±1.237 a

Different letters mean significant (*P*<0.05)

PCOS=polycystic ovary syndrome, BMI=Body mass index, FBS = fasting blood sugar, R1BS=Reading 1 blood sugar, R2BS= Reading 2 blood sugar, R3BS= Reading 3 blood sugar, HCY=homocysteine

As for the GTT, there were significant increased (*P*<0.05) levels in FBS, R1BS, and R3BS of obese PCOS than control group. In contrast, non significant differences (*P*>0.05) were noticed in levels of FBS, R1BS, and R3BS among normal weight PCOS, overweight PCOS, and control groups. Also, there were non-significant differences (*P*>0.05) in levels of FBS, R1BS, and R3BS among normal weight PCOS, overweight PCOS, and

obese PCOS group. Significant increment (P<0.05) was observed in R2BS levels in obese PCOS when compared with control group. Also, significant elevation (P<0.05) was observed in R2BS levels of obese PCOS as compared to normal weight PCOS group. Non-significant differences (P>0.05) were assessed in R2BS among normal weight PCOS, overweight PCOS and control group. Additionally, there was no significant difference (P>0.05) between obese PCOS and overweight PCOS groups**.** Another comparison among these groups was assessed in homocysteine levels as illustrated in the same table. No significant differences were (P>0.05) recorded among PCOS subgroups and control subject in homocysteine levels. A comparison among infertile PCOS subgroups according to their duration of infertility including; group (A) with duration of infertility range from 1-4 years; group (B) with duration of infertility range from 5-8 years; and group (C) with duration of infertility 9 ≤ years as shown in the table (3). From the same table, non-significant differences (P>0.05) were recorded in the age and BMI among the three groups, but significantly differences (P<0.05) were reported for duration of infertility among the three groups. As for GTT compared among PCOS subgroups (table 3). Significant differences (P<0.05) in FBS levels were observed between groups (B) and (C), which was higher in group (C) than group (B) while non-significant differences was noticed (P>0.05) for the group (A) as compared to both group (A) and (B). Significant elevation (P<0.05) in the levels of R1BS were observed in the group (C) when compared to the group (A) and group (B). While no significant differences (P>0.05) were recorded between groups A and B. In addition, R2BS levels were significantly increased (P<0.05) in the group (C) than the group (A). However, non-significant differences (P>0.05) were observed in R2BS levels of group (B) with the group (A) and group (C). Moreover, no significant differences (P>0.05) were observed among the three group in R3BS levels**.** Concerning to the comparison in HCY levels among infertile PCOS subgroups according to their duration of infertility, significant (P<0.05) levels of homocysteine was observed in the group (C) when compared with the group (B), but not significant (P>0.05) with the group (A). Also, no significant differences (P>0.05) in homocysteine levels were assessed between the group (A) and group (B).

TABLE 3: Comparison in parameters among PCOS subgroups according to their duration of infertility

Parameters	Group(A)(1-4 years)(n=23) 45.10%	Group(B)(5-8 years)(n=20) 39.22%	Group(C) (≥9years) (n=8) 15.68%
	Mean ± S.E	Mean ± S.E	Mean ± S.E
Age (Years)	27.70±1.780a	28.65±1.632 a	30.63±1.889 a
BMI (Kg/m^2)	29.62±1.381a	29.57±1.096 a	32.29±3.286 a
D. of infertility (years)	2.11±0.253 a	5.95±0.246 b	11.38±0.680 A
FBS (0 time) (mg/dl)	94.41±3.216 ab	90.93±2.303 a	111.76±20.481 b
R1BS(30min)(mg/dl)	150.13±6.475a	144.55±6.416 a	199.06±27.032 b
R2BS(60min) (mg/dl)	141.67±7.726a	156.13±9.929 ab	192.99±41.432 b
R3BS(90min)(mg/dl)	132.25±7.657a	136.88±8.538 a	166.81±39.040 a
HCY (nmol/ml)	4.17±1.027ab	3.03±0.913 a	12.58±9.716 b
Different letters mean significant (P<0.05)			

PCOS=polycystic ovary syndrome, BMI=Body mass index, D= Duration of infertility, FBS = fasting blood sugar, R1BS=Reading 1 blood sugar, R2BS= Reading 2 blood sugar, R3BS= Reading 3 blood sugar, HCY=homocysteine

DISCUSSION

When PCOS women divided into subgroups according to BMI values (Table 2), higher prevalence of PCOS women (36.06%) of females were observed had higher BMI (BMI=35.92±1.126 kg/m^2) and considered obese, about 26.23% of females with PCOS had mild higher BMI (BMI=27.30±0.304 kg/ m^2) and considered overweight, and about 21.31% of subjects with PCOS considered normal weight with (BMI= 23.38± 0.275 Kg/m^2) that means PCOS case frequently higher in overweight and obese women, and the obesity play an important role in pathophysiology of this syndrome[14]. Higher BMI (BMI≥30 kg/m^2) value is a marker for obesity which is a common finding in women with PCOS, but it is not part of the diagnostic criteria[15]. Non-significant differences in HCY levels for obese and overweight patients in comparison to PCOS subgroups and control subjects in spite of elevation an of HCY (HCY>3 nmol/ml) in these subgroups (Table 2). This result disagreement with Rekha *et al*., they concluded that obesity is not an independent risk factor to increase plasma homocysteine levels in PCOS women, and they added that HCY level increased in non-obese PCOS women in comparison to obese ones [16]. Moreover, homocysteine level significantly elevated in PCOS subgroup (C) with duration of infertility >9 years (table 3), where they had insulin resistance according to higher OGTT, this result in concordance with Hemati *et al*. They concluded significant correlation between insulin resistance and homocysteine[17]. Furthermore, PCOS subgroup (C) with proportion (15.68%) were obese (BMI >30 kg/m^2). In this case, hormone resistin may play an important role in insulin resistance[18], therefore there was a relation between obesity and homocysteine level and this result agreement with[19]. Also, higher GTT in obese subgroups refers to insulin resistance that may due to resistin hormone leading to insulin resistance, while serum resistin level increase in the presence of obesity in mice, an anti-diabetic agent rosiglitazone decreased levels and that administration of recombinant resistin to mice caused impairment in glucose tolerance and insulin effect[18].

Also, Rekha *et al*. concluded that IR was common in PCOS women and was practically more common in obese PCOS subgroups[16] and this conclusion in line with the present study. Moreover, the mid test of OGTT provided an enough information for hyperglycemia in PCOS women and this suggestion in line with Motala *et al.*, they performed mid test of OGTT with one year as baseline, and found the most significant predictive risk factor for subsequent progression to diabetes was the 90 min plasma glucose (mid-test level), a surprising finding was that neither fasting nor 2-hr plasma glucose were significant [20]. Certainly, homocysteine level may play an important role in fertility problem which related to the duration of infertility, this suggestion agreement with Al- Rubae'[21], which concluded that hyperhomocysteinemia was observed in women suffering from infertility, HCY increased with increase in BMI and obesity in infertile women, but unfortunately there were no studies illustrated the role of homocysteine in duration of infertility[21]. Furthermore, non-significant differences were recorded among PCOS patients and control group may due to small sample size, in spite of an elevation were assessed in homocystein and GTT in PCOS group.

CONCLUSION

From the results of the present study revealed that the pathophysiology of PCOS was common multiple causes. Homocysteine levels associated with insulin resistance and obesity. Also, it had a relation with duration of infertility.

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