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Utility of plasma free metanephrines for detecting insulin resistance in patients with PCOS

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ABSTRACT

Polycystic ovary syndrome (PCO) is one of the most public endocrine disorders affecting women. Metanephrines are O-methylated metabolites of catecholamines. Measurements of plasma, normetanephrine and metanephrine, provide a sensitive test for diagnosis of pheochromocytoma in adults but have not been evaluated in women with PCOS.This study was carried out at Karbala hospital for gynecology and obstetrics,the Kamal AL-Samarai Hospital. Forty five women fulfilling the clinical and biochemical criteria for polycystic ovary syndrome and hyperinsulinemia were enrolled before using any drugs. In addition Body Mass Index (BMI), fasting insulin, fasting blood sugar and HOMA levels were performed. FSH, LH, prolactin, insulin, free metanephrines and epinephrine were measured by enzyme-linked immunosorbent assay, fasting blood glucose were measured by colorimetric methods. There was a significant increase ($p<0.001$) in PCOS patients when compared to the control group in fasting serumprolactin, insulin, free metanephrines and Epinephrine. In conclusion,there was a significant an increase in free metanephrine and Epinephrine in women with PCOS before using any treatments. These results in the present study are shown the first time as our knowledge, these factors may be useful in following developments in insulin resistance in subjects with polycystic ovary syndrome. Further studies are needed to certify these factors in other populations with metformin treatment or with other insulin sensitizers or when treated with diet and exercise.

KEYWORDS

Polycystic ovarian syndrome;
Freemetanephrines;
Insulin resistance;
Epinephrine;
Prolactin.

INTRODUCTION

PCOS is a very complicated endocrine disorder. Polycystic ovarian syndrome (PCOS) is an particularly common disorder affecting 4% to 10% of women of reproductive age[1,2].Even though being heterogeneous in nature, the symbols of the disease are hyperandrogenism and chronic anovulation[3,4]. The in-

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cidence of PCOS greatly depends on which diagnostic criteria are used to define it[3]. PCOS is much more than a disorder of excess hair and anovulation. PCOS has profound implications for self-esteem and mood, interferes with fertility, and associated with serious complications[5]

The exact a etiology of PCOS remains unknown, but hyperandrogenism was believed to be a main underlying factor[6]. The mechanisms connecting PCOS and increased cardiovascular risk profile are not understood. In addition to insulin resistance(IR), it has been suggested that hyperandrogenemia contributes to vascular damage in women[7]. Furthermore, endothelial dysfunction seems to be associated with both insulin resistance and androgen level[8]. PCOS is associated with insulin resistance (IR) and hyperinsulinemia and proves an increased incidence in diabetes, hypertension, dyslipidemia, and atherosclerosis[9]. Through a accord conference on IR supported by the American Diabetes Association, a panel of experts defined the disorder as an impaired metabolic response to whichever exogenous or endogenous insulin. Catecholamine is the name of a group of aromatic amines (noradrenaline, adrenaline, dopamine, and their derivatives) which act as hormones and neurotransmitter, respectively. Adrenaline and noradrenaline are formed from dopamine. They act on the cardiac musculature and the metabolism (adrenaline) as well as on the peripheral circulation (noradrenaline) and help the body to cope with acute and chronic stress[10].Free metanephrines and normetanephrine (collectively referred to as metanephrines) are the 3-methoxy metabolites of epinephrine and norepinephrine, respectively. The metanephrines are stable metabolites and are cosecreted directly with catecholamines by pheochromocytomas and other neural crest tumors. This results in sustained elevations in plasma free metanephrine levels, making them more sensitive and specific than plasma catecholamines in the identification of pheochromocytoma patients[10].Metanephrine and normetanephrine are both further metabolized to conjugated metanephrines and vanillylmandelic acid[11].

The present work aimed to assess serum free metanephrines and Epinephrine levels in women with PCOS and its relationship to body mass index (BMI), IR and hormonal profile in these women.

MATERIALS AND METHODS

The present study includedforty five women with PCOS with mean age (27.27±4.35 years) and their mean BMI(31.05±5.05 kg/m²); was calculated according to standard WHO classification[12]. They were diagnosed by Rotterdam criteria, when two of the following three features were present: oligo- and/or anovulation polycystic ovaries on ultrasound examination (the presence of R12 follicles measuring 2–9 mm in diameter and/or ovarian volume >10 cm3),and clinical and/or biochemical signs of hyperandrogenism[13]. Forty healthy women volunteers without PCOS, with matched age were included as a control group, their healthy status was determined on the basis of medical history, physicaland pelvic ultrasonography. Patients were selected from the outpatient's infertility clinic at the Department of Obstetrics and Gynecology of Karbala University Hospital and Kamal AL-Samarai Hospital. Woman with hypertension, diabetes mellitus, chronic diseases, was excluded. All participant women were not under recent medications (ovulation induction agents, antiandrogens, insulin sensitizers).Physical examination including determination of BMI calculated as weight (kg)/ height squared (m²). After overnight fasting, venous blood samples were aspirated at 08:00-10:00 am during the third and sixth days of the menstrual cycle (early follicular phase) for those of normal cycle.The samples were transferred into clean plain tubes and centrifuged within 30 minutes of collection, then serum from all blood samples were separated, glucose levels were measured using the enzyme colorimetric methods supplied by Giesse Diagnostic, the rest was stored at -18 ^{0} C for determination of FSH, LH, prolactin, insulin,free metanephrines and epinephrine until the time of assay.

The FSH, LH, prolactin, insulin,free metanephrines and epinephrine hormones were determined by ELISA methods. The homeostasis model assessment (HOMA) was u s e d to calculate an index of insulin resistance for each patient using fasting glucose in mmol/L and insulin in μIU/ml, the index for insulin resistance, HOMA IR, was defined as (insulin ×glucose) /22.5[14].In addition to abdominal and pelvic ultrasound.

The women gave their oral and written consent before entering the study. All investigations were conducted in accordance with guidelines in the Declaration

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of Helsinki.

All statistical analyses in studies were performed using SPSS version 17.0 for Windows (Statistical Package for Social Science, Inc., Chicago, IL, USA). Descriptive analysis was used to show the mean and standard deviation of variables. The significance of difference between mean values was estimated by Student T-Test. The probability P< 0.05 = significant, P> 0.05 = non-significant. Correlation analysis was used to test the linear relationship between parameters.ANOVA test was used to show the differences between variables of differentiated groups

RESULTS AND DISCUSSION

The results were observed as follows offorty five PCOS patient women, 30 normal woman were serve as control group. Demographic distribution of groups of women according to definite characteristics were shown in TABLE 1.

TABLE 1: The mean and standard deviation of Age, weight ,height and BMI in group A[PCOS patients],group B[healthy women].

Characteristic	Group A[n=45]	Group B[n=30]	P Value
Age[year] Mean ±SD	28.27±4.21	27.70±3.28	>0.05
Range	27.00-39.00	22.00-36.00	
Weight[Kg] Mean ±SD	77.51±11.43	65.43±7.33	<0.01
Range	62.50-120.00	62.00-82.00	
Hight [m] Mean ±SD	1.59±0.52	1.62±0.37	<0.01
Range	1.55-1.67	1.55-1.68	
BMI [Kg/m^2] Mean ±SD	31.05±5.05	24.90±1.97	
Range	23.55-44.21	21.25-29.41	<0.001

Obesity is one of the clinical features of the PCOS beside with oligomenorrhea, hirsutism, and infertility. In the present study a significant increase in BMI [p<0.001] has been showed in PCOS patients, TABLE 1.The reason of obesity in the PCOS remainders unknown, [15] obesity may play a pathogenic character in the development of the syndrome in prone individuals[16]. The current study showed that women in patents with PCOS have a higher body weight than their European andUnited statescounterparts[17,18].

There were A highly significant increase in mean levels of insulin,HOMA,Glucose/Insulin ratio,prolactin, epinephrine and free metanephrinesin PCOS group comparing to healthy women P<0.001) as shown in table 2,while there were no significant different in FSH, LH:FSH ratio and free metanephrines/Insulin ratio between studied groups,TABLE 2.

TABLE 2 : The mean and standard deviation of FBG ,insulin , HOMA,globulin,Alb/Glu.ratio, Glucose/Insulin ratio,LH ,FSH, LH:FSH ratio,prolactin, Epinephrine,free metanephrines, and freemetanephrines/insulin ratio A[PCOS patients],group B [healthy women].

Characteristic	Group A[n=45]	Group B[n=30]	P Value
FBG[mmol/L]	4.93 ± 0.65	4.72 ± 0.55	>0.05
Insulin[µu/ml]	19.72 ± 5.86	6.98 ± 1.04	<0.001
HOMA	4.01±2.16	1.61±0.21	<0.001
Glucose/Insulin ratio	5.25±1.59	11.23±1.64	<0.001
LH[IU/L]	6.67 ±2.11	7.85±1.90	<0.05
FSH[IU/L]	4.81 ± 1.10	5.45± 0.79	>0.05
LH:FSH ratio	1.44 ± 0.51	1.38 ± 0.28	>0.05
Prolactin [ng/ml]	29.56±9.56	10.24±4.65	<0.001
Epinephrine[ng/ml]	86.67±7.27	38.33±5.34	<0.001
P.freemetanephrines[Pg/dl]	91.64 ± 15.27	35.54 ±6.34	<0.001
Freemetanephrines/Insulin ratio	6.01 ± 2.25	6.29 ± 2.49	>0.05

There are a number of methods of evaluating insulin sensitivity. The gold standard for calculation of insulin sensitivity is the hyperinsulinemic-euglycemic clamp technique. But, because this technique is both labor intensive and expensive, it is not appropriate for daily clinical use. Other methods, such as fasting insulin levels[19], HOMA[20], G/I ratio[21], are easier and much less expensive to manage and have been shown to correlate well with clamp techniques[22]. The mean serum level of fasting insulin was significantly elevated in the PCOS patients when compared to control group [P<0.001] TABLE 2. In the present study,it had been found that 89%of patients are insulin resistance,these result would agreement with another study in Iraq (92.5%)[23] and more than other study that found as many as 70% of PCOS women are insulin resistant[24,25]. In PCOS women with normal glucose metabolism initially, the rate of alteration to abnormal glucose metabolism can be 25% over just three years[26]. Other upsetting, insulin abnormalities are highly predominant in adolescents with PCOS[27].

The results indicate that a fasting G:I ratio is a good indicator of insulin sensitivity in PCOS patients. Fasting hyperinsulinemia has been used as a measure of insulin action[28]. Basal and glucose-stimulated hyperinsulinemia

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were healthy reported in obese PCOS women[29].

The mean level of S. FSH was no significant in the PCOS patients when it was compared to that found in the control group [p>0.05] as shown in TABLE 2, a result which was compatible with another studies[30], and it fit the criteria for the diagnosis of PCOS

There was a highly significant increase in serum prolactin level of PCOS patents group when compared to control as shown in TABLE 2. Other studies showed mild hyperprolactinemia was reported in 13% to 16.5% of patients with PCOS[31,32]. Presence of hyperprolactinemia in PCOS could be explained by many theories. The first theory referred to increased steroid feedback on lactotrope[33] or to the possible role of estrogen mainly estron E1 which couses hyperprolactinemia[34]. The second theory specified that association of estrogen and dopaminergic system enhanced prolactin release in PCOS[35]

There were A highly significant increase in mean levels ofEpinephrine, and free metanephrines in PCOS group comparing to control group (P<0.001) as shown in TABLE 2.There were A significant positive correlation between free metanephrines and HOMA as shown in figure 1.

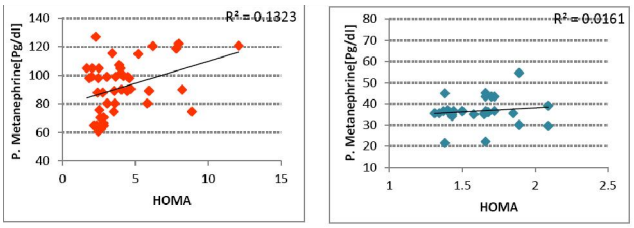

Figure 1 : Correlation between freemetanephrines and HOMA in patients and control group.

Free metanephrines is used to describe the chemical by product of the catecholamine hormone epinephrine after it is broken down. Epinephrine is also known as adrenaline. The main organ that produces epinephrine is the adrenal gland[36].

The present result showed that in normal population, plasma free metanephrines and normetanephrine levels are low, but in patients with PCOS, the concentrations may be significantly elevated. As our knowledge no previous study showed to these result.This is may be due to the relatively long half-life of these compounds, continuing secretion by the cell and, to a lesser degree, peripheral conversion of cell-secreted catecholamines into free metanephriness.Catecholamines interfere with carbohydratemetabolism by stimulation of adrenoreceptorswith subsequent modulation of pancreaticinsulin and glucagon release[37].

The present result showed that elevated epinephrine,free metanephrinesand insulin concentrations in patients with PCOS may further enhance adrenal and ovarian androgen production, reduction the therapeutic effectiveness of glucocorticoids, and contribute to development of polycystic ovary syndrome and/or the metabolic syndrome and their complications.

CONCLUSION

The finding that obese women with PCOS are more likely to be IR as compared to a cohort of women with out PCOS is not novel. However, one question that needs to be more carefully addressed is: Are the level of free metanephrinesused to define development the complication of disease begin IR in women with PCOS? Instead of other parameter to determine whom IR. The findings in present study indicate that plasma free metanephriness provide a sensitive tool for detection of insulin resistance in women with PCOS. Free metanephrines may be useful in following developments in insulin resistance in subjects with polycystic ovary syndrome. Further studies are required to confirm these factors in other populations with metformin treatment or with other insulin sensitizers or when treated with diet and exercise

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