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Sex Hormone-Binding Globulin in Obese Type 2 Diabetics

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ABSTRACT

Background: Obesity is one of risk factors for type 2 diabetes because of its association with insulin resistance and poor glycemic control. Sex hormone–binding globulin (SHBG) and adipose tissue hormones have a role in development of insulin resistance, hyperlipidemia and type 2 diabetes. Serum SHBG has a role in glucose homeostasis and low levels are associated with development of diabetes, cardiovascular diseases, insulin resistance and hyperinsulinemia. **Aim of work: t**o study the relationship between serum SHBG, obesity, and metabolic parameters in type 2 diabetes in both sex.

Patients and Methods: forty obese type 2 diabetic patients and ten obese non-diabetic as controls were included in this study. Blood was taking from all subjects for estimation of glucose, Lipid profile, insulin and SHBG. **Results:** there was highly significant decrease in mean serum SHBG concentration in diabetic group compared with control non diabetic group. There were significant negative associations between serum SHBG and age, disease duration, BMI and glucose. On the other hand, there were non significant correlations between SHBG and waist circumference, fasting insulin, HOMA-IR, cholesterol and triglyceride levels.

Conclusion: Low serum SHBG is associated with hyperglycemia in both sexes, independent of insulinemia

Key Words: SHBG, type 2 diabetes, insulin, obesity.

INTRODUCTION

Hyperglycemia characterizes diabetes mellitus is due to insulin defects (either secretion or action or both). It is associated with organ failure [1]. World Health Organization (WHO) recorded in 2014 that among adults aged 18 years and above, the diabetes world prevalence was near to 10%[2]. The prevalence of diabetes in Egypt according to WHO survey was 6.0% and Egypt will have about 8.6 million adults with diabetes in year 2030. Among the most important causes of premature mortality in Egypt is diabetes mellitus[3]. Early diagnosis of diabetes mellitus is limited to blood glucose and glycated hemoglobin (HbA1c) but blood glucose test is affected by sample processing and of lower reproducibility. HbA1c use for screening and diagnosing diabetes mellitus can reflect glycemic control in retrospect manner and are affected by haemolytic anemia[4]. Many factors contribute to physiologic changes responsible for T2DM as polycystic ovarian syndrome in women and hypoandrogenism in men. They are related to insulin resistance, suggesting that modulations in normal sex steroids may play a role in the pathogenesis of diabetes. SHBG is identified as a contributing factor in the pathophysiology of T2DM. Many studies showed a relation between decreased serum SHBG and T2DM, and genetic studies revealed that transmission of specific SHBG gene polymorphisms influence the risk of T2DM[5]. There are multiple interactions between SHBG and its receptors in many target tissues suggesting physiologic roles for SHBG that are more complex than transport of sex hormones in serum[6]. The involvement of SHBG in impairment of glucose metabolism may occur through modulation of sex hormones bioavailability and activation of SHBG specific receptor[7]. Plasma membranes of different cell types can bind specifically with high affinity to SHBG. These data support the role of SHBG in the pathophysiology of T2DM and insulin resistance. The concentrations of SHBG tend to be more reliably determined than those of sex steroids[8]. Previous studies identified the influence of hormone genes and lifestyle-related factors on circulating SHBG levels. The main factor in regulation of SHBG synthesis is estrogen/ androgen balance[7,8].

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The regulation of SHBG is multifactorial and nonsteroidal factors plays an important role in its circulating levels. SHBG levels were found to be decreased in obese subjects compared with normal weight people and weight loss through calorie restriction is found to increase serum SHBG as well as insulin sensitivity supporting that body composition is involved in the determination of SHBG levels[9].

PATIENTS AND METHODS

Participants in this case control study were recruited from inpatient and outpatient clinic of Internal Medicine Department, AL-Zahraa University Hospital. Cairo, Egypt. Study participant (n = 50) were classified into, **Group I**: forty obese subjects with T2DM. They were defined to have diabetes according to American Diabetes Association criteria that fasting plasma glucose levels ≥126 mg/dL, 2-h postprandial glucose levels ≥200 mg/dl [10] or they are on current treatment by oral glucose lowering agents. They further classified into: Male group: sixteen obese diabetic patients and Female group: twenty four obese diabetic females.

Group II: Ten obese subjects without history of hormonal disturbance or diabetes mellitus who fasting blood glucose (FBG) was less than 126 mg/dl and were matched for age and body mass index (BMI) were included as control group. Females on contraceptive pills or hormonal replacement therapy, patients with chronic liver or renal diseases and those on medication such as insulin, steroids, anticonvulsants, beta blockers, thiazides were excluded. After taking informed consent, all patients were subjected to:

Full medical history and physical Examination: including age, disease duration, and drug treatment. Waist circumferences were measured and BMI was calculated by dividing weight (in kg) by the square of height (in meters) (Kg/m^2). Obesity was defined as BMI> 30 and waist circumferences > 120 cm for men and > 88 cm in women. Clinical investigations include (pelvi-abdominal ultrasonography).

Sampling:

Five ml of fasting (12-14 hours) venous blood samples were taken from each subject participating in the study and divided into 2 samples: The 1st 2 ml was added to a tube containing EDTA for determination of complete blood picture on Coulter Counter T890 (Coulter Counter, Harpenden, UK) and HbA_1c by cation exchange resin . The 2nd (1.4 ml) was left to clot and the serum was separated by centrifugation for 15 minutes at 1000 xg and fasting blood glucose was determined immediately on Hitachi auto analyzer (Hitachi 912) by glucose oxidase method. The rest of the serum was stored at -20°C for determination of the followings: lipid profile, insulin and SHBG. 2 ml of venous blood was taken on fluoride for postprandial (PP) blood glucose two hours after meals and was determined on Hitachi auto analyzer 912.

Laboratory investigations:

Total cholesterol and triglyceride, were performed on Hitachi auto analyzer 912 (Roche Diagnostics GmbH, D-68298 Mannheim, USA) by colorimetric techniques. Complete urine analysis was done. Fasting serum insulin was determined using RIA[11]**.** Measurement of serum SHBG was performed by electro chemiluminescence immunoassay on Elecsys 2010 auto analyzer **(**Roche Diagnostics)[12].

The insulin resistance was calculated using homeostasis model assessment of insulin resistance (HOMA-IR) (HOMA-IR = [insulin µ IU/ml× glucose mg/dl]/405) and is considered elevated at HOMA-IR >3 [13].

Statistical Analysis:

Results were collected, tabulated, and statistically analyzed by personal computer and statistical package SPSS version 10 (Chicago, USA).Two types of statistics were performed: Descriptive statistics - for example, mean *(X)* and SD - and analytic statistics. Quantitative variables were compared using unpaired t-test. Spearman correlation coefficient (r) is a test used to measure the association between two quantitative variables. The P value of less than 0.05 was considered as statistically significant.

RESULTS

Forty obese T2DM patients were included in the study, their ages were 52.35±6.163 and their disease duration was 8.97±4.40 years. BMI was 32.92±3.88 and waist circumference was 98.3±7.06. There were no significant differences regarding age or anthropometric measures

between patients and controls ($p > 0.05$) (Table 1).

There were highly significant increase in fasting glucose (FG), two hours postprandial glucose (PP), fasting insulin, HOMA-IR, HbA_1c, cholesterol and triglyceride in diabetic patients compared to control group ($p < 0.001$) (Table 1).

There was highly significant decrease in mean serum SHBG in diabetic group compared with control group (Table 1).

There were significant negative correlation between mean serum concentration of SHBG levels and age, disease duration, BMI, FBS and PP; while there were non significant correlations between SHBG and waist circumference, fasting insulin, HbA_1c, HOMA-IR, cholesterol and triglyceride levels (Table 2).

There were no gender differences observed with clinical or laboratory parameters (Table 3). There was only significant negative correlation between SHBG and BMI only in female diabetic patients and FS in male diabetic patients ($p < 0.001$) (Table 4).

DISCUSSION

Sex hormone-binding globulin is a glycosylated homodimeric plasma transport protein that is predominantly synthesized in the liver. Several mechanism accounts for the association between SHBG, insulin and type 2 DM. SHBG was shown to be inversely related to insulin resistance and low SHBG concentrations potentially contribute to the pathophysiology of T2DM[14]. Many studies suggested that decreasing total protein secretion leads to suppression of hepatic SHBG production in human hepatic cells. Others suggest that increased carbohydrate consumption and levels of fasting glucose, are the main determinants of liver SHBG production through down regulation of hepatocyte nuclear factor 4-α (HNF-4α) activity. Regardless of the mechanism, SHBG seems to be frequently associated with insulin resistance and development of T2DM in overweight populations[15]. The contribution of circulating SHBG in biological functions has been related to its regulation of sex hormone concentrations. Both testosterone and estradiol regulate SHBG levels and have been associated with the development of type 2 diabetes[16]. Low testosterone leads to a decrease in muscle mass and increase in circulating free fatty acids which mediate the development of insulin resistance and the development of overt type 2 DM. So sex hormones may partially explain the link between SHBG and diabetes[15]. However, **Chen *et al*.**[8], concluded that a large portion of SHBG's influence on diabetes is independent of free or total sex hormone concentrations, suggesting that the relation between SHBG and diabetes could not be linked to sex hormone concentrations. Our aim was to investigate the relationship between SHBG concentration and type 2 diabetes in both sexes. No gender differences were observed with regard to clinical and laboratory parameters

In this study; diabetic patients had significantly lower levels of SHBG than non diabetics. Analysis of 23 cross-sectional studies found that significantly lower SHBG levels in T2DM both in male and female compared with non-diabetic [17].

In agreement with **Peter *et al*.**[18]**,** we found a strong negative correlation between SHBG, fasting and postprandial glucose levels. **Colangelo *et al*.**[19]**,** observed a significant negative association between SHBG levels and fasting glucose levels in type 2 diabetes of various ethnicities. While **Simó *et al*.**[20], demonstrated that increased intake of monosaccharide leads to lower human SHBG production by reducing hepatocyte HNF4-α a key transcription factor that regulates SHBG expression in the liver. HNF4α-binding sites are involved in the metabolism of glucose, lipids, and amino acids.

Chaoyang *et al*.[21], used HOMA-IR to assess the association between SHBG concentration and insulin resistance and they found that decreased SHBG is associated with an increased HOMA-IR in both sexes. In this study, the association between SHBG, insulin and HOMA-IR was insignificant. Fasting insulin did not reach statistical significance at the 0.05 level. This is in agreement with **Sutton-Tyrrell *et al*.**[22], studies who found that decreased circulating SHBG was not associated with increased insulin levels.

Selva and colleagues[23], demonstrated that hepatic SHBG transcription is mainly responsive

to levels of monosaccharide's rather than to insulin. These finding were against the hypothesis that hyperinsulinemia is responsible for the decrease in SHBG levels in humans and support that glucose has a major role in controlling SHBG levels. This finding differs from that of **Osuana *et al*.**[24], who who found negative correlation between SHBG and fasting insulin level in men.

In this study SHBG was negatively correlated with age of the diabetic patients, this agrees with study of **Onat *et al*.**[25] who found age-related decrease in SHBG. There was significant negative correlation between SHBG and BMI but not waist hip ratio (WHR). BMI is a major determinant of blood SHBG concentrations in both sexes and decreased SHBG levels in overweight subjects are a biomarker for the metabolic syndrome and T 2 DM[26]. Obesity is a state of chronic low-grade inflammation, an inverse correlation was found between SHBG and C-reactive protein levels[27]. Macrophages produces tumor necrosis factor -α (TNF-α) in obese subjects and its levels correlate with insulin resistance and degree of adiposity. Studies demonstrated that deletion of TNF-α or its receptor results in improving insulin sensitivity suggesting the role of TNF-α in obesity related insulin resistance[19]. **Selva *et al*.**[23], demonstrated that liver fat determined circulating SHBG levels and not total body fat.

Peter *et al*.[18], investigated the relation between changes in regional obesity following changes in lifestyle and circulating levels of SHBG among individuals at high-risk of T2DM. He found stronger correlations with serum SHBG changes than total body or visceral fat mass

In a previous study by **Chubb *et al*.**[28], a negative correlation between total cholesterol, triglyceride levels and SHBG level was seen. In this study we could not find significant correlation. This is in agreement with **Sá EQand his colleague**[29] who found no significant associations between SHBG levels and fasting serum triglycerides or between SHBG levels and HDL. SHBG is related to dyslipidemia, possibly by regulating hepatic lipoprotein lipase activity and reducing the release of fatty acids from adipocytes[30].

CONCLUSION

low SHBG concentration is associated with hyperglycemia independently of insulinemia in T2DM. SHBG showed significant decreased level in both male and female. Our finding supports the association of circulating SHBG with type 2 diabetes.

Declaration of interest:

Authors declare that they have no conflict of interest.

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Parameters	T2DM group (n=40)	Non-diabetic Control group (n = 10)	P value
Age (year)	52.35±6.163	51.8±7.480	N.S.
FS (mg/dl)	152.55±37.449	93±4.082	< 0.001
PP (mg/dl)	219.45±51.482	117.3±10.122	< 0.001
HbAlc (%)	7.15±1.159	5.71±0.288	< 0.001
Fasting insulin (µ iu)	34.3±7.800	17.98±2.439	< 0.001
Total cholesterol (TC) (mg/dl)	193.1±47.781	120.3±8.782	< 0.001
Triglyceride (mg/dl)	195.075±46.448	132.5 ±8.972	< 0.001
HDL-cholesterol (mg/dl)	36.925±8.678	47.3±4.373	< 0.001
LDL (mg/dl)	137.675±18.570	60.9±9.562	< 0.001
SHBG (nmol/L)	18.95±3.423	35.17±8.229	< 0.001
HOMA-IR	154.05±36.408	73.9±10.12	< 0.001
BMI (kg/m2)	32.92±3.88	32.33±2.30	N.S.
Waist Circumference	98.3±7.06	97.7±9.3	N.S.

Table 1: **Comparison of variables between diabetic patients and control group:**
FS = fasting sugar, HOMA = homeostatic model assessment, IR = insulin resistance, HDL=high density lipoprotein, LDL= low density lipoprotein, SHBG=sex hormone-binding globulin, BMI=body mass Index
P value < 0.05 *, p value < 0.001**, p value > 0.05 = N.S.

Table 2: Correlation of SHBG with clinical and laboratory parameters in diabetic group:

Variable	r value	p value
Age	-0.150	< 0.05
Disease duration	0.197	< 0.05
BMI	-0.364	< 0.05
Waist circumference	-0.120	N.S.
FBS	-0.645	< 0.001
PP	-0.403	< 0.001
HbAlc	0.129	N.S.
Fasting insulin	-0.114	N.S.
HOMA-IR	0.114	N.S.
Total cholesterol	0.026	N.S.
Triglyceride	0.162	N.S.
LDL	0.097	N.S.

Table 3: **Comparison of variables between male and female diabetic patients**

	Female (n=24)	**Male (n=16)**	**P- value**
	Mean ±SD	Mean ±SD	
Age (year)	49.58±7.31	51.50±3.79	N.S.
SHBG (nmol)	19.85±3.34	17.59±3.18	N.S.
Fasting Insulin (µ iu)	34.03±7.45	34.71±6.96	N.S.
BMI. (Kg/m2)	31.88±4.42	31.99±3.02	N.S.
HOMA- IR	154.17±37.62	153.88±41.41	N.S.
Waist circumference (cm)	97.13±8.13	96.31± 6.62	N.S.
Total cholesterol (TC) (mg/dl)	186.29±44.67	203.31±63.23	N.S.
Triglyceride (mg/dl)	201.79±49.27	185.00±66.20	N.S.

Table 4: Correlation of SHBG with variables in male and female diabetic patients:

	Male patients n=16		**Female patients n=24**	
Variable	**r value**	**P value**	**r value**	**P value**
Age	-0.259	0.333	-0.062	N.S.
FBS	-0.702	0.002*	-0.514	N.S.
Insulin	0.084	0.757	0.118	N.S.
HOMA-IR	0.303	0.254	0.094	N.S.
Waist circumference	-0.234	0.272	0.027	N.S.
BMI	0.155	0.568	-0.613	< 0.001
HbA1c	-0.103	0.704	0.029	N.S.