TO STUDY THE RELATIONSHIP BETWEEN SERUM TESTOSTERONE, HSCRP AND CAROTID Atherosclerosis IN MEN FROM NORTHWEST PUNJAB WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT

BACKGROUND

The average life expectancy of Women is at least five years more than Men across all age groups and in most nations. Traditionally, this difference has been attributed to the protective role played by Oestrogen in Women. However, the role of Serum Testosterone has not yet been as extensively evaluated. Initially it was believed that serum testosterone has no protective role and recent studies have provided evidence contrary to that assumption.

The objective is to study the relationship between Serum hsCRP, Testosterone and Carotid Atherosclerosis in Men with Type 2 Diabetes Mellitus.

MATERIALS AND METHODS

We randomly assessed 100 patients of type 2 Diabetes Mellitus attending various OPDs and wards of the hospital for levels of Serum Testosterone. We then proceeded to compare the various atherosclerotic markers between the two groups (with normal Serum Testosterone vs. Low Serum Testosterone). The results were then statistically analysed.

RESULTS

Prevalence of Low Serum Testosterone was 34% in our study. A higher BMI (28 +/- 2.5 kg/m²) seen in the Low Serum Testosterone group versus the normal Testosterone group (25.9 +/- 2.6 kg/m²). Increased Serum Cholesterol seen in the Low Serum Testosterone group (191.6 +/- 50.5 mg/dL) versus the normal Serum Testosterone (172 +/- 39.1 mg/dL) group. Raised Triglycerides seen in the Low Serum Testosterone group (157.1 +/- 58.7 mg/dL) than the normal Serum Testosterone group (137.6 +/- 31.9 mg/dL). Non-HDL Cholesterol in the Low Serum Testosterone group (155.2 +/- 49.1 mg/dL) was higher compared to the normal Testosterone Group (134.4 +/- 38 mg/dL). HbA1c levels were marginally higher in the Low Serum Testosterone group (7.9 +/- 1.1%) when compared to the normal Testosterone Group (7.5 +/- 0.9%). Increased hsCRP levels were seen in the Low Serum Testosterone group (3.2 +/- 1.0 mg/dL) than in the Low Serum Testosterone Group (1.9 +/- 0.8 mg/dL). CIMT in the Low Serum Testosterone group (0.82 +/- 0.1 mm) was significantly more than the normal Testosterone Group (0.65 +/- 0.1 mm). IHD was more frequent in the Low Serum Testosterone group (29%) versus the normal Testosterone Group (20%).

CONCLUSION

To conclude, a Low Serum Testosterone can serve as an adverse prognostic marker contributing to an increased atherosclerotic burden. Serum Testosterone should be screened for in all newly diagnosed patients of Type 2 Diabetes Mellitus.

KEYWORDS

CIMT, Intima Media Thickness, HbA1c, Ischaemic Heart Disease, Cerebrovascular Accident.


Financial or Other, Competing Interest: None.
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DOI: 10.14260/jemds/2017/411

BACKGROUND

The average life expectancy of Women is at least five years more than Men across all age groups and in most nations.

Traditionally, this difference has been attributed to the protective role played by Oestrogen in Women. However, the role of Serum Testosterone has not yet been as extensively evaluated. Initially it was believed that serum testosterone has no protective role and recent studies have provided evidence contrary to that assumption.

Aims and Objectives

To study the relationship between Serum hsCRP, Testosterone and Carotid Atherosclerosis in Men with Type 2 Diabetes Mellitus.

MATERIALS AND METHODS

The present study was conducted in 100 cases attending various OPD/ Indoor admissions of Guru Nanak Dev Hospital,
Amritsar and allied group of hospitals attached to Government Medical College, Amritsar after obtaining an informed consent. Diagnosis of Diabetes was made as per American Diabetes Association criteria 2014.(1) Detailed history and thorough physical examination were done. Routine investigations like Haemoglobin, TLC, DLC, Urea, Creatinine, S. Bilirubin, SGOT, SGPT, Total Serum Protein, Serum Albumin, HbA1c, Lipid Profile, ECG, Fasting and Random Blood Sugar were done. Serum hsCRP was measured by Quantra CRP US. It is a turbidimetric immunoassay for ultrasensitive determination of C-Reactive protein based on the process of latex agglutination.(2) Serum Testosterone was measured using Testosterone ELISA ALPCO kit for quantitative determination.(3)

Blood pressure measurements were obtained after minimum 5 minutes of rest in supine or semi-recumbent position and value obtained to the nearest 2 mm of mercury mark. Measurements for testosterone were obtained after overnight fasting, venous sample was obtained between 8:00 a.m. to 11:00 a.m. and stored at 4°C. Test for HbA1c, lipid profile, and serum C-reactive protein was performed. Carotid Doppler was used to measure degree of intimal thickness.

**Inclusion Criteria**
- Diabetes Mellitus type 2 patients.
- Male patient.
- Positive screening questionnaire for androgen deficiency.

**Exclusion Criteria**
- Diabetes mellitus type 1.
- Female patient.
- Renal or hepatic disease, infarction, systemic inflammation and infection.
- Secondary hypogonadism.
- Treatment by testosterone or testosterone replacement therapy 3 months prior to therapy.
- ACS/Stroke/TIA in last 3 months.

Carotid Doppler ultrasonography was performed by a single operator and the CIMT was measured using B-mode ultrasound and a 7.5 MHz transducer. Intimal-media thickness was defined as the distance between the leading edge of the first echogenic line (lumen-intima interface) and the second echogenic line (media-adventitia interface) of the far wall. Three measurements were taken at 0.5, 1 and 2 cm below the carotid bifurcation of the common carotid artery on each side, and their arithmetic averages were calculated. The intimal-media thickness of both sides (right and left) was also calculated and the average of these two values was calculated. In this study, an atherosclerotic plaque is defined as a local thickness of intima greater than 1 mm or two times more than its adjacent normal layer.

**Sample Size Estimation**
On basis of the results of the study done by El Saghier EO et al(1) taking alpha error as 0.5 power analysis was done and sample size was estimated to be 100.

**Statistical Analysis**
Simple random sampling was done. The statistical software SPSS (Statistical package for Social Sciences) Ver. 21 was used for statistical analysis. The mean ± standard deviation was calculated. Pair-wise comparison between the cases and controls was performed for all parameters using Student’s Unpaired t-test. The values of P <0.05 were considered as significant. The qualitative variables were compared using the chi-square test.

**RESULTS**
The present study was undertaken to evaluate the relation between Serum Testosterone, hsCRP and Carotid atherosclerosis in patients of Diabetes Mellitus in North West Punjabi population. In the present study, 100 patients with Diabetes Mellitus were taken from the various OPD/Indoor Admissions from Guru Nanak Dev Hospital after taking informed consent. Detailed history was taken and general and systemic examination was done. These patients were then systematically tested and the results were statistically analysed.

The mean age of the population under study was 60.17 years with a standard deviation of 13.23 years and a minimum of 23 to a maximum of 86 years. Mean duration of Diabetes of the population was 6 years.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-30</td>
<td>3</td>
</tr>
<tr>
<td>31-40</td>
<td>6</td>
</tr>
<tr>
<td>41-50</td>
<td>14</td>
</tr>
<tr>
<td>51-60</td>
<td>27</td>
</tr>
<tr>
<td>61-70</td>
<td>35</td>
</tr>
<tr>
<td>71-80</td>
<td>11</td>
</tr>
<tr>
<td>81-90</td>
<td>4</td>
</tr>
</tbody>
</table>

*Table 1. Age Distribution*

The population under study was divided into two groups on basis of levels of Serum Testosterone. The Age difference between the two groups was not statistically significant.

Low Serum Testosterone was found in 34% of the population.

**BMI and Serum Testosterone**
The mean BMI was 26.6 +/- 2.75 kg/m² with a minimum of 20.1 kg/m² and maximum of 34.5 kg/m².

The BMI of Low Testosterone group was significantly higher (28 kg/m²) than the normal Testosterone group (25.9 kg/m²).

<table>
<thead>
<tr>
<th>Serum Testosterone (900)</th>
<th>&lt;8</th>
<th>&gt;8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.9(SD=2.61)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td></td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*Table 2*

**Serum Testosterone vs BMI**

Mean Cholesterol (CHO) and Serum Testosterone
Mean Cholesterol was 179 mg/dL with a standard deviation of 44 mg/dL with a minimum of 96 and a maximum of 290 mg/dL. As given in Table No.3, the Mean Cholesterol in the Low Serum Testosterone group (191.6 +/- 50.5 mg/dL) was significantly higher than the normal Serum Testosterone group (172.8 +/- 39.1 mg/dL). This suggests an increased mean Cholesterol with lower serum Testosterone levels.

<table>
<thead>
<tr>
<th>Serum Testosterone (nmol/L)</th>
<th>&lt;8</th>
<th>&gt;8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Cholesterol (mg/dL)</td>
<td>191.6 (SD=50.5)</td>
<td>172.8 (SD=39.1)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

Table 3

Serum Triglyceride levels and Serum Testosterone
Mean Serum Triglyceride of the population was 144.23 +/- 43.6 mg/dL with a minimum of 96. The mean Serum Triglyceride in the Low Serum Testosterone group (157 +/- 58.7 mg/dL) was more than the normal Serum Testosterone group (137.6 +/- 31.9 mg/dL) indicating worse triglyceride profile in patients with lower levels of Total Serum Testosterone (shown in table no. 4).

<table>
<thead>
<tr>
<th>Serum Testosterone (nmol/L)</th>
<th>&lt;8</th>
<th>&gt;8</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG (mg/dL)</td>
<td>157 (SD=58.7)</td>
<td>137.6 (SD=31.9)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.03</td>
<td></td>
</tr>
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</table>

Table 4

Serum Testosterone and Serum HDL
Mean Serum HDL of the group was 37.7 mg/dL with SD of 7.3 mg/dL. The Serum HDL in low Testosterone group was 36.4 mg/dL and in normal Serum Testosterone group was 38.4 mg/dL. The difference was not statistically significant as shown in the Table No.5.

<table>
<thead>
<tr>
<th>Serum Testosterone (nmol/L)</th>
<th>&lt;8</th>
<th>&gt;8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum HDL (mg/dL)</td>
<td>36.38(SD=8.0)</td>
<td>38.41(SD=6.9)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.214</td>
<td></td>
</tr>
</tbody>
</table>

Table 5

Serum Non-HDL Cholesterol (Non-HDL CHO) and Serum Testosterone
Mean Non-HDL Cholesterol levels were 141.4 mg/dL with a standard deviation of 43 mg/dL. The mean Non-HDL in Low Testosterone group was 155.2 mg/dL while in the normal Testosterone group was 134 mg/dL as shown in Table No. 6. This suggests a higher dyslipidaemia burden in patients with lowered serum Testosterone.

<table>
<thead>
<tr>
<th>Serum Testosterone (nmol/L)</th>
<th>&lt;8</th>
<th>&gt;8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-HDL Cholesterol (mg/dL)</td>
<td>155.2(SD=49.1)</td>
<td>134.4(SD=38.0)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.02</td>
<td></td>
</tr>
</tbody>
</table>

Table 6

Serum hsCRP and Serum Testosterone
Mean serum hsCRP of the population was 2.35 ng/mL with a SD of 1.09 ng/mL. The hsCRP levels among the low Serum Testosterone group was 3.2 ng/mL and in the normal Testosterone group was 1.9 ng/mL (shown in Table no. 7). The difference was statistically significant indicating higher vascular inflammation in patients with lowered serum Testosterone.

<table>
<thead>
<tr>
<th>Serum Testosterone (nmol/L)</th>
<th>&lt;8</th>
<th>&gt;8</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsCRP (mg/dL)</td>
<td>3.2 (SD=1.0)</td>
<td>1.9(SD=0.8)</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Table 7

CIMT and Serum Testosterone
Mean CIMT of the population was 0.71 mm with a standard deviation of 0.12 mm as displayed in Table No. 8, the CIMT of the low Testosterone group was 0.82 mm and of normal Testosterone group was 0.68 mm. The difference was statistically significant. This suggests increased atherosclerosis in patients with lower Total Testosterone levels.

<table>
<thead>
<tr>
<th>Serum Testosterone (nmol/L)</th>
<th>&lt;8</th>
<th>&gt;8</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIMT (mm)</td>
<td>0.82 mm (SD=0.1 mm)</td>
<td>0.65 mm (SD=0.1 mm)</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Table 8

Prevalence of IHD and Serum Testosterone
IHD occurred in 29% of cases with low Serum Testosterone and 19% of cases with normal serum Testosterone (Table No. 9).
A lower serum Testosterone was associated with 1.5 times more frequent incidence of IHD.

<table>
<thead>
<tr>
<th>Serum Testosterone (nmol/L)</th>
<th>&lt;8</th>
<th>&gt;8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individuals without IHD</td>
<td>24</td>
<td>53</td>
</tr>
<tr>
<td>IHD</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>P-value</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

**Table 9**

**Incidence of IHD vs Serum Testosterone**

HbA1c and Serum Testosterone

Mean HbA1c of the population was 7.63% with a SD of +/- 0.99%. HbA1c in the low Testosterone group was 7.9 +/- 1.1% whereas in the normal Testosterone group was 7.5 +/- 0.9% indicating a worse glycaemic control in patients with low serum Testosterone. The same has been depicted in Table No.10.

**DISCUSSION**

In our study, we found that a low serum testosterone is more prevalent among diabetics than the general population. We used a criterion of Serum Testosterone < 8 ng/mL as our cut-off. A similar finding was noted by Barrett-Conner E(5) Al Hayek AA, Kapoor D(6) and Ayman A. Al Hayek et al(8)

**Testosterone and BMI**

The BMI was found to range from 20.1 kg/m² to 34.5 kg/m² with a mean of 26.6 +/- 2.75 kg/m². The BMI of the low Testosterone group was 28.0 +/- 2.5 kg/m² and that of the normal Serum Testosterone group was 25.89 +/- 2.6 kg/m². In the study conducted by W.E. Roudebush et al(9) (done on healthy adult males in 2005), the mean serum Testosterone was 19.6 nmol/L in Normal weight (BMI < 25), 14.9 nmol/L in overweight (BMI 25 to 29.9) and 14.4 nmol/L in obese (BMI more than 30).

**Testosterone and Dyslipidaemia**

The Mean Cholesterol of the study group was found to be 179.2 +/- 43.95 mg/dL. Mean cholesterol of the low serum testosterone group was 191.6 +/- 50.5 mg/dL and in the normal testosterone group was 172.8 +/- 39.1 mg/dL. Mean triglyceride levels of the total population were 144.2 +/- 43.7 mg/dL. Mean triglyceride of the low testosterone group was 157.1 +/- 58.7 mg/dL and in the normal testosterone group was 137.6 +/- 31.9 mg/dL. Mean Non-HDL of the population was 141.4 +/- 43.0 mg/dL. Mean Non-HDL in the low testosterone group was 155.2 +/- 49.1 mg/dL and in the normal Testosterone group was 134.4 +/- 38.0 mg/dL. In the study done by Simon D. et al(10) patients who had lower Serum Testosterone levels had increased serum Triglycerides (1.35 vs 1.0 nmol/mL), increased Total Cholesterol, increased LDL and decreased HDL cholesterol.

**Testosterone and hsCRP**

The mean hsCRP of the population under study was 2.35 +/- 1.09 mg/dL with a maximum of 6.3 mg/dL and a minimum of 0.5 mg/dL. Mean hsCRP of the low testosterone group was 3.2 +/- 1.0 mg/dL and of normal testosterone group was 1.9 +/- 0.9 mg/dL. In the study performed by Gannage-Yared et al(11) they studied 201 men and found that Total Testosterone was inversely associated with serum hsCRP and BMI. The study of Schneider HJ et al(12) concluded that obesity, metabolic syndrome, and acute inflammation was associated with hypogonadal testosterone levels. In the study by Javier M. F. et al(13) hsCRP levels were 2.74 +/- 1.37 mg/dL in low serum T group and 0.89 +/- 0.93 mg/dL in the normal Serum T group.

**Testosterone with CIMT**

The CIMT was found to range from 0.48 mm to 0.96 mm with a mean of 0.71 mm +/- 0.12 mm. The CIMT of low Serum Testosterone group was 0.82 +/- 0.1 mm and of Normal Serum Testosterone group was 0.65 +/- 0.1 mm (p<0.05). In the study performed by Javier M.F. et al, they studied the effect of low Serum Testosterone (<12 nmol/L) on CIMT. On multiple logistic regression analysis, they found that a lower level of Serum Testosterone was independently associated with a greater IMT but not with atherosclerotic plaque. In the study done by Soisson V. et al(14) they found that among men with low grade inflammation (CRP >2 mg/dL) serum hsCRP and Testosterone had increased serum Triglycerides and decreased HDL cholesterol.

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was 7.9% and in the normal Serum Testosterone group HbA1c was 7.5%. The difference was small but statistically significant. In the study conducted by Hackett et al. HbA1c was 7.8% in low Serum Testosterone group and 7.2% in normal Serum Testosterone group. In the study done by Prajesh Kurup et al., they found that increase of HbA1c and Fasting plasma glucose correlated negatively with Serum Testosterone levels. A study by Fukui M et al. also found the ratio of HbA1c vs. bioavailable testosterone as 2.94 +/- 0.38. They concluded a negative correlation exists between the same.

CONCLUSION

Summary
Prevalence of Low Serum Testosterone was 34% in our study. A higher BMI (28 +/- 2.5 kg/m²) seen in the Low Serum Testosterone group versus the normal Testosterone group (25.9 +/- 2.61 kg/m²). Increased Serum Cholesterol seen in the Low Serum Testosterone group (191.6 +/- 50.5 mg/dL) versus the normal Serum Testosterone (172 +/- 39.1 mg/dL) group. Raised Triglycerides seen in the Low Serum Testosterone group (157.1 +/- 50.7 mg/dL) than the normal Serum Testosterone group (137.6 +/- 31.9 mg/dL). Non-HDL Cholesterol in the Low Serum Testosterone group (155.2 +/- 49.1 mg/dL) was higher compared to the normal Testosterone Group (134.4 +/- 38 mg/dL). HbA1c levels were marginally higher in the Low Serum Testosterone group (7.9 +/- 1.1%) when compared to the normal Testosterone Group (7.5 +/- 0.9%). Increased hsCRP levels were seen in the Low Serum Testosterone group (3.2 +/- 1.0 mg/dL) than in the normal Testosterone Group (1.9 +/- 0.8 mg/dL). CIMT in the Low Serum Testosterone group (0.82 +/- 0.1 mm) was significantly more than the normal Testosterone Group (0.65 +/- 0.1 mm). HDL was more frequent in the Low Serum Testosterone group (29%) versus the normal Testosterone Group (20%). Hence, to conclude, a Low Serum Testosterone can serve as an adverse prognostic marker contributing to an increased atherosclerotic burden. Serum Testosterone should be screened for in all newly diagnosed patients of Type 2 Diabetes Mellitus.

REFERENCES