DETERMINATION OF SERUM SEX HORMONE BINDING GLOBULIN IN POLYCYSTIC OVARIAN SYNDROME & HEALTHY WOMEN

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ABSTRACT

Background

Sex hormone-binding globulin is a glycoprotein synthesized by liver cells; it binds to sex steroid hormones & regulate their bioavailability. Polycystic ovarian syndrome is a neuroendocrine disorder characterized by anovulation or oligoovulatione, hyperandrogenism, and signs of insulin resistance. Recently reverse correlation between SHBG & obesity, PCOS (Polycystic ovarian syndrome) and insulin resistance has been recorded.

Objectives

This study aimed to compare serum SHBG levels between PCOS & healthy women and, to determine the effect of BMI on its level. In addition to that, to provide information about the effect of age on serum SHBG levels in both groups of the study.

Patients and Methods

This was a case-control study involved one hundred women, 60 women with PCOS were subfertile (study group) & 40 healthy normal (control group). Five ml of blood obtained from all the patients & control women at the follicular phase of the menstrual cycle. Serum level of SHBG, free testosterone, estrogen & AMH were measured by using the ELISA technique. The studied women were divided into subgroups according to their ages & the women with PCOS were subdivided into four subgroups according to their clinical & biochemical markers.

Results

There was no statistically significant difference in the serum level of SHBG between subfertile PCOS & control women. Its levels were significantly lower in women with a BMI of ≥25 than normal-weight women in both groups. Serum level of SHBG in normal weight (BMI <25) subfertile PCOS women was significantly lower than normal-weight healthy women p=0.009. In the control group, a weak negative correlation between age &SHBG observed r=-0.33, while this correlation was insignificant in subfertile PCOS women. No correlation between SHBG & other hormonal parameters recorded. Also, the insignificant difference in SHBG level among PCOS subgroups found F=2.061,p=0.116.

Conclusions

Both obesity & PCOS affect the serum level of SHBG but the effect of obesity is more potent. Serum levels of SHBG decline with age but the hormonal disturbances in PCOS patients becloud this physiological decline.

Keywords: *SHBG, Sex hormone-binding globulin, PCOS, Obesity, Age, Sex Steroid Hormones.*

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INTRODUCTION

Sex hormone-binding globulin (SHBG), a homodimeric glycoprotein of 95 kilo Dalton, it is synthesized in the liver with a half-value time of 7 days in plasma. Its main function is to transport sex hormones in the circulation and to extravascular target [1]. SHBG plays a role in regulating the bioavailability of sex steroid concentration through competition of available binding sites & limiting its diffusion to the target cells [2, 3]. Eighty percent of circulating testosterone is bound to SHBG, 19% is bound to albumin only 1% is free in the circulation[4]. SHBG is influenced by androgen/ estrogen balance, nutritional status, BMI, sex & insulin resistance [5].

PCOS is a common disorder characterized by oligo-ovulation- or anovulation, signs of androgen excess, and multiple small ovarian follicles. These signs and symptoms may vary widely between women as well as with individuals over time. The diagnosis of PCOS was based on Rotterdam Consensus Meeting on PCOS in 2003. It defines the syndrome of PCOS in the presence of at least two of the following criteria:-

- 1-Ultra sound appearance of polycystic ovaries
- 2-Menestural disturbance
- 3-Evidence of hyperandrogenism, acne, and hirsutism [6]

SHBG concentration increased in older men, pregnancy, hormone replacement therapy, liver cirrhosis, hyperthyroidism & hypogonadism. While, it has a negative correlation with the bioavailability of testosterone in premenopausal women [7, 8]. There is a link between PCOS hallmarks and serum SHBG level, where its level is lower in obese, hyperandrogenic women, but it shows a significant improvement after treatment [8]. Hyperandrogenemia in PCOS patients contribute to lowering SHBG level. It had been found that SHBG correlate positively with 17-OHP [9]. Recently it found that leptin hormone also involved in the pathogenesis of PCOS.Its level in obese PCOS patients is higher than normal-weight patients. There is a reserve strong correlation between leptin and SHBG [10, 11]. In spite of that, both testosterone & SHBG are related to obesity and insulin resistance, but many other factors may be located in between, higher testosterone levels & lower SHBG levels were associated with lower adiponectin & higher leptin levels in normal women, suggesting that adipose tissue hormones may involve in regulating both hormones [12]. None the less, no association between BMI & total testosterone were observed in PCOS Kurdish Iraqi [13] women suggesting that SHBG may be more sensitive to obesity than androgens.

More than half of PCOS patients are insulin resistance [14], but the extent of this resistant is unrelated to obesity, it depends on body fat distribution [15]. Yet, insulin resistance and hyperandrogenemia in PCOS patients are interconnected. SHBG is the main serum transporter of testosterone meanwhile, serum insulin found to inhibit SHBG [16]. It had been believed that obesity lower SHBG but the detailed mechanism beyond this lowering remains not fully exposed. Recently reverse association between SHBG & central obesity has observed in Chines men. They explained their results by results of another previous in vivo study which demonstrates increase lipid level in hepatocyte cells causes downregulation of hepatocyte nuclear factor 4 that reduce SHBG gene expression [17, 18]. Obesity-related type 2 diabetus, insulin resistance, hyperlipidemia, and hyperglycemia.

Serum insulin had found to inhibit SHBG production in liver cells. Moreover, in vitro studies illustrated that insulin decrease SHBG in cultured liver cells. Treatment with Diazoxide had found to elevate serum SHBG levels [19]. Recently it believes that SHBG is not just steroid transporter but it also has several receptors in different target tissue suggesting that the physiological role of SHBG is more complex than what is believed previously [20]. In the same line, Azrat et al., 2012 [21] found that intra-abdominal adipose tissue in healthy premenopausal women is correlated independently with SHBG & its level not affected by insulin resistance Locally Kadhim *et al.* [22] found that level of SHBG showed no significant variations between obese women with and without metabolic syndrome of obesity. In 2013 Wild man *et al* [12] demonstrated that there are other factors rather than steroid hormones that may affect the SHBG level, they found that SHBG is correlated strongly & positively with high molecular weight adiponectin and soluble form of the leptin receptor in healthy mid-life women. Furthermore, they found that SHBG negatively correlated with leptin hormone. In another study in Egypt, they found that the level of SHBG is lower in diabetic obese patients as compared with obese subjects they also found that its level was not correlated with insulin but it correlated with Fasting blood sugar & BMI [20].

The aim of this study is to compare serum SHBG levels between PCOS & healthy women. As well as,

to determine the effect of BMI on its level. In addition to that, to investigate the effect of age on serum SHBG levels in both groups of the study.

PATIENTS AND METHODS

This case-control study conducted at the family planning center (infertility clinic) in Azady teaching hospital, Kirkuk governorate / Iraq, from April 2018 to March 2019. The study involved 100 women with a mean age of 29.66±6.84years. Sixty patients were subfertile (ovarian cause), who were unable to conceive after a year of regular unprotected intercourse for those bellow 35 years old and, 6 months for those patients above 35 years old. Forty patients were fertile with regular menstrual cycles. The 60 infertile patients were diagnosed as Polycystic ovarian syndrome patients.

Menstrual disturbances involved amenorrhea which is defined as the absence of menstrual cycle for more than 6 months and oligomenorrhea which defined as a delay in the menses of > 35 days to 6 months.

The Ultrasound study was performed in Azady teaching hospital/ ultrasound department. Transvaginal ultrasonic screening with 3.5 MHz vaginal transducer) was performed to all infertile women. Ovaries are described as polycystic ovaries if there were 10 or more follicles with 2-10 mm in one or both ovaries. Ethical permission was taken from all study participants.

All the patients involved in the study fulfilled the following criteria:

- 1-The patients agreed to participate in the study.
- 2-The patients have five basic investigations:- Ovulatory assessment, Hysterosalpingography, seminal fluid analysis, laparoscopy & post-coital test.

Patients with abnormal investigations apart from ovulatory assessment were excluded from the study. Furthermore, women with hyperprolactinemia, thyroid disorder & Cushing syndrome also excluded from the study.

The fertile & infertile PCOS women subdivided into four subgroups according to their ages:-

1. 20-24 years old
2. 25-29 years old
3. 30-33 years old
4. 35-40 years old

The PCOS patients subdivided into four subgroups according to their clinical & biochemical markers.

A: Ovulatory dysfunction + hirsutism or hyperandrogenism+ polycystic ovary (PCO)
B: Ovulatory dysfunction + hirsutism or hyperandrogenism
C:Ovulatory dysfunction + PCO (no hirsutism, normal androgen)
D: Hirsutism or hyperandrogenism +PCO with normal menstrual cycle

Five ml of blood obtained from all patients and healthy women by vein puncture at the follicular phase of the cycle, all the samples incubated at room temperature for two hours for completing the clotting process. Serum separated by centrifugation for 20 minutes at 3000 RPM, then it transferred to plain tubes and stored at – 40^{o} C until the assay process. All the study parameters (SHBG, Estradiol, Free testosterone, AMH, LH & FSH) measured by enzyme-linked immunosorbent assay (ELISA).

Values presented as mean & ±SD. The Goodness-of-fit test used to test the normality of distribution. Student T-test used for comparison of two group means. One-way analysis of variance (ANOVA) done to estimate the difference among groups, followed by Tukey's post hoc test to evaluate the relationship between two groups. Correlation between SHBG & other parameters found by the calculation of correlations & r-value.

RESULTS

This study involved one hundred women with a mean age of 29.66±6.84. Sixty patients were subfertile & diagnosed as PCOS women, with forty control women. The demographic data of involved women demonstrated in Table 1. There was primary infertility in 37 of PCOS women. The duration of marriage in 43, infertile PCOS women were above 5 years, Table 2.

There was no statistical difference in serum levels of SHBG between PCOS & healthy women (Table 3). The normal & subfertile PCOS women were subdivided into two subgroups according to their BMI. The first group involved women with BMI ≥ 25 & the second group were those women with BMI < 25. In PCOS, women's serum SHBG level was significantly higher in women with BMI < 25 (Table 5). In control of women's serum, Estrogen was significantly higher in overweight women, while the serum level of SHBG was significantly higher in women with BMI <25 (Table 6).

SHBG levels in non-obese infertile PCOS women were significantly lower than non-obese healthy women (Table 7). No significant variation in SHBG levels

between overweight (BMI ≥25) PCOS & healthy women observed (Table 8).

The infertile PCOS & fertile women subdivided into four subgroups according to their ages. In infertile PCOS women, no correlation between SHBG and (age, FSH, AMH) found, while in healthy women a weak reverse significant correlation between age & SHBG observed, Table 9.

In infertile PCOS women, there was no significant correlation between serum SHBG and other study parameters except a very weak correlation with BMI (r=- 0.162) Table 10 and Figure 1.

The PCOS women were also subdivided into four phenotypes according to their clinical & biochemical parameters. The frequency of each phenotype were as follow:

Phenotype A: 47%
Phenotype B: 12%
Phenotype D 26 %
Phenotype C 15 %

There were no significant differences in the SHBG level among the PCOS phenotypes (F=2.061 p=0.116), Table 11.

Table 1. shows the distribution of study groups according to the demographic data.

	Infertile women with PCOS	Control
Number	60	40
Mean age	30	31
Residence		
Inside Kirkuk	35	54
Outside Kirkuk	25	6
Ocupation		
Employer	15	36
Nonemployee	45	4

Table 2. shows distribution of subfertile women according to the clinical features.

	PCOS Women
Type of infertility	
1-Primary	37
2-secondary	23
Abortion	8
Pariety	15
Duration of Marriage	
More than 5 years	43
Less than 5 years	7
Menstruation	
Amenorrhea	6
Oligomenorhea	38
Polymenorhea	6
Regular	10
Hirsutisim	
Positive	22
Negative	28
Durration of subfertility	
More than 2 years	31
Less than 2 years	29

Table 3. Shows the difference between the biochemical markers & BMI in PCOS & Control Women.

Parameters	Subfertile PCOS women (Mean &SD)	Control (Mean &SD)	P Value
Number of the subjects	60	40	
BMI (Kg/m2)	26.05±3.76	25.93±3.7	NS
SHBG (nmol/L)	77.86±55	80±13.2	NS
Free Testerone ng/ml	0.66±0.75	0.37±0.22	<0.05
Estrogen pg/ml	47.76±42	45.85±15	<0.05
FSH IU/ml	5.04±1.33	6.25±2	< 0.05
LH IU/ml	8.92±5.47	4.57±1.02	<0.05

Table 4. illustrates the comparison in serum (SHBG, Estrogen, free testerone) between overweight & normal weight PCOS infertile Women.

Parameters	PCOS BMI≥25	PCOS BMI<25	P Value
Number of the subjects	37	23	
SHBG nmol/L	66.09±5.8	72.4±4.64	< 0.05
Estrogen pg/ml	41.38±30.8	52.25±34	NS
Free testerone ng/ml	0.67±0.54	0.81±1.23	NS

Table 5. illustrates the comparison in serum (SHBG, estrogen, free testesterone) between overweight & normal weight fertile women.

Parameters	Fertile women BMI ≥25	Fertile women BMI > 25	P value
Number of the subjects	23	17	
SHBG nmol/L	69±5.2	78±5.2	< 0.05
Estrogen pg/ml	77.1±23	45±30.1	< 0.05
Free Testesterone ng/ml	0.25±0.31	0.31±0.28	NS

Table 6. Shows the comparison in serum level of (SHBG, Estrogen, free testerone) between normal weight (fertile & infertile PCOS women).

Parameters	PCOS women BMI < 25	Fertile women BMI < 25	P value
Number of the subjects	23	17	
SHBG nmol/L	72.4±4.63	78.5.2	0.009
Estrogen pg/ml	52.25±34	45±30.1	NS
Free testerone ng/ml	0.81±1	0.31±0.28	NS

Table 7. shows the comparison in serum level of (SHBG, Estrogen, free testerone) between overweight fertile & infertile PCOS women.

Parameters	PCOS women ≥25	Fertile women BMI≥25	P value
Number of the subjects	37	23	
SHBG nmol/L	66.09±5.8	69±5.2	NS
Estrogen pg/ml	41.38±30.8	77.11±23	0.003
Free testerone ng/ml	0.67±0.54	0.25±0.31	0.006

Table. 8 illustrates the between SHBG & (AMH, FSH & age) in subfertile PCOS women.

Parameters	SHBG R value	P value
Age (years)	-0.187	NS
FSH MIU/ml	-0.02	NS
AMH pg/ml	-0.01	NS

Table 9. illustrates the between SHBG & (AMH, FSH & age) in healthy women.

Parameters	SHBG R value	P value
Age (years	-0.33	0.02
FSH MIU/ml	-0.13	NS
AMH pg/ml	-0.11	NS

Table 10. shows the correlation between SHBG with other biochemical markers in infertile PCOS women

	R value	P value
BMI kg/m2	-0.162	0.1
FSH MIU/ml	0.054	0.33
Free testerone	-0.151	0.12
Estrogen pg/ml	0.035	0.39
LHMIU/ml	0.001	0.49

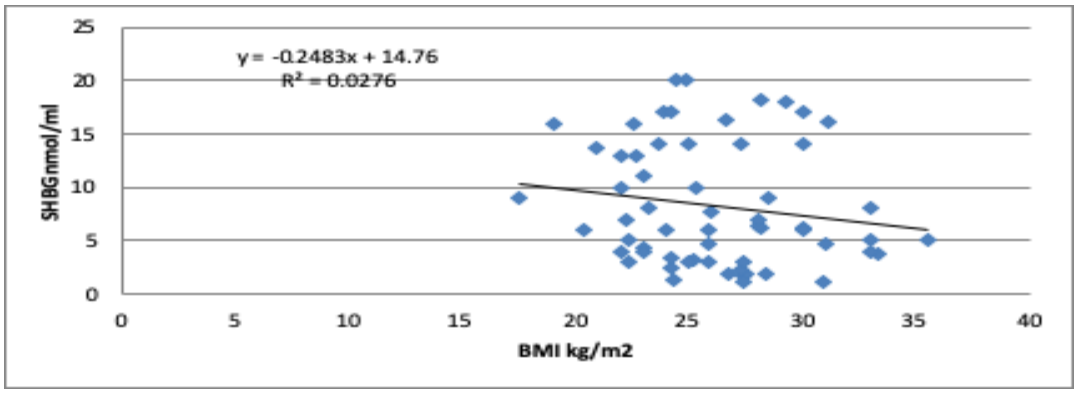

Figure 1 Shows the correlation between BMI & SHBG in infertile PCOS patients groups

Table 11 illustrates serum SHBG level in PCOS patients subgroups.

PCOS Subgroups	Number of the patients	SHBGnmol/ml (Mean &SD)	F value
A	28	80±33.2	F=2.061
B	7	100±20.2	p=0.116
C	16	70±14	
D	9	80±25	

DISCUSSION

In the present study, there were insignificant variation in serum SHBG levels in PCOS & control women, Table 3.

Dissimilar to our result locally, Jamil et al., 2015 [23] found that serum SHBG levels were significantly lower in PCOS patients in comparison with healthy women. In the same line, a meta-analytic study done by Deswal et al 2018 [24] that, analyzed the results of 22 cross-sectional and 14 case-control studies around the world, in almost all this studies the level of SHBG in PCOS patients were lower than control women. In the same line with these two previous studies Nadaraja et al.,2018 [25] stated that all androgenic parameters except SHBG were higher in PCOS women. In this study, there were no significant variants in BMI of involved women that is why insignificant differences were observed. Another reason for this insignificancy maybe since the involved PCOS women in this study stopped taking medication for three months before they participate in the study. SHBG is very sensitive to insulin-sensitizing agents like (metformin, troglitazone) that had been found to elevate SHBG in PCOS patients [26, 27].

When the study participants divided according to their BMI, the serum level of SHBG in women with BMI ≥25 was significantly lower than women with BMI <25 in both fertile & infertile (PCOS) women (Table 4 and 5). These results agree with Akin et al., 2008 (28) who demonstrated that premenopausal women with low SHBG were those who had BMI more than 30 kg/m^2. Also, Dahan & Goldstein, 2006 [9] who measured the serum level of SHBG in obese PCOS & healthy overweight women, they didn't find a significant difference. In the same line, Franik et al 2018 found that PCOS patients with WHR (Waist to hip ratio) more than 0.8 showed lower SHBG serum levels than those with WHR less than 0.8 [29].

In our results, SHBG levels were lower in PCOS lean (BMI<25) patients as compared with lean fertile patients, Table 6. PCOS is a heterogeneous neuroendocrine disorder, factors rather than those with insulin resistance & obesity may participate in lowering SHBG level, for instance, genetic factors, where mutant alleles of the rs727428 has been found to lower serum SHBG in PCOS women [30].

SHBG correlates weakly but significantly with age r= - 0.33 in healthy women ((Table 9), but no correlation

observed in infertile PCOS patients (Table 10). This result agrees with Metter et al 2001 [31] who found that SHBG level decline with steroid decline. SHBG is lower in women's in thirties than those in twenties. Also, there is a notable decline in its serum level in premenopausal women [7, 32, 33]. The major alteration in SHBG level occurs beyond age 40 exactly at 45 years old, for that reason a weak correlation between age & SHBG observed in this study.

No correlation between AMH &SHBG observed in both fertile & PCOS women (Table 10). Song et al., 2017 [34] also didn't record any correlation between them. Both AMH & SHBG levels decline with aging, AMH decreases steadily with age, it shows a significant decrease at age 25 and a dramatic decline at age 35. While SHBG level changes after age 40. Androgens, insulin resistance, and basal follicular status affect AMH in PCOS women. However, it mostly increased due to an increase of follicles beyond 5 mm. Different mechanisms cause alteration in SHBG & AMH levels.

In the present study, there were no statistical differences in serum SHBG levels among different PCOS subgroups (Table 11). Our result didn't agree with Song et al 2018 & Danilowicz et al., 2014 [34, 35], they found that normal androgen lean PCOS women had higher SHBG levels in comparison with hyperandrogenism women.In the same line Jamil et al., 2015 [23] suggest that the alteration in the hormonal & other biochemical markers is self-evident in hyper androgenized PCOS women. On the other hand, Women with normal androgen may have low SHBG levels. SHBG is a marker for androgen bioactivity, low SHBG and normal testosterone may indicate peripheral androgen activity [35].

In conclusion, sex hormone-binding globulin SHBG is an important biochemical marker for diagnosis of PCOS, its levels decline with advancing age and is strongly affected by obesity.

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