Original Article

DOI: 10.7860/NJLM/2021/48297:2532

Biochemistry Section

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.

Keywords: Cardiovascular risk, Diabetes mellitus, Erectile dysfunction, Hypogonadism

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 to7.40 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].

Age group (years)	Cases (Diabetics) n=60	Control (Non-Diabetic) n=40	Total
30-39	3 (5)	1 (2.5)	4
40-49	10 (16.7)	9 (22.5)	19
50-59	31 (51.7)	25 (62.5)	56
60-69	15 (25)	5 (12.5)	20
70-79	1 (1.7)	0	1
Total	60 (100)	40 (100)	100 (100)

[Table/Fig-1]: Age distribution of study population (N=100).

Parametres	Group	N	Mean
FPG mg/dL	Diabetic	60	178.450
	Non diabetic	40	84.725
PPG mg/dL	Diabetic	60	279.20
	Non diabetic	40	115

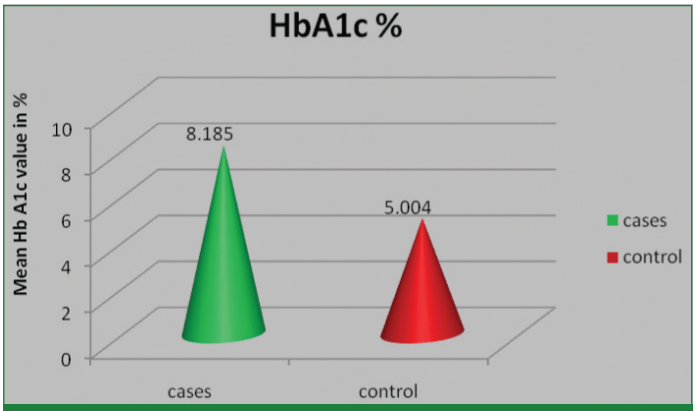
[Table/Fig-2]: Mean values of fasting and postprandial plasma glucose.
FPG: Fasting blood glucose; PPG: Postprandial plasma glucose

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.

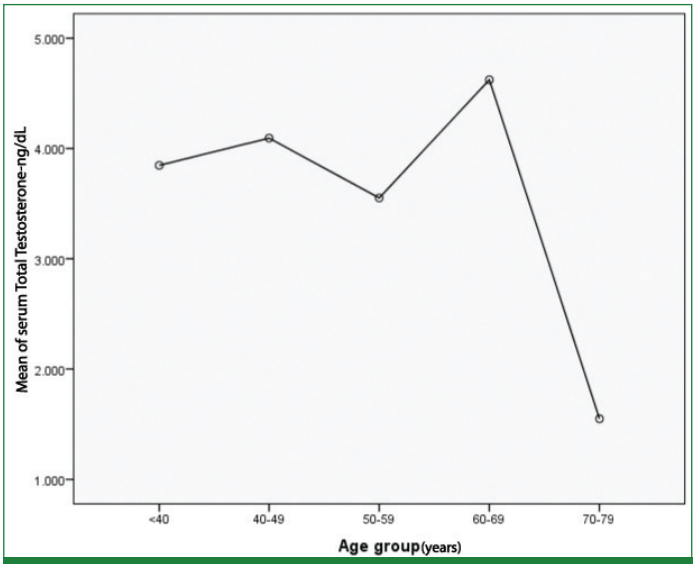
A 75% of case population (Diabetic) has ED [Table/Fig-6] and remaining 25% population doesn't have ED, whereas 5% of non diabetic controls have ED but 95% doesn't have ED. It is evidenced as statistically significant (p-value <0.001).



[Table/Fig-3]: Comparison of HbA1C among cases and controls.
HbA1c: Glycated haemoglobin

Total testosterone in ng/dL			Group: Cases (Diabetic) n=60	Group: Controls (Non-diabetic) n=40
Testosterone level	0-3.5	Number	27	3
		Percentage (%)	45	7.5
	>3.5	Number	33	37
		Percentage (%)	55	92.5

[Table/Fig-4]: Cross tabulation of testosterone low level and normal level.
Chi-square (χ^2) 16.07; p=0.00006100; Total N=100



[Table/Fig-5]: Age group wise mean testosterone value ANOVA mean plots.

Variables			Group: Cases (Diabetic) n=60	Group: Controls (Non-diabetic) n=40
ED	No	Number	15	38
		Percentage (%)	25	95
	Yes	Number	45	2
		Percentage (%)	75	5

[Table/Fig-6]: Erectile Dysfunction (ED) distribution between cases and controls.
Chi-square 47.210; p-value: 0.000 (<0.001); Total N=100

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

			Group	
			Cases (Diabetic) n=60	Controls (Non-diabetic) n=40
PME	No	Count	42	35
		% within group	70	87.5
	Yes	Count	18	5
		% within group	30	12.5

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

Hs-CRP levels are grossly elevated in diabetic population [Table/Fig-8] than 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].

Age groups (years)	n	Hs-CRP (mg/L)				
		Mean	Std. Deviation	Std. Error	Minimum	Maximum
<40	3	5.633	2.7319	1.5773	2.6	7.9
40-49	10	3.370	0.9832	0.3109	2.1	4.6
50-59	31	3.797	2.3035	0.4137	1.7	15.0
60-69	15	4.067	1.9540	0.5045	1.8	9.7
70-79	1	3.200	-	-	3.2	3.2
Total	60	3.875	2.0556	0.2654	1.7	15.0

[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

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.

Hs-CRP (mg/L)	Values	Diabetic (n=60)	Control (n=40)	Total
<1	Count	0	15	15
Low risk group	% within gp	0	37.5%	15.0%
1-3	Count	19	22	41
(moderate risk group)	% within gp	31.7%	55.0%	41.0%
>3	Count	41	3	44
(high risk group)	% within gp	68.3%	7.5%	44%

[Table/Fig-9]: Association of Hs-CRP risk with diabetic and non diabetic group.
Chi-square, p-value=0.005

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; suggestive of low Testosterone is common in diabetic study groups. The prevalence of low Testosterone below cut off is 45% in the present study cases. Grossmann M et al., study had a prevalence of 43% low testosterone in type 2 diabetics which is similar to present study [3]. Bhatia V et al., study reveal that one third of male 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 upto 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.

Thejaswini KO et al., described a higher level of Hs-CRP in type 2 diabetic patients [7]. In the study, among diabetic cases, 31.7% are 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 this study, the diabetic patients fall under intermediate and high risk categories for CVD. Non diabetic controls are distributed predominantly in low and intermediate sub groups with only 7.5% control in high risk category. It shows that diabetic patients are prone for future Cardiovascular Events (CVE). Cardoso CR et al., concludes that type 2 diabetic patients are usually considered to have intermediate-to-high cardiovascular risk [4].

Nevertheless, the prognostic importance of CRP in type 2 DM is still controversial, a previous study revealed that it was associated with CVEs [8]. This in turn suggests that CRP may be more useful on risk stratification for secondary rather than primary cardiovascular disease prevention.

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 possibility 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]. Meta-analysis by Yao QM et al., study states that higher testosterone level can reduce the risk of type 2 DM in males and an important protective factor against T2DM in men [14].

Further study or follow-up study is needed to explore, ageing related longitudinal changes and various scales of sexual assessment which is absolutely necessary to strengthen the present study effectively.

Limitation(s)

Longitudinal change of serum testosterone level could not be observed as the patients were not followed-up with repeated tests. As the sample size was small, the expanded variations of 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

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.

Acknowledgement

The author would like to thank all the participants for their cooperation in this study.

REFERENCES

[1] Burtis, Ashwood, Bruns. Tietz Textbook of clinical Chemistry and Molecular Diagnostics, Reproductive Endocrinology and Related Disorders, 5th Edition Elsevier; 2012:789-94;1507-8;1945-51.
[2] Chejara RS, Nawal CL, Agrawal MK. Study to evaluate serum free testosterone and hs-CRP concentration to predict low hematocrit in type 2 diabetes mellitus. International Journal of Research in Medical Sciences. 2017;3(6):1501-04.
[3] Grossmann M, Thomas MC, Panagiotopoulos S, Sharpe K, MacIsaac RJ, Clarke S, et al. Low testosterone levels are common and associated with insulin resistance in men with diabetes. The Journal of Clinical Endocrinology & Metabolism. 2008;93(5):1834-40.
[4] Cardoso CR, Leite NC, Salles GF. Prognostic Importance of C-Reactive protein in high cardiovascular risk patients with type 2 diabetes mellitus: The Rio De Janeiro type 2 diabetes cohort study. Journal of the American Heart Association. 2016;5(11):e004554.
[5] American Diabetes Association. Standards of medical care in diabetes 2016. Diabetes Care. 2016;39(suppl 1):S01-106.
[6] Bhatia V, Chaudhuri A, Tomar R, Dhindsa S, Ghanim H, Dandona P. Low testosterone and high C-reactive protein concentrations predict low hematocrit in type 2 diabetes. Diabetes Care. 2006;29(10):2289-94.
[7] Thejaswini KO, Roopakala MS, Dayananda G, Chandrakala SP, Prasanna Kumar KM. A study of association of ankle brachial index (ABI) and the highly sensitive C-reactive protein (hs-CRP) in Type 2 diabetic patients and in normal subjects. Journal of Clinical and Diagnostic Research: JCDR. 2013;7(1):46-50.
[8] Lachine NA, Elnekiedy AA, Megallaa MH, Khalil GI, Sadaka MA, Rohoma KH, et al. Serum chemerin and high-sensitivity C reactive protein as markers of subclinical atherosclerosis in Egyptian patients with type 2 diabetes. Therapeutic Advances in Endocrinology and Metabolism. 2016;7(2):47-56.
[9] Malik PK, Rani A. Prevalence of testosterone deficiency in patients of diabetes mellitus less than 40 years of age. J Evid Based Med Healthc. 2016;3(9):275-78.
[10] Fedele D, Bortolotti A, Coscelli C, Santeusanio F, Chatenoud L, Colli E, et al. Erectile dysfunction in type 1 and type 2 diabetics in Italy. International Journal of Epidemiology. 2000;29(3):524-31.
[11] Hadeed NN, Thanoon IA, Al-Mukhtar SB. Total testosterone levels and the effect of sildenafil on type 2 diabetics with erectile dysfunction. Oman Medical Journal. 2014;29(1):46-50.
[12] Isidori AM, Giannetta E, Gianfrilli D, Greco EA, Bonifacio V, Aversa A, et al. Effects of testosterone on sexual function in men: Results of a meta-analysis. Clinical Endocrinology. 2005;63(4):381-94.
[13] Cheung KK, Luk AO, So WY, Ma RC, Kong AP, Chow FC, et al. Testosterone level in men with type 2 diabetes mellitus and related metabolic effects: A review of current evidence. Journal of Diabetes Investigation. 2015;6(2):112-23.
[14] Yao QM, Wang B, An XF, Zhang JA, Ding L. Testosterone level and risk of type 2 diabetes in men: A systematic review and meta-analysis. Endocrine Connections. 2018;7(1):220-31.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Dec 28, 2020
- Manual Googling: Jun 22, 2021
- iThenticate Software: Jul 09, 2021 (11%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Dec 27, 2020**
Date of Peer Review: **Mar 06, 2021**
Date of Acceptance: **Jun 22, 2021**
Date of Publishing: **Oct 01, 2021**