Role of Serum Total Testosterone and High Sensitivity C-Reactive Protein Levels in Type 2 Diabetic Males

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ABSTRACT
Introduction: Diabetes Mellitus (DM) is a chronic metabolic disorder associated with low level of Total Testosterone (TT) and Hypogonadism. Estimation of High sensitivity C-Reactive Protein (Hs-CRP) is useful for assessing the cardiovascular risk. Aim: To estimate serum TT and Hs-CRP levels in Diabetic patients and hence, the association of Diabetes with Erectile Dysfunction (ED) and Cardiovascular risk.
Materials and Methods: This was a case-control study with a case-control design, conducted at a tertiary care centre on 100 subjects, 60 DM males as cases and 40 healthy male volunteers of same age, as controls, from June 2016 to May 2017. All the relevant demographic and clinical details i.e. age, height, weight, body mass index (BMI), Systolic and Diastolic Blood Pressure were noted for all subjects. All relevant tests i.e. serum TT by Electrochemiluminescence immunoassay analyser Enzyme Chemiluminescence Immunoassay (ECLIA) method, serum Hs-CRP by immunoturbidimetry method, fasting and post-prandial glucose measurements, HbA1c were all done and results were tabulated and analysed. Chi-square test was used to calculate p-value. Analysis of Variance (ANOVA) descriptive analysis was used to find out the mean, standard deviation, standard error and 95% confidence interval.
Results: The mean of HbA1c among the case group was 8.185% and 5.004% among the controls. The mean value of TT among cases was 3.891 ng/dL, which was significantly reduced (p-value <0.005) as compared to healthy controls i.e. 5.339 ng/dL. Low level of TT (below 3.5 ng/dL) was seen in 45% of the cases among the diabetic study population which was statistically significant (p-value <0.0001). Seventy five percent of diabetics had erectile dysfunction. The mean value of Hs-CRP among diabetic population was 3.875 and controls were 1.457 mg/L (p-value 0.001). Based on Hs-CRP values, the subjects were sub-categorised into low, intermediate and high risk for CVD. A 31.7% of diabetics were in the intermediate group and 68.3% were in the high risk group for CVD.
Conclusion: There was significant reduction in TT in Type 2 Diabetic men. Patients with low TT had increased Hs-CRP value among which 75% falling under high risk category and 25% under intermediate risk category. All the cases had serum Hs-CRP value of either intermediate or high risk category. Low level of TT was also associated with ED and PME.

INTRODUCTION
The DM is a chronic metabolic disorder, due to insufficient insulin secretion, action or both, leading to hyperglycaemia and affects the carbohydrate, protein and lipid metabolism which may lead to micro and macro vascular complications [1]. Testosterone is the principal androgen in men [2]. There is an inverse relationship in between testosterone and insulin resistance in Type 2 DM which is a strong risk factor for both micro and macro vascular complications, people with low TT levels are prone for developing type 2 diabetes, moreover, testosterone replacement improve insulin sensitivity [3]. Hypogonadism is seen in few diabetic patients, even without associated ED. Testosterone values below 3.5 ng/dL is considered as low and defined as hypogonadism [2]. The Hs-CRP is a marker of endothelial dysfunction in turn responsible for inflammatory process in atherosclerosis. About one-third of type 2 diabetic male patients have low serum Testosterone level but Hs-CRP was found to be high. It helps to assess the future cardiovascular risk and to prevent mortality and reduce morbidity. Hs-CRP values of <1, 1-3 and >3 mg/L is categorised as low, intermediate and high cardiac risk [1]. There was only one study available [4] covering both DM and sexual function, sub categorisation of Hs-CRP with DM in assessing CVS risk adds benefit in the follow-up of DM patients. The author estimated the TT, Hs-CRP levels in both DM cases and controls and checked the association of Hs-CRP with cardio vascular risk. HbA1c was done to decide the glycaemic status Association of TT with ED and Premature Ejaculation (PME) was also assessed.

MATERIALS AND METHODS
This case-control study was conducted on 60 DM males as cases and 40 healthy male volunteers of same age group during their routine medical health check-up as controls, in a tertiary care centre (Chennai Medical College Hospital and Research Center) from June-2016 to May-2017. Ethical clearance from hospital Ethical Committee Approval no. (133/26.11.2015) was obtained. Inclusion criteria: Diabetic and healthy volunteer males aged 40-70 years , who came to the selected tertiary care centre during the study time period, were included in the study after taking written informed consent. Exclusion criteria: Those patients who had acute inflammatory disorders, liver disease, cardiac illness, h/o alcoholism, known case of hypogonadism, diabetics, on testosterone replacement therapy were excluded.
Study Procedure
A 5 mL of venous blood was collected in the early morning between 6-8 am, after overnight fasting and two hours postprandial plasma glucose measurements were taken. Serum TT (ECLIA- Cobas e immunoassay analyser- 20-49 years- males-2.49 to 8.36 ng/mL, ≥50 years-1.93 to 7.40 ng/mL) [1], serum
Hs-CRP was estimated (immunoturbidimetry- Mind ray auto analyser- Ref-range:0-3 mg/L) [1]. Other parameters like plasma fasting: (Hexokinase method, reference range: 70-100 mg/dL [5]), post prandial glucose: (Hexokinase method, reference range: <140 mg/ dL) [5] and HbA1c: (Immuno turbidimetric method, reference range: non diabetic-4.5-5.6, prediabetic-5.7-6.4, diabetic ≥6.5) [5] were analysed by Cobas c 311 chemistry analyser. Diabetic status of the participants (cases) were confirmed by ADA [5] criteria with fasting plasma glucose of 126 mg/dL (≥7mmol/L), 2 hours plasma glucose of 200 mg/dL (≥11.1 mmol/L) or HbA1c of (≥6.5% 48 mmol/mol) [5]. Height, weight, systolic and diastolic blood pressure were also recorded for all the study population.

STATISTICAL ANALYSIS
The statistical analysis of the measured values was done using the latest software version Statistical Package for Social Sciences (SPSS) version 22.0. The parameters were grouped according to age group and cross tables were tested with Chi-square test. When the p-value is less than 0.005, it is taken as significant. ANOVA descriptive analysis was used to find out the mean, standard deviation, standard error and 95% confidence interval for mean.

RESULTS
A 60 DM cases and 40 controls were included in the study. Age distribution among the diabetic cases and the controls was comparable as given in [Table/Fig-1]. BMI among the DM cases and normal controls were statistically insignificant, evidenced by Chi-square value -1.667 and p-value-0.644. The mean value of fasting and postprandial plasma glucose of present cases and controls are given in [Table/Fig-2].

In the present study, mean of HbA1c among the case group was 8.185 and 5.004 among the controls [Table/Fig-3]. The glycaemic control was not achieved in all the cases and it was well within normal limits in controls. Among 60 diabetics 31.7% had hypertension which was statistically insignificant (p-value=0.084).

The mean of TT in DM cases were 3.891. It is significantly reduced when compared to non diabetic controls (mean-5.339). It is statistically significant by the p-value of 0.005. Values below 3.5 ng/dL is considered as low and define as hypogonadism and was observed in 45% of the cases [Table/Fig-4] among the diabetic study population which is statistically significant. (p-value <0.0001) whereas 7.5% in case of non-diabetic control population.

One-way ANOVA analysis of testosterone values among various age group showed low mean testosterone below the cut-off value in 8th decade [Table/Fig-5]. As it was only a single case in that decade. The mean value in the 6th decade was close to the arbitrary cut-off value.

A 75% of case population (Diabetic) has ED [Table/Fig-6] and remaining 25% population doesn’t have ED, whereas 5% of non diabetic controls have ED but 95% doesn’t have ED. It is evidenced as statistically significant (p-value <0.001).

The PME among cases was 30% [Table/Fig-7] which is considered statistically significant as evidenced by the p-value of 0.042.

The mean of Hs-CRP among the diabetics was- 3.875 mg/L [Table/ Fig-8] and non diabetic controls was 1.457 mg/L which is significant, as evidenced by the p-value of 0.001 (below 0.05). It indicates that
Hs-CRP levels are grossly elevated in diabetic population [Table/Fig-8] compared to controls.

All the decades of diabetic patients have the mean value of Hs-CRP above 3 mg/L which is the high risk for cardiovascular diseases, statistically well-established [Table/Fig-8].

The mean value of Hs-CRP related to duration of DM is in the risk category of above 3 mg/L, except 15-20 years duration because of smaller population in that category. Among DM patients 31.7%, (19) were in the intermediate group and 68.3% (41) [Table/Fig-9] were in the high risk group for CVD risk. It is statistically significant as evidenced by p-value of 0.005.

In the present study, serum TT in diabetic male patients was lower when compared to control group; whereas in controls, it was within reference range. Type-2 DM is associated with low testosterone values and vice versa. The reference range of normal testosterone is 2.6-10 ng/dL [1]. Low testosterone when symptomatic, it is associated with symptoms of hypogonadism, mainly ED and PME. All the diabetic patients with DM type 2 have low serum free testosterone level. It is also associated with Hs-CRP levels [6].

The Hs-CRP is superior in assessing the risk of cardiovascular diseases in diabetics. Chejara RS et al., says that diabetic patients have low serum testosterone, also have high Hs-CRP concentration similar to the present study [2]. Mean of Hs-CRP is 3.875 mg/L among diabetic patients and 1.457 mg/L among controls, usual normal reference range of Hs-CRP is <1.5 mg /L. It may go up to 2 mg/L in healthy normal individuals.

The Hs-CRP among the diabetics is elevated than non diabetic controls which is significant by the p-value of 0.001 (<0.05). All the decades of diabetic patients have the mean value of Hs-CRP above 3 mg/L which is also statistically well established.

The mean value of Hs-CRP related to age groups in cases. Hs-CRP: High sensitivity C-reactive protein; p-value calculated by ANOVA test; p-value <0.001

The mean of Hs-CRP is 3.875 mg/L which is also statistically well established. Mean of Hs-CRP is 3.875 mg/L in the intermediate group and 68.3% are in the high risk group for CVD. It is statistically significant as evidenced by p-value <0.005.

In present study, testosterone has a positive correlation with Triglyceride (TAG) and VLDL cholesterol and negative correlation with Age, BMI, Total Cholesterol/High Density Lipoprotein (TC/ HDL) ratio; Malik PK and Rani A in their study confirmed that there is a correlation of BMI which has significant effect on serum testosterone levels [9]. Patients with higher BMI had negative correlation with testosterone [1]. ED was present in 75% of the cases, whereas five percent in the controls in present study. The prevalence of ED in diabetic males was between 30-90% [10]. This correlates with results of the present study.

A 31% of ED patients also have PME. Among cases with ED, 30% of them (18 out of 45) had low testosterone in the present study. Hadeed NN et al., also concluded that hypotestosteronemia was found in 36% of type 2 diabetic males with ED [11]. This result correlates with this study. Isidori AM et al., meta-analysis also demonstrated that approximately one-third of men with ED have androgen deficiency [12]. The 45% (27 out of 45) patients with ED had testosterone above the cut off value. Cheung KK et al., study says that testosterone levels can be low in the presence of inter current infection, infarction and injury including the postsurgical period and can be higher the morning after sexual intercourse [13].

In present study, testosterone has a positive correlation with Hs-CRP risk with diabetic and non diabetic group. Chi-square, p-value=0.005

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**DISCUSSION**

In the present study, serum TT in diabetic male patients was lower when compared to control group; whereas in controls, it was within reference range. Type-2 DM is associated with low testosterone values and vice versa. The reference range of normal testosterone is 2.6-10 ng/dL [1]. Values below 3.5 ng/dL is considered as low and defined as hypogonadism [2]. There is also an age dependent fall of Testosterone which is about 0.5-2 % per year from 4th decade onwards [1]. Low testosterone when symptomatic, it is associated with ED, PME, change in mood and loss of libido [5].

The mean of TT was 3.891 in diabetic cases and 5.339 in control; whereas in controls, it was within reference range. Type-2 DM is associated with low testosterone levels or Hs-CRP could not be studied. Lastly, 1:1 ratio of cases and controls could not be obtained.

**CONCLUSION(S)**

The present study revealed that low testosterone is associated with DM with a definition of <3.5 ng/dL, most of the diabetic has symptoms of hypogonadism, mainly ED and PME. All the diabetic males may undergo hormonal study evaluation in addition to routine

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**Table/Fig-7:** PME between cases and controls. Chi-square 4.15, p-value=0.042; PME: Premature ejaculation; total N=100

**Table/Fig-8:** Mean of Hs-CRP in regard to age groups in cases. Hs-CRP: High sensitivity C-reactive protein; p-value calculated by ANOVA test; p-value <0.001

<table>
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<tr>
<th>Age groups (years)</th>
<th>n</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
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<th>Maximum</th>
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<td>&lt;40</td>
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<td>2.6</td>
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<td>10</td>
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<td>0.3109</td>
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<td>15.0</td>
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<tr>
<td>60-69</td>
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<td>1.9540</td>
<td>0.5045</td>
<td>1.8</td>
<td>9.7</td>
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<tr>
<td>70-79</td>
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<td>3.200</td>
<td>-</td>
<td>-</td>
<td>3.2</td>
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</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>3.875</td>
<td>2.0566</td>
<td>0.2654</td>
<td>1.7</td>
<td>15.0</td>
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**Table/Fig-9:** Association of Hs-CRP risk with diabetic and non diabetic group. Chi-square, p-value=0.005

<table>
<thead>
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<th>Hs-CRP (mg/L)</th>
<th>Values</th>
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<th>Control (n=40)</th>
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<td>15.0%</td>
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<td>22</td>
<td>41</td>
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<tr>
<td>(moderate risk group)</td>
<td>% within gp</td>
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<td>55.0%</td>
<td>41.0%</td>
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<td>&gt;3</td>
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<td>44</td>
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<tr>
<td>(high risk group)</td>
<td>% within gp</td>
<td>68.3%</td>
<td>7.5%</td>
<td>44.0%</td>
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blood parameters to find out the prevalence of low testosterone and in turn prevent future macro vascular complications. With the Hs-CRP cardiovascular risk stratification about 70% of cases had high and 30% of them had an intermediate risk for future CVEs. Hence, Hs-CRP can be estimated in all cases of diabetics at an earlier date to assess the risk of cardiovascular diseases.

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REFERENCES