

Sex Hormone-Binding Globulins and Testosterone Levels as a Risk Marker for Type 2 Diabetes Mellitus among Postmenopausal Women

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ABSTRACT **Background:** Endogenous sex hormones and sex hormone-binding globulins (SHBG) determine the risk of occurrence of Type 2 diabetes mellitus (T2DM) in postmenopausal (PM) women. **Aims:** To investigate the association between sex hormones (estradiol and testosterone) and SHBG with plasma glucose, fasting insulin levels, HbA1c, and homeostasis model assessment insulin resistance (HOMA-IR) and also to investigate independent role of sex hormones in the occurrence of T2DM among PM. **Settings and Design:** Cross-sectional case-control study. **Subjects and Methods:** The present study was conducted in Endocrinology department Guwahati, Medical College, Assam, India. The participants included cases – PM women with T2DM ($n = 100$) and controls – Healthy PM women ($n = 86$). The medical history, clinical examination, and investigations including total testosterone, serum estradiol, SHBG, free testosterone index, high sensitivity C-reactive protein (hs-CRP), lipid profile, fasting insulin, fasting plasma glucose (FPG), and postprandial plasma glucose (PPPG) were done and analyzed. HOMA-IR was calculated. **Statistical Analysis:** Pearson correlation between sex hormone level and SHBG with plasma glucose, HbA1c, fasting insulin, hs-CRP, and HOMA-IR was seen. Multivariate logistic analysis was done to find the independent association between sex hormones/SHBG and the occurrence of T2 DM. $P < 0.05$ was considered statistically significant. **Results:** Among the cases, a significant positive correlation was found between total testosterone/free testosterone index with waist circumference, FPG PPPG, HbA1c, fasting insulin, and HOMA-IR, and a significant negative correlation was found between SHBG and FPG, PPPG, HbA1c, fasting insulin, and HOMA-IR ($P < 0.01$). The logistic analysis showed total testosterone levels and SHBG are independently associated with the occurrence of T2 DM among PM ($P < 0.01$). **Conclusion:** SHBG and testosterone levels in PM can be a risk marker for the development of T2DM.

KEYWORDS: Insulin resistance, menopause, sex hormone-binding globulins, testosterone, type 2 diabetes mellitus

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INTRODUCTION

The traditional risk factor for type 2 diabetes mellitus (T2DM) such as obesity, high blood pressure, high triglyceride levels, low high-density lipoprotein-cholesterol (HDL-C), physical inactivity, and family history are known.^[1] Sexual-dimorphic relationship between testosterone and the risk of development of T2DM has been studied. Lower testosterone levels are associated with diabetes in men, whereas higher

testosterone levels correlated with increased diabetes risk in premenopausal women.^[1] Postmenopausal (PM) women are at risk of diabetes mellitus.^[2] The risk of developing T2 DM among PM women is considered to

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be multifactorial. Alteration of body composition and occurrence of metabolic syndrome among PM women have been thought to be the cause of T2 DM. The role of sex hormones in the genesis of T2DM is evolving and conflicting.^[3] Although the relationship between sex hormone-binding globulin (SHBG) and T2D has been known, the role of sex hormones, such as endogenous estrogen and testosterone, with T2D is scarce. Moreover, relationship between traditional risk factor, sex hormones, and T2 DM has not been well studied in India and hence we embark on the study.^[4,5]

SUBJECTS AND METHODS

The present study was a cross-sectional case-control study conducted in the Gauhati Medical College Hospital, Guwahati, Assam, India over a period from September 2015 to August 2019. The study was approved by the institutional ethics and informed consent was taken from each study participants. To aim of the study was to investigate the association between sex hormones (estradiol and testosterone) and SHBG with plasma glucose, fasting insulin levels, HbA1c, and homeostasis model assessment insulin resistance (HOMA-IR). Second, also to investigate the independent role of sex hormones in the occurrence of T2DM among PM. The study participants were divided into cases and control. Cases defined as PM women of age between 50 and 75 years attending the Endocrinology outpatient department of Gauhati Medical College Hospital with diabetes mellitus as by American Diabetes Association recommendation to define diabetes mellitus.^[6] Controls were age and body mass index (BMI) matched healthy PM. PM state was defined as cessation of menses for more than 12 months in the presence of natural menopause. Patients with Type 1 diabetes mellitus, pancreatic diabetes, maturity onset diabetes of young, latent autoimmune disease of adulthood, history of other endocrine diseases such as thyroid illness, hypopituitarism, hepatic disease, renal dysfunction, on drugs that can cause hyperglycemia, lipid-lowering medications, hormone replacement therapy, diabetes patients on insulin, smokers, alcoholism, history of oophorectomy, head and neck surgery, or chemo-radiation were excluded from the study. T2 DM was diagnosed clinically based on phenotype, family history and after excluding other types of diabetes mellitus. Patients who fulfilled the study criteria underwent a detailed medical history and clinical examination.

We defined metabolic syndrome as per the IDF criteria for Asian populations.^[7] The waist circumference for defining abdominal obesity is ≥ 80 cm in the Asian

population women. Waist and hip circumference ratio >0.8 women suggestive of android obesity and BMI >25 for obesity. All study participants underwent a detailed history and thorough physical examination, including recording of weight, height, BMI, waist and hip. Venipuncture for serum estradiol, total testosterone, SHBG, high-sensitive C-reactive protein (hs-CRP), fasting insulin, glycated hemoglobin (HbA1c), fasting plasma glucose (FPG), and lipid profile were performed in the morning after the participants had fasted for at least 8 h from both the cases and controls. Seventy-five g oral glucose was given after collecting fasting samples and 2 h later for postprandial plasma glucose (PPPG) was collected. The samples were immediately centrifuged, and the serum was stored at -20°C until assayed. Serum estradiol was estimated by Elecsys Estradiol II assay (ROCHE COBAS) which employs a competitive test principle using a polyclonal antibody specifically directed against 17β -estradiol. Intra-assay and inter-assay coefficient of variation varies from 5.6% to 6.5% and 3.1% to 5.0%. Testosterone assay (ROCHE COBAS) was also based on a competitive test principle using a monoclonal antibody specifically directed against testosterone. Intra-assay and inter-assay coefficient of variation varies from 5.2% to 6.8% and 3% to 4.5%. SHBG assay (ROCHE COBAS) employed sandwich assay using two monoclonal antibodies specifically directed against human SHBG. Intra-assay and inter-assay coefficient of variation varying from 2.1%–2.7% to 2.5%–4% for SHBG. Serum insulin was estimated by insulin assay (ROCHE COBAS) which employs a competitive test principle. Intra-assay and inter-assay coefficient of variation varies from 2.2% to 4.1% and 2.4% to 4.7%. FPG and PPPG were measured using the glucose oxidase method. HbA1C was estimated using a National Glucose Standardization Program-certified high-performance liquid chromatography system (BIORAD-D10, United States). Lipid profile was measured by enzyme calorimetry, using Vitros 5600 auto analyzer. Low-density lipoprotein cholesterol (LDL-C) was calculated using Friedewald formula. Non-HDL cholesterol was calculated as the difference between total cholesterol and HDL cholesterol, has been found to be equivalent to LDL-C in predicting cardiovascular disease. Triglycerides (TG) to HDL-C ratio were calculated as an additional predictor of cardiovascular disease risks. hs-CRP was measured from stored serum samples using a solid phase chemiluminescent assay with the immulite 1000 (Siemens Healthcare Diagnostics) and intra-assay coefficient of variation of 5.0%. HOMA-IR was calculated from the standard formula using FPG in mmol/L and insulin levels in microunits/ml.

Statistical analysis

The mean and standard deviation were calculated for normally distributed data. Pearson analysis was done to find the correlation between sex hormones/SHBG and plasma glucose, HbA1c, insulin and HOMA-IR. Multivariate logistic analysis was done to find the independent association between sex hormones/SHBG and the occurrence of T2 DM. $P < 0.05$ was considered significant. Results were analyzed with the SPSS 16.0 statistical package (SPSS, Chicago, IL, USA).

RESULTS

As shown in Table 1, the mean age of the cases and control were 64.27 ± 5.81 and 65.03 ± 5.24 years, respectively ($P = 0.24$). There were no significant differences between the mean BMI, LDL-C and serum estradiol levels among the cases and controls. However, the cases had significantly higher mean waist circumference, W/H ratio, total cholesterol, TG, fasting insulin, total testosterone, free testosterone index, E2/T ratio, FPG, 2hr PPPG, HbA1c, systolic and diastolic hypertension, levels, hs-CRP, and HOMA-IR. There was also a significant low HDL-C and SHBG levels between the cases in comparison to controls. The mean duration type 2 DM among the cases was 6.6 ± 3.4 years. Systemic

hypertension was detected among 72 of the cases. Among the hypertensive participants, 50% ($n = 36$) of the women were on antihypertensive (30 were on single antihypertensive and 6 were on dual antihypertensive). Twelve participants among the cases had a documented history of coronary artery disease (CAD) and three had cerebro-vascular accident. None of these 15 participants were on lipid-lowering agents despite being prescribed statins, but 10 were on single antiplatelet. Thirty-two cases of PM women with diabetes mellitus were on anti-diabetic medications which included glibenclamide ($n = 18$), glimepiride ($n = 16$), gliclazide ($n = 6$), and metformin ($n = 2$). None of the women had a history suggestive of polycystic ovarian disease (PCOD). A presumptive diagnosis of PCOS in menopausal women was made based on the presence of a well-documented long-term history of menstrual irregularities in the form of oligomenorrhea and features of hyperandrogenemia like hirsutism or acne and/or radiological or hormonal documented evidence of PCOS during reproductive years.

Among the PM women with type 2 DM, a significant positive correlation was found between total testosterone and free testosterone index with waist circumference, W/H ratio, FPG, 2hr PPPG, HbA1c, fasting insulin, hs

Table 1: Baseline characteristics of cases ($n=100$) and controls ($n=86$)

Variable	Mean±SD		P
	Cases ($n=100$)	Control ($n=86$)	
Age (years)	64.27±5.81	63.05±5.24	0.24
BMI (kg/m ²)	25.98±3.86	25.36±4.1	0.43
WC (cm)	86±5.2	82±5.8	<0.01
WHR	0.81±0.04	0.78±0.08	<0.01
SBP (mm Hg)	140.65±8.2	120.38±10.1	<0.01
DBP (mm Hg)	96.5±9.88	82.1±4.2	<0.01
Total cholesterol (mg/dl)	201.55±40.1	190.03±22.8	0.02
LDL-C (mg/dl)	125.62±36.8	120.65±28.11	0.20
HDL-C (mg/dl)	36.75±8.08	40.34±9.12	<0.001
TG (mg/dl)	221.5±88.60	122.92±50.1	<0.01
E2 (pg/ml)	11.18±2.39	11.23±2.42	0.7
Total testosterone (ng/dl)	20.08±2.94	12.53±2.64	<0.01
E2/T ratio	0.10±0.72	0.11±0.08	0.06
SHBG (mcg/ml)	3.08±0.48	3.85±0.39	<0.01
FPG (mg/dl)	130.52±14.32	78.32±8.2	<0.01
PPPG (mg/dl)	248.75±11.38	124±9.26	<0.01
HbA1c	7.84±1.45	5.24±0.32	<0.01
Fasting insulin levels (microunits/ml)	14±4.8	4.5±2.2	<0.01
HS-CRP (mg/L)	5.92±4.08	2.70±0.9	<0.01
Free testosterone index (%)	2.50±0.52	2.11±0.69	<0.01
HOMA-IR	4.52±2.84	0.9±0.2	<0.01

BMI: Body mass index, WC: Waist circumference, WHR: Waist hip ratio, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, TG: Triglyceride, SHBG: Sex hormone binding globulins, FPG: Fasting plasma glucose, PPPG: Postprandial plasma glucose, HbA1c: Hemoglobin A1c, HS-CRP: High sensitivity C reactive protein, HOMA-IR: Homeostasis model assessment-insulin resistance, SD: Standard deviation, E2: Estradiol

Table 2: Correlation between sex hormones (total testosterone levels, estradiol and sex hormone binding globulins) with waist circumference/waist/hip ratio/fasting plasma glucose/2 h postprandial plasma glucose/hemoglobin A1c/ fasting insulin/high sensitivity C reactive protein/homeostasis model assessment-insulin resistance among the cases (n=100)

	Pearson correlation coefficients (n=100) (P)		
	Total testosterone and free testosterone index	E2	SHBG levels
WC	$r1=0.64, r2=0.79$ $P<0.01, P<0.01$	-0.72 $P=0.01$	0.14 $P=0.59$
WHR	$r1=0.55, r2=0.50$ $P<0.01, P<0.01$	-0.64 $P=0.01$	0.12 $P=0.66$
FPG	$r1=0.68, r2=0.81$ $P<0.01, P<0.01$	0.07 $P=0.34$	-0.53 $P=0.01$
2 h PPPG	$r1=0.68, r2=0.80$ $P<0.01, P<0.01$	-0.21 $P=0.6$	-0.49 $P=0.01$
Fasting insulin	$r1=0.54, r2=0.64$ $P<0.01, P<0.01$	-0.28 $P=0.8$	0.53 $P=0.01$
HbA1c	$r1=0.69, r2=0.62,$ $P<0.01, P<0.01$	-0.22 $P=0.7$	-0.53 $P=0.01$
HS CRP	$r1=0.48, r2=0.50$ $P<0.01, P<0.01$	-0.29 $P=0.4$	0.04 $P=0.61$
HOMA-IR	$r1=0.6, r2=0.56$ $P<0.01, P<0.01$	-0.16 $P=0.54$	-0.6 $P<0.01$

WHR: Waist hip ratio, PPPG: Postprandial plasma glucose, FPG: Fasting plasma glucose, HbA1c: Hemoglobin A1c, HS CRP: High sensitivity C-reactive protein, HOMA-IR: Homeostasis model assessment-insulin resistance, SHBG: Sex hormone-binding globulins, WC: Waist circumference

Table 3: Multivariable logistic analysis (n=186) showing the odds of occurrence of an type 2 diabetes mellitus in postmenopausal women after adjusting for age, body mass index, systemic hypertension, fasting insulin levels, lipid profile, and high sensitivity-C reactive protein levels

Variables	OR	95% (CI)	P
Model			
Multiple logistic regression			
E2	0.29	0.33-1.87	0.5
Testosterone	7.12	5.38-7.77	<0.01
SHBG	0.79	0.64-0.88	<0.01

OR: Odds ratio, CI: Confidence interval, SHBG: Sex hormone-binding globulins

CRP and HOMA IR ($P < 0.01$). Estradiol levels had a significant negative correlated with waist circumference and W/H ratio [Table 2].

SHBG level showed a negative correlation with FPG, 2 HR PPPG, HbA1c, hs-CRP, fasting insulin and HOMA-IR. No such correlations were seen among the controls. Linear regression analysis showed a significant association between total testosterone and free testosterone index with waist circumference, W/H ratio, FPG, 2hr PPPG fasting insulin, hs-CRP, and HOMA-IR ($P < 0.01$). Linear regression analysis also showed a significant association between serum estradiol and waist circumference and W/H ratio and association

between SHBG and FPG, 2 HR PPPG, HbA1c, hs-CRP, fasting insulin, and HOMA-IR ($P < 0.01$).

Multivariable logistic regression analysis [Table 3] showed that total testosterone levels (odds ratio [OR] 7.12 (confidence interval [CI] 5.38–7.77) $P < 0.01$) and SHBG (OR 0.79 [CI 0.64–0.88], $P < 0.01$) are independently associated with the occurrence of T2DM PM women after adjusting for age, BMI, systemic hypertension, fasting insulin levels, lipid profile, and hs CRP levels.

DISCUSSION

In our study, we could find that all PM women were obese (cases 25.98 ± 3.98 and controls 25.36 ± 4.1) and had central obesity (cases 86 ± 5.2 cm and control 82 ± 5.8 cm). Previous studies have shown that menopausal state is a relative change in body composition, particularly increased abdominal fat and decreased lean body mass.^[8,9] This change in body composition is attributed predominantly by menopausal transition and partly by senility.^[10] The development of metabolic syndrome due to menopause has its effects on lipoprotein levels.^[10-12] HDL-C levels tend to fall among women below the desirable levels after menopause (HDL-C was 36.75 ± 8.08 and 40.34 ± 9.12 mg/dl in the cases and controls). Changes in body composition and lower HDL-C were universal

seen in the study population. We were able to make certain significant remarkable derangement in metabolic profile among the PM women with T2DM compared to the healthy controls. Type 2 diabetic PM had significantly higher systolic and diastolic blood pressure, TG levels, fasting insulin and hs-CRP compared to control group. The cases hence fulfilled the criteria for metabolic syndrome as per the IDF criteria.

The baseline hormonal profile showed a significant higher total testosterone levels with lower SHBG among the cases. A clear association could be delineated between total testosterone levels and free testosterone index with WC, W/H ratio, FPG, 2-h PPG, hs-CRP, fasting insulin levels and HOMA-IR among the cases. The association of testosterone levels with central obesity and IR has been demonstrated here. Studies have supported the association between abdominal adiposity with relatively more androgenic sex hormone profile among PM women.^[13-15] Cases had a higher IR compared to healthy PM women (HOMA-IR cases 4.52 ± 2.84 vs. controls 0.9 ± 0.2 , $P < 0.01$).^[16] We found that among the cases serum testosterone levels and free testosterone index significantly correlated with HOMA-IR (correlation: $r_1 = 0.54$, $P < 0.01$; $r_2 = 0.64$, $P < 0.01$). Such relationship were not seen among healthy premenopausal women in previous studies, which was considered to be due to the positive effects of estrogen.^[17] Loss of estrogen and elevated testosterone in PM women decreases the insulin sensitivity. A HOMA-IR value of 2.5 and above is taken as an indicator of underlying IR in adults.^[18] We could also demonstrate an association between testosterone levels and hs-CRP. Comparing to healthy controls the levels were much significantly higher in type 2 diabetes PM women (cases 5.92 ± 4.08 vs. controls 2.70 ± 0.9). Pizarra *et al.* in his prospective study showed that people with baseline hs-CRP ≥ 3 mg/L developed T2DM.^[19] Here, we could relate between inflammatory markers, central obesity, and development of IR. Adipose tissue serves as an endocrine organ, secreting a variety of cytokines including leptin, adiponectin, plasminogen activator inhibitor-1, tumor necrosis factor- α , resistin, and interleukin-6. These cytokines have immunological, vascular, and metabolic actions.^[20,21] The above-mentioned inflammatory marker blocks the insulin downstream signaling pathway and hence involved in the genesis of IR.^[22] In short menopausal state leads to central obesity and an elevated testosterone levels in absence of estrogen in women can potentiate a state of chronic inflammation which may be a perpetrator for the development of IR and later occurrence of T2DM among PM women.

Our cases had higher blood pressure as compared to healthy controls. The loss of the protective effects of estrogen in women can predispose to endothelial dysfunction and hypertension.^[23] Moreover, elevated testosterone levels and chronic inflammation in PM women could be an additive cause for hypertension. The role of elevated chronic inflammatory markers as an independent marker of hypertension among the elderly irrespective of gender has been demonstrated.^[24] A recent study showed a significant association between androgen levels and systolic and diastolic blood pressure among PM women. Androgens also had an undesirable effect on lipid parameters, BMI, and insulin sensitivity. The present study concluded that higher testosterone level is associated with elevated risk for incident cardiovascular disease and CAD.^[25]

We also found that PM women with type 2 DM, a significant negative correlation was found between SHBG, FPG, 2 h PPP, fasting insulin, and HOMA-IR. SHBG has been known as a simple transport protein for sex steroid and later studies have shown it complex physiologic interactions with various target tissues. SHBG appears to be involved in glucose homeostasis. The effects of SHBG on glucose metabolism had been reasoned. The first being the indirect effects of SHBG and T2D may result from alterations in the sex hormone bioavailability at the target tissue. Second, the direct effects of SHBG as demonstrated by two recent studies demonstrated on SHBG SNP genotypes, circulating SHBG levels, and the risk of T2DM. These studies emphasized that altered SHBG physiology may be a primary defect in the pathogenesis of disease which is subsequently followed derangement in glucose metabolism.^[26,27] Finally, we did multi-variance logistic analysis to find the role of sex hormones and SHBG independently in the occurrence of T2 DM among the PM women. After adjusting for age, BMI, blood pressure, lipid profile, fasting insulin, and hs-CRP, we found that total testosterone levels (OR 9.66 [CI-5.38–17.77] $P < 0.01$) and SHBG (OR-0.79 [0.64–0.88], $P < 0.01$) are independently associated with the occurrence of T2 DM in PM women.

CONCLUSION

The postmenopausal state is a relative change in body composition, particularly fat mass of centripetal distribution. Further exacerbation of metabolic syndrome and chronic inflammation results from an elevation of serum testosterone levels and this later leads to IR. We also learned that an alteration in serum testosterone and SHBG levels in PM women can independently lead to dysfunction of glucose metabolism culminating to the occurrence of T2 DM.

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Conflicts of interest

There are no conflicts of interest.

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