ORIGINAL RESEARCH

Association of sex hormone profile with dyslipidemia in diabetic Indian men

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ABSTRACT

Introduction: The endogenous testosterone concentrations to be associated with more favourable cardiovascular profile, including higher HDL cholesterol and lower triglyceride concentrations, blood glucose, blood pressure and body mass index. The purpose of this study was to investigate the biological association between sex hormone profile and type 2 diabetes with dyslipidaemia in Indian men.**Methodology:** In this cohort study, total of 181 diabetic were enrolled as per WHO norms. As per their lipid profile, they were further divided into patients with dyslipidaemia and patients without dyslipidaemia. Along with clinic-demographical data, fasting blood glucose, Post-prandial blood glucose, HBA1c, insulin, lipid profile and Testosterone, LH, FSH were measured and compared. As per serum gonadal hormones assessment, LH and FSH showed significantly elevated levels in cases as compared to controls.**Results:**The majority of the patients in the case groups were aged between 31-40 years [82(45.30%)]. The FPG, HbA1c, total cholesterol, LDL, and triglycerides were significantly elevated in cases as compared to controls, except HDL and SHBG, showing non-significant differences. However, serum albumin ($p<0.0001$) and free testosterone ($p<0.0001$) were significantly lower in cases as compared to controls. The spearman correlation between Testosterone and different parameters, and all the correlations showed negative correlation.**Conclusion:**Significant association of dyslipidemia with hypogonadism in diabetic patients were observed. Low levels of testosterone are associated with visceral fat accumulation, metabolic syndrome, type 2 diabetes, increased inflammatory cytokines, hyperlipidemia and abnormalities of coagulation.

Key words: Type 2 Diabetes, testosterone level, hypogonadism, dyslipidemia

INTRODUCTION

Testosterone is the main sex hormone in man. A young man produces 3-10 mg of testosterone daily that results in serum levels of 300-1000 ng per 100 ml. Testosterone levelis generally lower in the late afternoon and is suppressed by glucose administration or foodintake [1]. Although considering specific testosterone threshold is likely to be unreliable,generally, the symptoms and signs of androgen insufficiency more likely occur with totaltestosterone level lower than normal for young healthy men (less than 280-300 ng/dl or 9.7-10.4 nmol/L [2].

Epidemiological studies have displayed that total and free testosteronelevels decline with aging. Clinical outcomes of male hypogonadism are well known.

Recent investigations have demonstrated that low testosterone levelsare associated with atherosclerosis in all major vessels [3].Moreover, animal experiments have indicatedthattestosterone inhibits atherosclerosis plaque formation inrabbits and rodents fed a high-fat diet [4]. In line with theseevidence, another study showed that men with ischemic heartdisease had lower levels of testosterone than controls and thatserum testosterone levels were conversely correlated to thedegree of coronary atherosclerosis [4]. However, lipidmetabolism disorder plays a major role in atherosclerotic plaque formation, whether testosterone deficiency is a riskfactor for dyslipidemia or whether the replacement oftestosterone is useful for men ≥60 years old or not is still oneof the major challenges that have not been answeredconclusively.

MATERIAL AND METHODS

The present case-control study was conducted at the Department of Biochemistry, Index Medical College Hospital and Research Centre, Indore. After ethical clearance (Approval No-MU/Research/EC/PhD/2019/34)and informed consent,patients aged 30-60 years of male diabetic patients diagnosed on the basis of WHO norms [blood sugar (≥126mg/dl) and 2 hour post-prandial blood

sugar (≥200mg/dl)] were included as cases (n=181). However, patients with chronic diseases like CKD, CVD, MI, cancer, etc, patients with infectious diseases like TB, HIV and Hepatitis, patients with metabolic disorders like Hypothyroidismand non-willing patients were excluded. Normal healthy subjects as per WHO norms of fasting blood sugar (<126mg/dl) and 2-hour post-prandial blood sugar (<200mg/dl), were included as controls (n=181).

Along with family history and Medical history of subjects, demographic details including BMI, Waist to Hip ratio, Systolic Blood pressure, and Diastolic Blood pressure were measured. American Diabetic Association (ADA) criteria were followed forImpaired Fasting Glucose (IFG) as a fasting plasma glucose value of 100- 125 mg/dl (5.6-6.9 mmol/L) in the absence of a previous diagnosis of diabetes.

Minimum 5ml of blood will be drawn from each group under the aseptic condition in a suitable vial and used for the investigation of fasting blood glucose, Postprandial blood glucose, HBA1c, insulin, lipid profile and Testosterone, LH, FSH as per manual protocol.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA) for Windows program (21.0 version). When required, the continuous variables were presented by mean (Standard deviation) or range value and analysed using the Student t-test. The dichotomous variables were presented in number/frequency and analysed using the Chi-square test. The correlation was done using Spearman correlation analysis. All the analysis was done at 95% confidence level and p-value of < 0.05 or 0.001 was regarded as significant.

RESULTS

The mean age of the patients in the case and control groups were 45.51±5.35 and 43.79±12.69, respectively. A non-significant difference was observed [p=0.0938] between the mean age among the case and control groups of the study population.

The majority of the patients in the case groups were aged between 31-40 years [82(45.30%)], followed by 41-50 [56(30.94%)] and 18-30 [22(12.15%)]. However, in the control group, most of the patients were aged 31-40 years [77(42.54%)], followed by 41-50 [62(34.25%)] and 18-30 years [25(13.81%)]. A non-significant difference was observed [p=0.7831] between different ages among the study population's case and control groups. [FIGURE-1]

The mean SBP [142.72±25.36 and 127.35±22.68], DBP [83.16±13.42 and 80.46±14.25], WC [91.25±11.85 and 78.29±9.68], BMI [24.52±4.87 and 23.42±3.75]and Testicular volume [21.34±4.32 and 22.61±3.68] in the case and control groups showed a significant difference exceptDBP [p=0.0643]. [TABLE-1, FIGURE-2]

The FPG, HbA1c, total cholesterol, LDL, and triglycerides were significantly elevated in cases as compared to controls, except HDL and SHBG, showing non-significant differences. However, serum albumin (p<0.0001*) and free testosterone (p<0.0001*) were significantly lower in cases as compared to controls [TABLE-2, FIGURE-2].

As per serum gonadal hormones assessment, LH and FSH showed significantly elevated levels in cases as compared to controls.However, the insulin level was remarkably lower in cases than in controls (p<0.0001*) [TABLE-3].

The majority of the patients had 0-5 years[90(49.72%)] of T2DM duration, followed by 6-10 years [46(25.41%)] and 11-15 years [28(15.47%)] of enrolled patients in case groups [FIGURE-3]. The majority of the patients in the case groups were given Metformin + Glimepiride Medication [63(34.81%)] followed by Metformin + Glibenclamide [39(21.55%)] and Metformin Alone [28(15.47%)] respectively.

The spearman correlation between Testosterone and different parameters, and all the correlations showed negative correlation. However, Testosterone Vs. Testicular Volume (ml) [r=0.2981], Testosterone Vs. HDL cholesterol (mmol/l) [r=0.04884] and Testosterone Vs. Calculated Free Testosterone (mmol/L) [r=0.007494] respectively shows significant positive correlation.

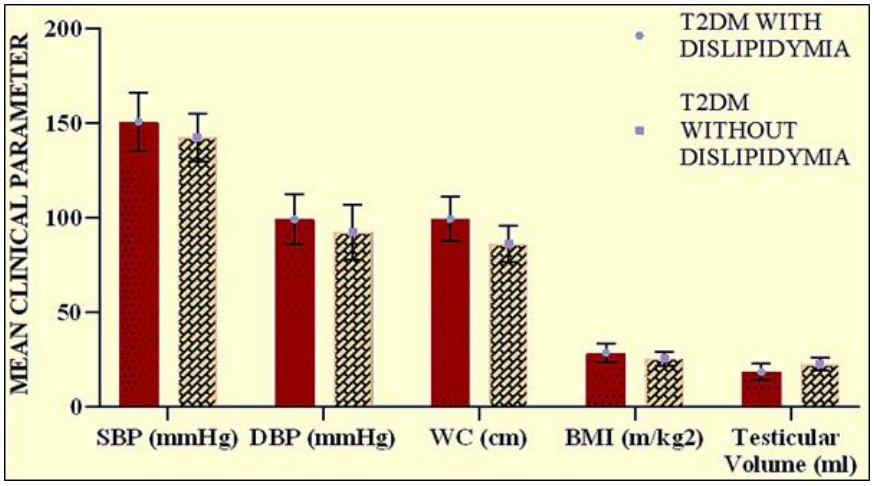

Figure1: Graphical representation of clinical parameter of the enrolled patients among the T2DM with dislipidymia and T2DM without dislipidymia groups

Table1: Clinical parameter of the Enrolled Patients among the T2DM with Dislipidymiaand T2DM without Dislipidymia Groups

Clinical Parameter	T2DM withDislipidymia [n=72]	T2DM withoutDislipidymia [n=109]	P-Value
	MEAN	MEAN	
SBP (mmHg)	150.71±15.34	142.33±12.64	t=4.006 p<0.0001*
DBP (mmHg)	99.14±13.41	92.41±14.27	t=3.180 p=0.0017*
WC (cm)	99.28±11.84	86.27±9.69	t=8.086 p<0.0001*
BMI (m/kg^2)	28.54±4.86	25.41±3.78	t=4.859 p<0.0001*
Testicular Volume (ml)	18.36±4.31	22.65±3.65	t=7.197 p<0.0001*

Student t-test, Significant

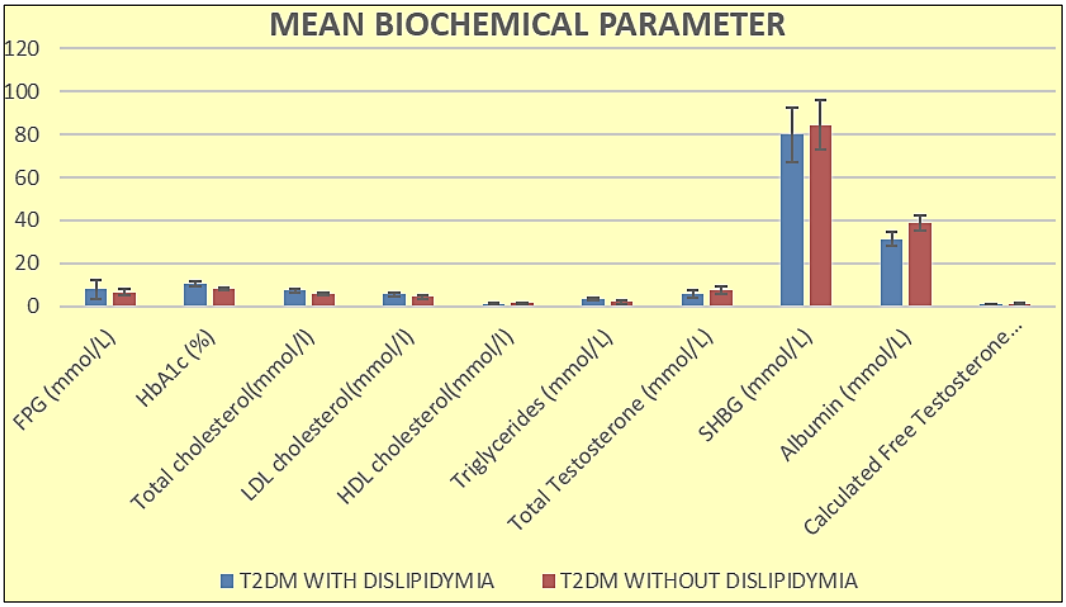

Figure2: Graphical representation of Biochemical parameters of the enrolled patients among the T2DM with dislipidymia and T2DM without dislipidymia groups

Table3: Serum Gonadal Hormones of the Enrolled Patients among the T2DM with Dislipidymiaand T2DM without Dislipidymia Groups

Serum Gonadal Hormones	T2DM withDislipidymia [n=72]		T2DM withoutDislipidymia [n=109]		P-value
	MEAN	SD	MEAN	SD	
LH (MIU/ml)	9.74	3.57	7.97	3.59	t=3.254 p=0.0014*
FSH (MIU/ml)	19.84	5.34	13.22	4.61	t=8.873 p<0.0001*
Insulin (IU/ml)	4.78	1.21	8.02	1.59	t=14.70 p<0.0001*

Student t-test, Significant

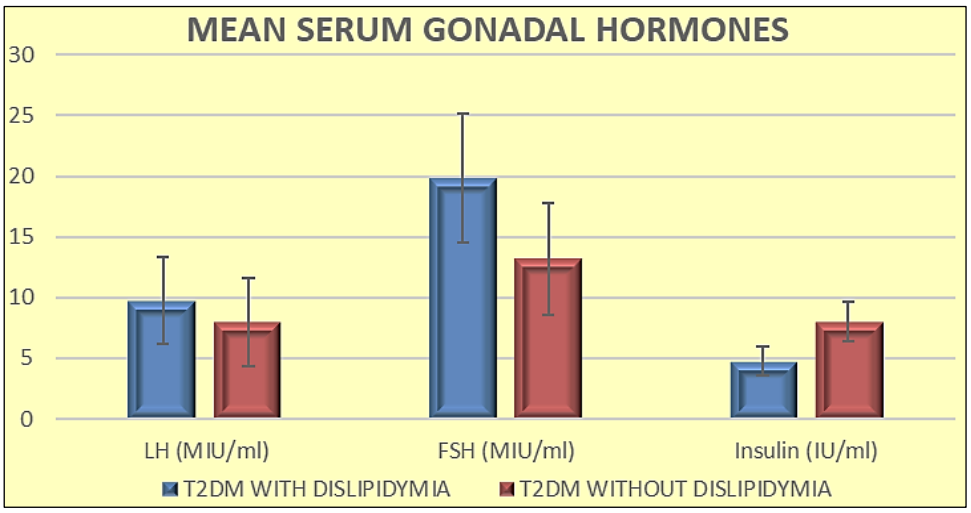

Figure3: Graphical Representation of Serum Gonadal Hormones of the Enrolled Patients among the T2DM with Dislipidymiaand T2DM without Dislipidymia Groups

DISCUSSION

In our study, the mean age of patients in the case and control groups was 45.51±5.35, and 43.79±12.3 respectively. We observed a non-significant difference [p=0.0938]. The majority of patients in the case groups were between the ages of 31 and 40 [82(45.30%)], followed by 41 to 50 [56(30.94%)] and 18 to 30 [22(12.15)]. However, the majority of patients in the control group were aged 31-40 [77(42.54%)], followed by 41-50 [62(34.25%)] and 18-30 [25(13.81%)]. A non-significant difference [p=0.7831] was noticed between the ages of the case and control groups in the study population. In contrast, Mattack N *et al*. [5] demonstrated that the majority of test group patients were aged 51 to 60 years. The group with diabetes had a mean age of 52.57 years, which was not substantially older than the control group [p=0.061]. In our study, the case group had a mean BMI of [28.54±4.86] and the control group had a mean BMI of [25.41±3.78], with a significant difference between the groups [p<0.0001*]. Similarly, Mattack N. *et al.* [5] observed a significant difference between the mean BMI of the test group [23.42±2.25] and the control group [25.598±2.148] [p<0.0001]. This result is consistent with those of Chandel*et al*. [6] and Shah *et al*. [7]. In our study, the systolic and diastolic blood pressure readings were [150.71±15.34; 142.33±12.64] [99.14±13.41; 92.41±14.27], and both SBP and DBP values were significantly higher in the case group compared to the control group [p<0.0001*; p=0.0017*] respectively. Significantly greater waist circumference was observed in the case group [99.2811.84] than in the control group [86.279.3] [p<0.0001*]. Similar to our research, Mattack N. *et al*. [5] observed that both systolic and diastolic blood pressure were significantly higher in the test group, with a significant difference between the two [p=0.007; p=0.02]. In contrast, Mattack N. *et al*. [5] found that the waist circumference was greater in the case group [82.82±10.38] and that the difference was statistically significant [p=0.0029]. Significantly greater testicular volume was seen in the control group compared to the experimental group [p<0.0001*]. The mean fasting plasma glucose in the case group was [7.85±4.59] and in the control group it was [6.64±1.45], with a significant difference between the two groups [p=0.0110*]. The mean HbA1C was determined and found to be statistically significant [p=0.0001*] between groups. Fasting blood glucose and glycated haemoglobin were significantly higher in the test group with a significant difference [p<0.0001], corroborated by Abate *et al*. [8] and Zhang *et al*. [9] According to the research of Ueshiba[10], males with metabolic syndrome whose testosterone levels were increased had a reduction in their fasting

blood glucose levels. Therefore, they concluded that administering testosterone improves insulin resistance. The mean total cholesterol (mmol/l) was greater in the case group [7.35±0.95] than in the control group [5.740.69], and this difference was statistically significant [p<0.0001*]. This finding is consistent with those of Andersson *et al*. [11] and Haffner *et al*. [12] in that LDL and triglycerides were significantly higher in cases as compared to controls, but HDL and SHBG were significantly elevated in control groups. SHBG is a primary driver of sex hormone clearance and modulates active testosterone availability in target tissues. Recent molecular epidemiologic investigations have demonstrated that genetically determined levels of SHBG are inversely linked with the incidence of type 2 diabetes, supporting the role of SHBG in its aetiology [13,14]. In addition, in the study conducted by Mattack N *et al*. [5], the mean blood total cholesterol and mean LDL cholesterol were considerably greater than in the control group. These findings are consistent with those of Samatha*et al*. [15] In the study by Mattack N *et al*. [5], the mean triglyceride level in the control group was 117mg/dL, which was considerably lower than the mean of 148.38mg/dL in the test group (p=0.023). Diabetes type 2 is frequently accompanied with dyslipidemia, which increases cardiovascular risks. Changes in triglyceride-rich lipoprotein metabolism play a crucial role in the development of this dyslipidemia. According to Krauss *et al*. [16], alterations include both an increase in hepatic VLDL secretion and a decrease in the clearance of VLDL and intestinally produced chylomicrons. This elevation in VLDL causes a surge in tiny, dense LDL particles. Low testosterone levels are associated with insulin resistance, hyperglycemia, hypertension, dyslipidemia, and an elevated risk of cardiovascular disease [17]. Similarly, Adrekani*et al*. [18] and Mattack N *et al*. [5] found that the mean serum total testosterone (TT) concentration of the control group was considerably higher than that of the test group [p<0.0001]. In our investigation, the mean total testosterone was significantly greater in the control group [p<0.0001*]. Low testosterone levels are associated with insulin resistance, hyperglycemia, hypertension, dyslipidemia and an elevated risk of cardiovascular disease [22]. The mean calculated free testosterone was significantly higher in control group [1.21±0.26] as compared to case group [0.84±0.12]. Similar to our study, Mattack N *et al*. [5] and Haffner *et al*. [12] noted calculated FT and found that the control group was significantly higher than that of the test group [p=0.0051].

Andersson *et al*. [11] could not identify any change in FT between the two groups, in contrast to our findings. In our investigation, LH and FSH levels in blood gonadal hormones were considerably increased in cases compared to controls. However, insulin levels in patients were significantly lower than in controls [p<0.0001]. The majority of enrolled patients in case groups had T2DM for 0-5 years [90(49.72%)], followed by 6-10 years [46(25.41%)] and 11-15 years [28(15.47%)]. The majority of patients in the case groups were administered Metformin + Glimepiride [63(34.81%)], followed by Metformin + Glibenclamide [39(21.55%)] and Metformin Alone [28(15.47%)]. In this investigation, age was found to be negatively linked with serum TT, cFTand SHBG. The spearman association between Testosterone and other factors revealed a negative correlation for all parameters. However, Testosterone vs Testicular Volume (ml) [r=0.2981], Testosterone versus HDL cholesterol(mmol/l) [r=0.04884] and Testosterone versus Calculated Free Testosterone (mmol/L) [r=0.007494] exhibit positive correlations. Similar to Vikan*et al*. [19] and Mattack N *et al*. [5] observed a negative correlation between Serum TT and cFT with respect to age and BMI. Through the action of aromatase, visceral fat accelerates the conversion of testosterone to oestrogen. This estrogen in turn inhibits the release of GnRH and LH, further reducing testosterone levels [20].

Low testosterone causes a loss in muscle mass and an increase in free fatty acids in circulation [21]. The development of insulin resistance andultimately, type 2 diabetes is mediated by free fatty acids [22]. In addition, it was observed that non-diabetic rats treated to subtherapeutic or supratherapeutic amounts of testosterone developed insulin resistance [21]. SHBG is more strongly related with metabolic syndrome in older men than TT, according to Chubb *et al*. [23] This indicates that the relationship between TT and metabolic syndrome may be secondary to the relationship between SHBG and metabolic syndrome. According to Wang *et al*. [24], low TT and SHBG independently predict the onset of diabetes, regardless of age, race, or weight, with SHBG being a greater predictor of type 2 diabetes. Similar to our findings, Mattack N. *et al*. [5] found a negative correlation between blood LDL cholesterol and TT and SHBG levels. HDL cholesterol associated positively with testosterone levels [r=0.04884]. Low testosterone levels are linked to visceral fat accumulation, metabolic syndrome, type 2 diabetes, elevated inflammatory cytokines, hyperlipidemia, and coagulation problems [24]. According to epidemiological evidence, testosterone levels are inversely associated with total cholesterol, LDL cholesterol, and triglycerides, and favourably associated with HDL cholesterol. Similarly, testosterone replacement therapy has been demonstrated to enhance the lipid profile in males with dyslipidemia. However, the precise method through which testosterone levels alter lipid profile remains unknown. Nonetheless, low testosterone can be regarded as a new cardiovascular risk factor in males [25] in light of the overwhelming evidence that supports this hypothesis.

CONCLUSION

This study reveals a significant association of dyslipidemia with hypogonadism in diabetic patients. Serum testosterone and SHBG levels were significantly lower in case group and testosterone vs. testicular volume, HDL cholesterol and calculated free testosterone showed positive correlation. This suggests that Low levels of testosterone are associated with visceral fat accumulation, metabolic syndrome, type 2 diabetes, increased inflammatory cytokines, hyperlipidemia and abnormalities of coagulation. However, small samples and the single centric study was the limitation of the study. Author recommended further multicentric study with a large sample size to increase the reliability and generalizability of the present findings.

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