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Role of Serum Zinc alpha2-glycoprotein, SHBG and Leptin Levels in Prediction of Polycystic Ovary Syndrome in Overweight and Obese Women

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Abstract. Background: The most prevalent endocrine illness in women, polycystic ovarian syndrome (PCOS), can manifest with a variety of signs and symptoms, including changes in the reproductive, endocrine, and metabolic systems. The soluble protein zinc alpha-2-glycoproteins (ZAG), which is expressed in and released by subcutaneous and visceral adipose tissue, has been linked to metabolic syndrome and obesity. Sex hormone-binding globulin (SHBG), a plasma glycoprotein, binds androgens and estrogens that are physiologically active, controlling the bioavailability of these hormones to their target tissues. The obese (ob) gene encodes leptin, a peptide hormone that is secreted from adipose tissue and is frequently used as a marker for increasing obesity, which may be linked to PCOS. Objectives: This study aims to investigate the role of serum levels of Zinc alpha 2-glycoprotein, SHBG, and leptin in prediction of PCOS in overweight and obese healthy women as well as in normal weight healthy women. Subjects and methods: This case control study was carried out at the Department of Biochemical, College of Medicine, University of Baghdad, and at Kamal Al Samurai Hospital, Ministry of Health, Baghdad, Iraq, during the period from November 2021 to March 2022. It involved 120 women, age range 18-42 year, classified into three groups; group 1 (G1; obese PCOS) involved 40 overweight/obese women who diagnosed by Consultant Gynecologist to have had PCOS based on Rotterdam criteria, group 2 (G2; obese healthy women) included 40 overweight/obese healthy women were examined by Consultant Gynecologist that have no PCOS, and group 3 (G3; normal weight) involved 40 normal weight healthy women. Serum investigations included measurements of ZAG, SHBG, and leptin by ELISA technique, and testosterone and LH by miniVidas instrument. Results: The mean (±SD) values of ZAG and the median (±IQR) of SHBG were significantly decreased in obese healthy & obese PCOS than normal weight (p=0.001) along with significant decrease in obese PCOS than obese healthy (0.005). The mean (±SD) values of leptin was significantly increase in obese healthy & obese PCOS than normal weight (p=0.001) with significant increase in obese PCOS than obese healthy (p=0.005). The results revealed significant positive correlation in obese PCOS group between leptin and BMI (r=0.322, p=0.043) as well as significant positive correlation between Zinc alpha 2 glycoprotein and LH (r=0.317, p=0.046). Testosterone had the highest ROC and AUC value (0.956) at cutoff (0.55 ng/ml) along with leptin (ROC 0.876) at cutoff value (36 ng/ml) in predicting PCOS in obese healthy women. While, leptin at cutoff value (36.5 ng/ml) had ROC and AUC (1.000) and testosterone at cutoff value (0.55 ng/ml) had ROC and AUC (0.972) in prediction of pcos in normal weight healthy women. Conclusions: Serum testosterone at cutoff value (0.55 ng/ml) and leptin at cutoff value of (36 ng/ml) had the excellent utility in predicting PCOS in obese healthy women. Circulating leptin at cutoff value (36.5 ng/ml) and testosterone at cutoff value (0.55 ng/ml) were the best biochemical markers in differentiation between normal weight women and obese PCOS.

Keyword: Polycystic ovary syndrome, zinc alpha 2-glycoprotein, six hormone binding globulin, leptin

Introduction

Polycystic ovarian syndrome (PCOS), the most common endocrine disorder in women, can present as a number of phenotypes, including problems in the reproductive, endocrine, and metabolic systems. Nonetheless, PCOS is distinguished by hypothalamic-pituitary-ovary axis dysfunction and anovulation, in contrast to other types of ovulatory failure that entail constrained ovarian follicle growth or diminished gonadotropin production (or both) [1]. Obesity is one of the main risk factors for the development of PCOS because

35 to 80% of women with PCOS are reported to be overweight or obese [2].

Zinc alpha-2-glycoproteins (ZAG) are soluble proteins that are produced and released by subcutaneous and visceral adipose tissue. Obesity and the metabolic syndrome have been linked to circulating ZAG [3]. It functions as a lipid mobilizing factor that encourages lipolysis, inhibits lipogenesis, and controls the release of other adipokines [4]. Adenosine triphosphate is converted enzymatically by ZAG to cyclic adenosine monophosphate (cAMP), which starts the first step of lipolysis [5]. Serum ZAG levels were shown to be significantly lower in PCOS patients than in healthy women, and to be lower in overweight and obese patients than in people of normal weight [6].

Hepatocytes produce and release Sex hormone-binding globulin (SHBG), a plasma glycoprotein, into the circulation. Physiologically active androgens and estrogens are less bioavailable to their target tissues when SHBG is present [7]. The SHBG gene can be expressed at low levels in a number of human organs, including the ovary, uterus, breast, prostate, kidney, and brain [7], even though the liver is assumed to be the primary source of plasma SHBG. The link between obesity and PCOS is assumed to be due to the negative effects of obesity on SHBG secretion and synthesis, which in turn raises testosterone bioavailability. According to earlier research, women with PCOS exhibit metabolic problems related to obesity that are substantially correlated with decreased SHBG levels without effecting the indicies of hyper-androgenism [8].

Leptin is a peptide hormone that is secreted by adipose tissue and is encoded by the obese (ob) gene [9]. It has been well recognized as an indicator of increasing obesity, which may be linked to PCOS [10]. Between women's dietary state and reproductive health, leptin plays a key intermediate role. The indicators of PCOS patients have been discovered to be related with high levels of leptin [11].

This study aims: This study aims to investigate the role of serum levels of Zinc alpha 2-glycoprotein, SHBG, and leptin in prediction of PCOS in overweight and obese healthy women as well as in normal weight healthy women.

Subjects and method

The Department of Biochemistry, Faculty of Medicine, University of Baghdad, and Kamal Al Samurai Hospital, Ministry of Health, Baghdad, Iraq, carried out this case control study research between November 2021 and March 2022. It involved 120 women, age range 18-42 year, classified into three groups; group 1 (G1; obese PCOS) involved 40 overweight/obese women who diagnosed by Consultant Gynecologist to have had PCOS based on Rotterdam criteria [12], group 2 (G2; obese healthy) included 40 overweight/obese women who examined by Consultant Gynecologist to be do not have PCOS, and group 3 (G3; normal weight healthy) involved 40 normal weight apparently healthy women. Obesity was defined based on body mass index (BMI) obtained by global equation: BMI (Kg/m^2) = weight (Kg)/ height (m^2). Body mass index equal to or more than 25 Kg/m^2 was considered overweight and obese, while BMI between 18 and 24.9 Kg/m^2 was considered normal weight.

Five milliliter of peripheral vein blood sample was aspirated from each included woman, let to clot for 15 minute and separated for 10 minute at 2500 rpm to obtained serum sample which stored at – 20° C till the time of measurements of ZAG, SHBG, and leptin by ELISA technique, and testosterone and LH by miniVidas instrument.

Results

Table 1 shows the mean (±SD) values of age, BMI, and WC of the studied groups. The mean (±SD) value of the age did not differ significantly among the three groups. The mean (±SD) values of BMI of G1 & G2 were significantly higher than G3 (p=0.005), but there was no significant difference between G1 and G2. The mean (±SD) value of WC of G1 and G2 were significantly higher than G3 (p=0.005), without significant difference between G1 and G2.

Table 1. Mean (±SD) values of age, BMI, and waist circumference of the three studied groups

Parameter	Obese PCOS (n=40)	Obese healthy (n=40)	Normal weight healthy (n=40)
Age^{NS}	28.40 ± 3.49	29.03 ± 3.69	27.95 ±3.36
BMI (Kg/m^2)	32.37 ± 3.66*,**	31.75 ± 4.00*	21.62 ±1.63
WC(cm)	98.05 ±3.40*,**	98.43 ± 3.45*	74.32 ±3.21

ANOVA & t-test revealed; * significant increase in BMI and WC of G1 and G2 than G3 (P=0.005), **no significant difference between G1 and G2, NS: non- significant differences among three groups

Table 2 shows the mean (±SD) values of serum Zinc alpha 2 glycoproteins (ZAG) and leptin and the median and its IQR (Inter-quartile range) of serum sex hormone binding globulin (SHBG) of the G1, G2, and G3.The mean (±SD) value of serum ZAG of G1 was significantly lower than G2 (P=0.005) and G3 (P=0.001). In addition, G2 had significantly lower ZAG than G3 (P=0.005). The mean (±SD) value of serum leptin of G1 was significantly higher than G2 (p=0.005) and G3 (P=0.001) and G2 had significantly higher leptin than G3 (P=0.004). The median (±IQR) of serum SHBG of G1 was significantly lower than G2 (p=0.005) and G3 (p=0.001). Also, G2 had significantly lower SHBG than G3 (P=0.005).

Table 2. Mean (±SD) values of serum ZAG, Leptin, and Median (±IQR) of serum SHBG of the three studied groups

Parameter	Obese PCOS (n=40)	Obese healthy (n=40)	Normal weight healthy (n=40)
ZAG (µg/ml)	37.08±3.89♠	40.03± 3.83♠♠	68.48± 2.95
Leptin(ng/ml)	40.77±3.28 ●	34.88±3.62●●	13.60± 1.89
SHBG (nmol/l)	24.00± 4.75▲	27.00 ± 6.00▲▲	54.00± 6.75

ANOVA & t-test revealed; ♠ significant decrease in ZAG of G1 than G2 (0.005) and G3 (P=0.001), ♠♠ significant decrease in G2 than G3 (P=0.005), ●significant increase in leptin of G1 than G2 (P=0.005) and G3 (P=0.001), ●●significant increase in G2 than G3 (P=0.004).Kruskal-Wallis&Mann-Whitney U test revealed; ▲significant decrease in SHBG of G1 than G2 (0.005) and G3 (P=0.001), ▲▲significant decrease in G2 than G3 (P=0.005).

Table 3 shows the medians (±IQR) of serum LH and testosterone of G1, G2, and G3.The median and IQR of serum LH of G1 was significantly higher than G2 (p=0.005) and G3 (p=0.005), While there was no significant difference between G2 and G3. The median and IQR of serum testosterone of G1 was significantly higher than G2 (p=0.005) and G3 (p=0.005), but there was no significant difference between G2 and G3.

Table 3. Median (Inter-quartile range) IQR of serum LH and testosterone of the three studied groups

Parameter	Obese PCOS (n=40)	Obese healthy (n=40)	Normal weight healthy (n=40)
LH mIU/ml	8.95 ±3.10*	4.25± 2.78*	3.15 ± 1.67
Testosterone ng/ml	0.80±0.50*	0.30 ± 0.20*	0.20 ± 0.10

Kruskal-Wallis&Mann-Whitney U test revealed;significant increase in LH & testosterone in G1 than in G2 and G3 (P=0.005), * no significant difference between G2 with G3.

The results revealed significant positive correlation in obese PCOS group between leptin and BMI (r=0.322, p=0.043) as well as significant positive correlation between Zinc alpha 2 glycoprotein and LH (r=0.317, p=0.046), as shown in figures (1, 2).

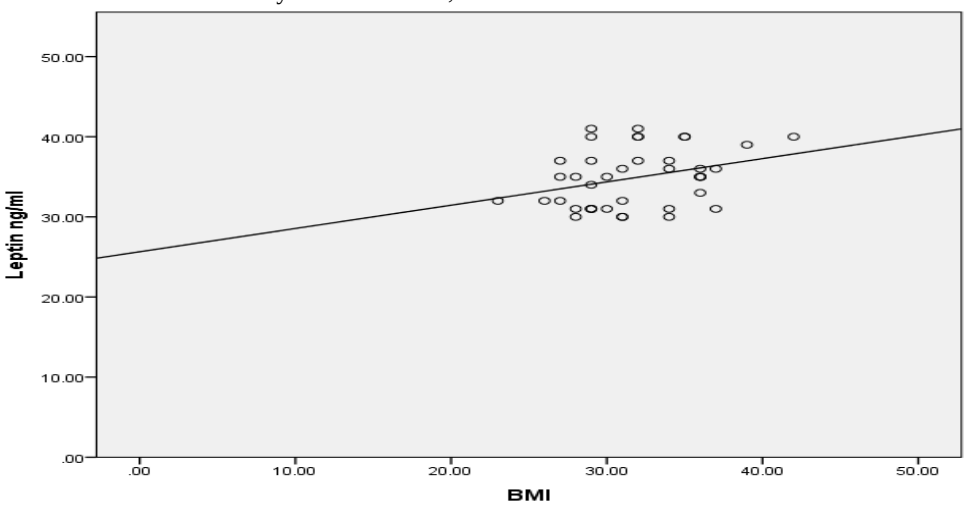

Figure 1. A scatter plot demonstrating the relationship between leptin and BMI is shown in obese healthy group.

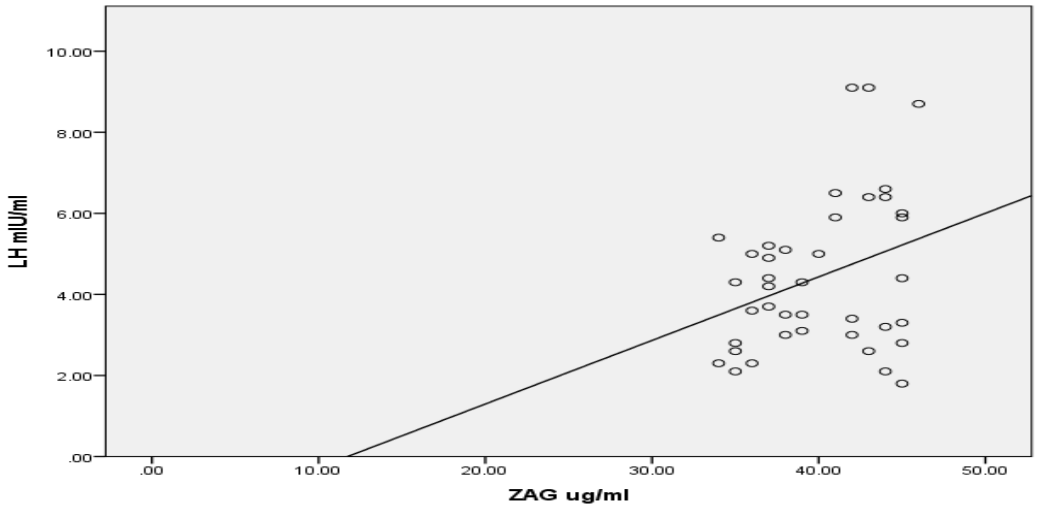

Figure 2. A scatter plot demonstrating the relationship between ZAG and LH is shown in obese healthy group.

The receiver operating characteristic curve (ROC) and area under the curve of the investigated parameters for PCOS prediction in obese healthy women are shown in Table (4). The results showed that serum testosterone levels had the highest ROC and AUC values (0.956) in differentiation between obese PCOS and obese healthy women at cutoff value (0.55 ng/ml) with sensitivity and specificity (SE=85.00, SP=97.5) and leptin had ROC and AUC value (0.876) in such differentiation at cutoff value (36.0 ng/ml) with sensitivity and specificity (SE=95.00, SP=67.5).

Table 4. ROC curve criteria and coordinates for obese pcos and obese healthy

Test Result Variable(s)	AUC	P-value	Cutoff value	Sensitivity	Specificity
ZAG µg/ml	0.717	0.0001	36	55.0%	77.5 %
SHBG nmol/l	0.778	0.0001	25	62.5%	70.0 %
Leptin ng/ml	0.876	0.005	36.0	95.0%	67.5 %
LH mIU/ml	0.948	0.005	10.8	25%	100.0 %
Testo. ng/ml	0.956	0.005	0.55	85.0%	97.5 %

Table (5) and illustrate the receiver operating characteristic curve (ROC) and area under the curve for the investigated parameters used in the diagnosis and separation of obese PCOS from normal weight healthy women. The results showed that AUC of serum leptin levels was (1.000) at cutoff value (36.5 ng/ml) with sensitivity and specificity (SE=95.00, SP=100.00), AUC of serum testosterone levels was (0.972) at cutoff value (0.55) with sensitivity and specificity (SE=85.5, SP=100.0). And AUC of serum ZAG levels was (1.00) at cutoff value (40.0 µg/ml) with sensitivity and specificity (SE=72.5, SP=100.0).

Table 5. ROC curve criteria and coordinates for obese pcos and normal weight healthy

Test Result Variable(s)	AUC	P-value	Cutoff value	Sensitivity	Specificity
ZAG µg/ml	1.000	0.0001	40	72.5%	100.0%
SHBGnmol/l	1.000	0.0001	26	75.0%	100.0%
Leptinng/ml	1.000	0.005	36.5	95.0%	100.5%
LH mIU/ml	0.973	0.005	10.8	25.0%	97.5%
Testo. ng/ml	0.972	0.005	0.55	85.5%	100.0%

Discussion

The current study's findings showed non-significant differences in mean values of age among the studied groups in order to eliminate the age factor effect (table 1). The present study designed that the mean values of BMI and WC of obese PCOS and obese normal did not differ significantly to avoid the effect of obesity on obtained parameter results (table 1). However, the results showed significant increase in mean values of BMI and WC of

obese PCOS, and obese healthy than normal weight (table 1); this finding is in agreement with another study by Barrea et al. which found that PCOS is usually more commonly found in obese women, obesity is reported in 50–70% of their women with PCOS [13].

The study showed that there was significant decrease in the mean value of ZAG with obesity and more obvious with the presence of PCOS (table 2); these findings were agreed with a study conducted by Lai et al., who found circulating ZAG levels had significantly lower in PCOS women, overweight/obese than women with normal BMI [6]. According to studies, ZAG is regulated by changes in the pro- and anti-inflammatory cytokines in adipose tissues. Furthermore, TNF (tumor necrosis factor alpha), which is known to reduce ZAG expression, may prevent ZAG expression by blocking PPAR (peroxisome proliferator-activated receptor gamma), which enhances ZAG expression [14]. Other studies show a negative correlation between ZAG mRNA levels in adipocytes and the expression of the leptin gene, another important adipokine, which is known to be elevated with the growth of adipose tissue in obesity and implicated in inflammatory responses [16]. Obese women with PCOS were shown to have decreased levels of ZAG, which may therefore result in reduced rates of lipolysis and consequent lipid buildup. It is known that the lipotoxicity that results causes insulin resistance in cells. Likewise, it is possible to propose that a decline in ZAG is related to the emergence of hyperandrogenism.

The study found a significant decline in the mean value of SHBG with obesity and more significant with PCOS (table 2). This finding was consistent with a prior research by Qu et al., which hypothesized that the negative effects of obesity on SHBG synthesis and secretion, which in turn increases testosterone bioavailability, may account for at least some of the link between obesity and PCOS [18]. Adiponectin promotes hepatic SHBG production, while TNF decreases it [19]. They discovered that TNF, the principal activator of SHBG gene expression, reduces the protein levels of hepatocyte nuclear receptor-4-alpha (HNF-4), which causes a reduction in SHBG synthesis [20]. Adiponectin, on the other hand, stimulates hepatic -oxidation and inhibits hepatic lipogenesis, both of which increase SHBG production via HNF-4 levels. Obese women may develop PCOS as a result of a cascade of excessive androgen production caused by decreased hepatic SHBG levels. Obesity-related hyperinsulinemia can also directly cause PCOS women's ovaries and adrenal glands to produce too many androgens [21].

Also, this study showed a significant increase in the mean value of leptin with obesity and PCOS (table 2). Owing to leptin resistance, obesity is linked to circulating hyperleptinemia, which implies that obese individuals are resistant to leptin's anorectic and weight-reducing actions [22]. The current study's findings concur with those of Peng et al., who discovered that elevated leptin is substantially related with PCOS and may be a key biochemical marker for identifying and treating PCOS [23].

The present study showed significant increase in the mean value of serum LH in obese PCOS women compared with obese normal and normal weight control groups (table 3). As a result, a high quantity of LH can result in irregular menstrual cycles and infertility, leading to PCOS. The present study showed a significant increase in the mean value of serum testosterone in obese PCOS subjects compared with obese healthy and normal weight healthy group (table 3), Obesity increases ovarian androgen synthesis, which expands functional ovarian hyperandrogenism and stimulates theca cells to LH stimulation [25]. These findings support the Malini et al. study's conclusion that an unexpected rise in LH and testosterone levels contributes to PCOS and infertility [24].

The result of this study observed a significant positive correlation in the obese healthy group between leptin and BMI. Leptin regulates food intake and body mass by the leptin signaling system to maintain body mass. This study also observed a significant positive correlation in an obese healthy group between ZAG and LH, The present study has demonstrated the significantly decreased serum ZAG levels in overweight/obese healthy and its positive association with LH, which decreases in the follicular phase in healthy women.

The present study found that measurement of serum testosterone (cutoff value 0.55 ng/ml), and leptin (cut-off value 36ng/ml) were the best biomarkers in predicting PCOS in obese healthy (table 4). Serum leptin at cutoff value (36.5 ng/ml) had excellent ability in differentiating between obese PCOS and normal weight women as well as testosterone at cut-off value (0.55 ng/ml) (table 5). In differentiation between obese healthy and normal weight healthy, serum leptin at cutoff value (30.5 ng/ml) was the best one as well as ZAG at cutoff value (44 µg/ml).

Conclusion

Serum testosterone at cutoff value (0.55 ng/ml) and leptin at cutoff value of (36 ng/ml) had the excellent utility in predicting PCOS in obese healthy women. Circulating leptin at cutoff value (36.5 ng/ml) and testosterone at cutoff value (0.55 ng/ml) were the best biochemical

markers in differentiation between normal weight women and obese PCOS.

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