



## ESTIMATION OF SERUM TESTOSTERONE & SHBG IN TYPE 2 DIABETES & THEIR CORRELATION WITH HbA1C & LIPID PROFILE IN TYPE 2 DIABETES CASES IN A TERTIARY CARE HOSPITAL IN KOLKATA.'

### Biochemistry

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### ABSTRACT

Type 2 diabetes mellitus is one of the major causes of mortality & morbidity worldwide & studies done in recent years has showed that androgen deficiency is associated with type 2 diabetes mellitus in male population. A case control study was done in Medical College & Hospital, Kolkata, Dept. Of Biochemistry with 180 male subjects of which 100 were type 2 diabetic, 80 were age matched healthy control. The diabetic group had significantly lower level of testosterone (9.14±3.14nmol/l) as compared to healthy control (20.61±7.35nmol/l). Same went with SHBG where diabetic group had significantly lower value (36.4±21.4 nmol/lit) than normal control (63.5±18.4 nmol/lit). Fasting blood sugar & glycated Hb were negatively correlated with free testosterone and SHBG. Total cholesterol & LDL were negatively correlated with total testosterone. To precisely establish the role of testosterone in type 2 diabetes, a more elaborate study with larger study group should be done & also effect of therapeutic testosterone should be seen in large cohort.

### KEYWORDS

Diabetes, Testosterone, SHBG, Cholesterol, HbA1C.

### INTRODUCTION :

Diabetes mellitus, more importantly type 2 diabetes mellitus is one of the major causes of mortality & morbidity worldwide, International diabetes federation estimates that 9% of the world's population (415 million) has diabetes in 2015, and this number is predicted to rise to 10% (642 million) by 2040, of whom 91% are diagnosed with type 2 DM (1). It is a heterogeneous & bipolar disease characterized by impaired insulin action and abnormal secretion and is usually associated with obesity, insulin resistance, and increased hepatic glucose production.

Insulin plays the most important role in regulating glucose homeostasis in our body through a highly organised fashion, which include promoting glucose uptake in peripheral tissues such as muscle and fat, decreasing hepatic glycogenolysis, and regulating lipid metabolism (2). Hormones like glucagon, growth hormone, cortisol, catecholamines, insulin like growth factor-1 also have a role in maintaining glucose homeostasis (3). Studies done in recent years has showed that androgen deficiency plays important role in the general health of men & also in certain common systemic illnesses, type 2 diabetes being one of them.

In general, most case control done worldwide has shown that there is evidence of low testosterone and SHBG associated with proneness to diabetes in both human study population as well as orchietomized rats, some studies have also reported higher endogenous testosterone concentrations to be associated with more prognostically good cardiovascular profile, like higher HDL cholesterol and lower triglyceride concentrations, blood glucose, blood pressure and body mass index, so overall lower incidence of metabolic syndrome. But, on the other hand, high doses of exogenous testosterone or other anabolic steroids have been associated with adverse health outcomes, including sudden cardiac death and hepatic disease (4). So it is an issue of debate whether low testosterone is involved in the pathogenesis of diabetes or it is just one of the biomarker coexisting with diabetes. There is a shortage of research in this field in India. Therefore, the objective of the present study is to evaluate the serum levels of testosterone & SHBG in type 2 diabetic men and find any significant correlation between the different parameters like HbA1C & lipid profile in those people in a tertiary care hospital in eastern India, i.e., in Kolkata.

### MATERIALS & METHODS :

The present study was carried out in the Department of Biochemistry of Medical College and Hospital, Kolkata from October 2018 to January 2019. This case control study was conducted in a study population comprising of 180 male subjects with age ranging from 27 to 70 years who sought health care in the endocrinology and medicine outpatient departments in Medical College and hospital, Kolkata and also the male attendants & male technicians & healthy non diabetic

patient parties who accompanied them. Of these 180 subjects, 100 were type 2 diabetic men and 80 were healthy non diabetic men.

### Exclusion criteria:

Subjects with documented hypogonadism, liver failure, renal failure, those with any history of acute illness, alcohol consumption or smoking and those under sex hormone replacement therapy or any medication (e.g., diuretic, statins, glucocorticoids, danazol) that could have an influence on lipid metabolism, blood pressure or body composition were excluded from the study. Patients with history of hypothyroidism or hyperthyroidism, acromegaly, cushing's disease & hyperprolactinemia were also excluded as these factors tend to alter body SHBG level.

Taking all aseptic and antiseptic precautions, 5ml of blood was drawn from the median cubital vein. Fasting samples were used for all the investigations. Separated serum was used and tests were done within eight hours of collection, or else the samples were preserved at -20°C for future use. Estimations of serum fasting glucose (5), total cholesterol (6), triglyceride (7) and HDL (8), serum albumin (9) and glycated haemoglobin (10) were done using Cobas autoanalyzer. VLDL and LDL were calculated using Friedwald's formula (11). Estimations of serum total testosterone (12) and serum SHBG (13) were done using Advia Centaur CLIA.

Estimation of serum free testosterone was done using the Vermeulen's formula using the values of serum total testosterone, SHBG & albumin (14).

After all the biochemical estimations were done, the results obtained were statistically analysed and compared between diabetic and non diabetic groups participating in the study. Normality were decided by Kolmogorov smirnov Goodness of fit test, the data were found to be not in normal distribution. So, Mann Whitney U-test were used whenever applicable to analyse differences in baseline characteristics between the control and the test groups. Correlations were observed using Spearman correlation coefficient. The results were considered significant when the probability (p-value) was less than 0.05 % of the observed values of "t" at a particular degree of freedom. Statistical analysis was done using SPSS software.

### RESULTS :

The control group comprised of 80 healthy non diabetic men ( age group 27 – 70 years), and case group comprised of 100 diabetic men ( age group 27-70 years), both were chosen keeping in mind the inclusion- exclusion criterias.

Comparison was done between case and control group, the factors

compared were lipid profile,fasting blood glucose,serum free testosterone, SHBG, HbA1C, body mass index,waist circumference & waist hip ratio.

Serum free testosterone & SHBG were found to be significantly lower in diabetic cases than the non diabetic control population. There were significantly more prevalence of diabetes in men with free testosterone less than . Men from the diabetic group was found to be having higher BMI, waist circumference, WHR,systolic and diastolic BP,FBG,total cholesterol,LDL,VLDL,TG & HbA1C.

**TABLE -1: Comparison of different characteristics between test & control group :-**

Parameters	Control group	Diabetic group	p- value
Age ( years)	45.75+11.37	56.24+10.48	0.063
BMI (kg/m2)	22.46+2.15	26.574+2.34	< 0.0001
Waist circumference (cm)	74.35+9.25	83.24+9.34	0.001
WHR	0.87+0.04	0.95+0.03	< 0.0001
Systolic BP(mm Hg)	126 +- 15	145+-10	0.004
Diastolic BP(mmHg)	80+-8	86+-7	0.03
FBG ( mg /dl)	88.7+-11.5	190.2+-20.5	<0.0001
Total Cholesterol(mg/dl)	155.4 +- 30.7	195.6+-20.9	<0.0001
LDL cholesterol (mg/dl)	80.4+-3.7	130.7+-11.8	<0.0001
VLDL (mg/dl)	22.6+-6.5	30.7+-11.1	0.03
TG (mg/dl)	115+-25.6	149.5+-24	0.003
HbA1C (%)	3.9+-1.1	8.3+-1.2	<0.0001
Total testosterone(nmol/L)	20.61+-7.35	9.14+-3.14	<0.0001
Serum SHBG(nmol/l)	63.5+-18.4	36.4+-21.4	<0.0001
Free testosterone(nmol/l)	0.253+-0.134	0.201+-0.127	0.003

**Table 2 : interrelation of low testosterone level & number of type 2 diabetic men –**

Serum testosterone levels	Total cases	Type 2 DM present Frequency (percentage)	Type2DM absent Frequency (percentage)	Odds ratio	95% Confidence interval	p - value
TT ≤ 7.5 nmol/l	60	52(46)	8(7)	6	2.2-14.05	0.002
FT ≤ 0.211 nmol/l	52	40(36)	12(11)	6.3	2.9-15.4	0.0005

This table shows that men with a total testosterone of less than 7.5 nmol/l has higher chances of type 2 diabetes.

There was strong negative correlation of age with free & total testosterone,waist hip ratios were negatively correlated with free & total testosterone,fasting blood sugar levels & glycated haemoglobin levels were negatively correlated with free testosterone and SHBG.

Among the lipid profile parameters , total cholesterol as well as LDL were negatively correlated with total testosterone.

**Table 3 : Correlation between different parameters in type 2 diabetic men –**

Parameters	Serum total testosterone		Serum free testosterone		Serum SHBG	
	Correlation Coefficient	p-value	Correlation Coefficient	p-value	Correlation Coefficient	p-value
AGE	-0.71 <0.0001	<0.0001	-0.57	< 0.0001	-0.25	0.18

BMI	-0.211	0.202	-0.174	0.32	0.06	0.82
WHR	-0.423	0.0034	-0.423	0.0075	-0.04	0.91
FBG	-0.45	0.0011	-0.43	0.0071	-0.29	0.091
HbA1 C	-0.24	0.0975	-0.15	0.51	-0.35	0.04
Serum CHOL	-0.32	0.0195	-0.214	0.22	-0.49	0.0052
LDL CHOL	-0.35	0.0154	-0.198	0.23	-0.52	0.0025
VLDL CHOL	-0.02	0.92	-0.007	0.98	-0.22	0.310
HDL CHOL	0.2	0.55	0.02	0.90	0.28	0.09
Serum TG	-0.04	0.86	-0.007	0.88	-0.38	0.04

**DISCUSSION:**

In this study, it was found that in the diabetic men group most of the patients were of the age group 55-65 y. The mean age of the diabetic group was 58.67 yr. The test group which is the group of diagnosed type 2 diabetic patients had mean BMI of 28.62 Kg/m2 and WHR of 0.96 ,these values were significantly higher than in the control group with a p-value of <0.0001. This finding corroborates with the findings of (15)(16)Dhindsa et al,Shah et al. According to Yang et al,(17)the visceral obesity shown by the raised WHR can be explained by insulin resistance which is a characteristic of type 2 diabetes. Both systolic and diastolic BP were significantly higher in the test group with p values of 0.007and 0.02 respectively. (18)

The mean fasting serum glucose in the test group and control group were 190.2 mg/dL and 88.7 mg/dL respectively. Glycated haemoglobin too was significantly higher in the test group with a p-value of <0.0001.

The mean serum total cholesterol and the mean LDL cholesterol in the test group were 193.5 mg/dL and 126.7mg/dL respectively which were significantly higher than in the test group with p-values of <0.0001 and 0.0004 respectively.(19) The mean VLDL cholesterol in the control group was 22.6mg/dL which was significantly lower than that of the test group with a mean of 30.7mg/dL (p=<0.001). The mean triglyceride level in the control group was 115mg/dL which was significantly lower than in the test group with a mean of 149.5 mg/dL.

Dyslipidemia is often associated with type 2 diabetes and therefore increases cardiovascular risks. Development of this dyslipidemia can be attributed to altered metabolism of triglyceride rich lipoproteins. There is alteration in hepatic secretion of VLDL and impaired clearance of VLDL and intestinally derived chylomicrons. This increased VLDL ultimately increases small dense oxidized LDL particles.(20) These findings were in collaboration with studies of Samatha P et al & Krauss RM et al.

Mean serum total testosterone (TT) of the non diabetic group was 20.61nmol/L which was significantly higher than that of the diabetic group 9.14 nmol/l with a p-value of <0.0001 (21). Low levels of testosterone has been seen to be associated with with insulin resistance and plays important role in hyperglycemia, hypertension, dyslipidemia and an increased risk of vascular diseases(22). Calculated FT (Mean=0.253 nmol/L) of the control group was found to be significantly higher than that of the test group (Mean=0.201nmol/L) with a p-value of 0.003.(23) In this study, 40(36%) of the subjects having cFT less than 0.211 nmol/L suffered from DM as depicted in [Table/Fig-2. The odds ratio was 6.63 and the p value was extremely significant (p=0.0005).(24) These findings were in collaboration with studies of Adrekani MA et al, Traish AM et al, Haffner SM et al & Kapoor D et al.

The mean serum SHBG of the control group was 63.5nmol/L whereas that of the test group was 36.4nmol/L. The difference between the two groups was extremely significant with a p-value of <0.0001 . (25)SHBG is a major determinant of clearance of sex hormones and regulates the availability of active testosterone at target tissues. Recent studies in the field of molecular biology and epidemiology have shown that genetically determined levels of SHBG were inversely associated with the risk of type 2 DM, lending support to the role of SHBG in its development(26) . Serum SHBG was found to be negatively correlated with age but the correlation was not significant. BMI correlated

inversely with TT, cFT and SHBG but the correlations were not significant. But, WHR correlated inversely with TT and cFT in a significant manner. Whereas BMI is a measure of whole body obesity, WHR is a measure of central obesity & related to cardiovascular diseases. When there is increased visceral obesity there is increased break down of testosterone to estrogen by the action of aromatase. This estrogen in turn suppresses the release of GnRH and LH thereby further decreasing the levels of testosterone.(27)(28). These findings were in collaboration with studies of Andersson B et al, Goto A et al, Gupta R et al & Mohr BA et al.

As shown in [Table/Fig-3], FBG correlated inversely with both TT and cFT, the association being stronger with TT. According to some studies, the fasting blood glucose improved in men with metabolic syndrome after administration of testosterone in a Japanese male population. Thus, they concluded that testosterone administration improves insulin resistance. Their study explained that low testosterone leads to a decrease in muscle mass and an increase in circulating free fatty acids. This free fatty acids mediate the development of insulin resistance and ultimately the development of overt type 2 DM. (29)

Recent studies suggest that testosterone levels are associated negatively with total cholesterol, LDL cholesterol and triglycerides, and positively with HDL cholesterol. Similarly, trials of testosterone replacement have shown improvement in the lipid profile of dyslipidemic men. The association of testosterone and SHBG with an altered lipid profile is partly secondary to abdominal fat accumulation, but there also appears to be an independent relationship between low levels of testosterone and hyperinsulinemia and dyslipidemia. Low levels of testosterone have also predicted worsening abdominal obesity. Testosterone itself may have a central or permissive role in the pathogenesis of the metabolic syndrome and type 2 diabetes by increasing skeletal muscle tissue and decreasing abdominal obesity consequently improving insulin sensitivity. Overall or abdominal obesity increases glucocorticoid turnover and production, which disturbs regulation of the hypothalamic- pituitary-adrenal axis and may contribute to mild hypogonadism in men(30).

Testosterone itself may contribute to the pathogenesis of insulin resistance and diabetes by increasing skeletal muscle mass at the expense of fat mass and decreasing abdominal obesity through inhibition of lipoprotein lipase activity. Low levels of free or total testosterone have been consistently associated with overall or abdominal obesity, insulin resistance or hyperinsulinemia, hyperglycemia, and dyslipidemia.

### Conclusion :

In this study we found that testosterone levels correlated inversely with diabetic status in men, so there may be role of testosterone in development of type 2 diabetes. Serum testosterone and SHBG levels were significantly lower in type 2 diabetic men and levels of total testosterone correlated inversely with total cholesterol and LDL cholesterol. According to some studies like S. W. Ahn et al testosterone deficiency in mice resulted in Chronic perturbations in glucose metabolism and hyperinsulinemia leading to impairment of the Leydig cell steroidogenesis via insulin-mediated induction of DAX1 (dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1), thus causing diabetes in mice (31).

To precisely establish the role of testosterone in type 2 diabetes, a more elaborate study with larger study group should be done, as the limitation of our study was small study group.

Also elaborate cohort can be done to see the effect of therapeutic testosterone in diabetic male population.

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