ONLINE FIRST

ORIGINAL ARTICLE

Testosterone Levels in Male Type-II Diabetic Patients and its Association with $HBA1_C$ Levels and Duration of the Disease

Mubeena Laghari, Fozia Shaikh, Shazia Murtaza, Munazzah Meraj, Farah Shaikh

Dr. Mubeena Laghari *(Corresponding Author)*
Associate Professor
Department of Biochemistry
LUMHS, Jamshoro
Cell: 03340342073
Email: drmubeena2002@gmail.com

Dr. Fozia Shaikh
Lecturer
Department of Biochemistry
LUMHS, Jamshoro

Dr. Shazia Murtaza
Clinical trial unit Research Specialist
Prince Naif Health Research Centre King Saud University Medical City,
Riyadh KSA

Dr. Munazzah Meraj
Associate Professor (PhD Biochemistry)
Department of Biochemistry
Peoples University of Medical and Health Sciences for women,
Nawabshah
Email: munazzahmeraj@yahoo.com

Dr. Farah Shaikh
Lecturer
Department of Community Medicine
LUMHS, Jamshoro

ABSTRACT

OBJECTIVE: To measure serum testosterone levels in type-II diabetes mellitus male subjects and to correlate its association with blood glycated haemoglobin A1 ($HbA1_C$) levels and - duration of the disease.

METHODOLOGY: This cross sectional study was carried out at Biochemistry department, LUMHS, Jamshoro, from December 2015 to May 2016. One hundred male subjects including 50 DM patients and 50 age matched with no family history of DM, were enrolled by purposive sampling technique. Study population excluded those patients diagnosed as diabetic erectile dysfunction, diabetic nephropathy, diabetic renal failure, metformin therapy, familial dyslipidemia, and hypercholesterolemia. Serum testosterone (ELISA assay) and whole blood $HbA1_C$ levels (TINIA assay) were measured by standard laboratory methods using Hitachi Roche Diagnostic Chemistry Analyzer. The obtained results were analysed by SPSS version 21.0(IBM, Incorporation, and USA) and is reported as Mean± SD. The $p \leq 0.05$ was considered to indicate statistical significance.

RESULTS: The ages of diabetes mellitus patients and control subjects were 58.24±11.13 and 54.56±10.44 years respectively. Serum testosterone levels were observed to be more significant ($p<0.004$) in control subjects (3.53±0.74 mg /dL) and its levels decreased in diabetes mellitus patients (2.53±1.13 mg /dL). Similarly, $HbA1_C$ levels were significantly higher ($p<0.0001$) in diabetic subjects as compared to control subjects (8.40±1.27 vs 5.75±0.24%). Testosterone levels showed negative correlation with blood $HbA1_C$ levels and positive with duration of disease.

CONCLUSION: In type 2 diabetic male patients serum testosterone levels demonstrated negative correlation with blood $HbA1_C$ levels and positive with duration of DM.

KEYWORDS: Serum testosterone, Type 2 diabetes mellitus, Glycated $HbA1_C$

INTRODUCTION

Diabetes Mellitus (DM) is significant cause of morbidity and mortality[1]. In the world approximately 285 million people are affected by DM and hypothesized that in 2030 its prevalence will rise up to 438 million[2]. It considered that in most developed countries, DM is the 4th leading cause of death[3]. With the burden of Diabetes, Pakistan is at seventh position and it is estimated that if this situation is continuing it will move to the fourth position[4]. Fortunately, therapeutic regimens improvement and early diagnosis permit diabetic patients to spend healthy lives. Clinical biochemistry plays a crucial role in monitoring, diagnosis and treatment of diabetes. Glycemic control, the routine measurement of glycosylated haemoglobin (HbA1c), is generally recommended to limit end organ damage including increase in blood cholesterol and decrease in serum testosterone levels and mortality[5-7]. Researchers revealed the metabolic effect of testosterone reduction with age-associated metabolic changes such as markers of pre-diabetes, diabetes and abdominalobesity[8-10]. In 2010, Endocrine Society's Clinical Practice Guideline was recorded the low serum testosterone level in patients in advanced type 2 diabetes. The prevalence rate of said condition was high and it was investigated in the perspective of androgen deficiency syndrome in adult men. The Endocrine society recommends the proper measurement of morning serum total testosterone for those patients already suffered with type 2 diabetes mellitus (T2DM) and having the prominent symptoms of sexual dysfunction, weakness or weight loss[11]. This study will help to improve the fertility status of patients having type-II diabetics mellitus.

METHODOLOGY

This cross sectional study was conducted at the Biochemistry Department, Liaquat University Hospital Jamshoro from December 2015 to May 2016. One hundred subjects (n=100) were distributed in two groups; Group A (n=50): Diagnosed type 2 Diabetic patients (Case), Group B (n=50): Non-Diabetics and healthy normal subjects (Control). Study population included diagnosed T2DM patients with age 30-60 years and excluded those patients diagnosed as diabetic erectile dysfunction, diabetic nephropathy, diabetic renal failure, metformin therapy, familial dyslipidemia, and hypercholesterolemia.

Research bias was overcome by selecting the study population according to of inclusion and exclusion criteria. Diagnosed cases of T2DM (defined as per American Diabetes Association criteria) with age 30-60 years. Diabetic erectile dysfunction, Diabetic nephropathy, Diabetic Renal failure, Metformin therapy, Familial dyslipidemia, hypercholesterolemia, etc; were excluded from the study. Disposable syringe (BD, USA) was used for vein prick to collect 5 ml of venous blood, 2.5ml was shifted to EDTA containing glass tube for HbA1c estimation, remaining was centrifuged at 3000 rpm (10 minutes) to get the sera. The sera were used for the Biochemical analysis and the Analysis of parameters performed on Roche, Hitachi chemistry analyser at the Research and Diagnostic Laboratory, Liaquat University Hospital Jamshoro/Hyderabad.

Readings of Testosterone were recorded at 500nm on spectrophotometer for all samples. The HbA1c was estimated from the clotted Blood. The clotted Blood was analysed on the Chemistry analyser Hitachi, Roche, Cobas systems (501) based on the turbid metric inhibition immunoassay for the Haemolysed whole blood. Consent form was printed in trilingual format. A volunteer was eligible to enter in the research protocol once he signed the consent form or by his legal heir. Data analysis including paired and correlation analysis were computed using SPSS software version 21.0 (IBM, Incorporation, and USA) for windows. Paired t-test was used to determine the significance of changes. P-value ≤ 0.05 was considered to indicate statistical significance.

RESULTS

Total 100 male subjects with age between 30-60 years were evaluated to find correlation (Association) of glycated haemoglobin with serum cholesterol and testosterone levels in T2DM patients. The mean age of study subjects of Group A was 58.24±11.13 years with range 24(36-60) years. The mean age of Group B subjects was 54.56±10.44 years with range 28(31-59) years. The mean age distribution of Group A and Group B is presented in **Graph I**. The detailed descriptive statistics of age of both groups are presented in **Table I**. The mean glycated haemoglobin ($HbA1_C$) of study subjects of Group A was 8.40±1.27 while the mean $HbA1_C$ of study subjects of Group B was 5.75±0.24(%). The mean serum testosterone of study subjects of Group A was 2.53±1.13 mg/dL while the mean serum testosterone of study subjects of Group B was 3.53±0.74 mg/dL.

The detailed descriptive statistics of $HbA1_C$and testosterone of both groups are presented in **Table-II**. While the Comparison of serum HbA1c is in both Groups A and B is shown in **Graph II**. Stratification with respect to diabetes and higher levels of HbA1c was done to observe effect of these modifiers with low serum testosterone levels. These results exhibited that there was a significant association of low serum testosterone level with diabetes ($p<0.05$) and HbA1c ($p<0.05$), and it has also been described with the duration of Diabetes Mellitus type 2. It showed that as the duration of Type 2 diabetes mellitus increases there is significantly reduction in serum testosterone levels.

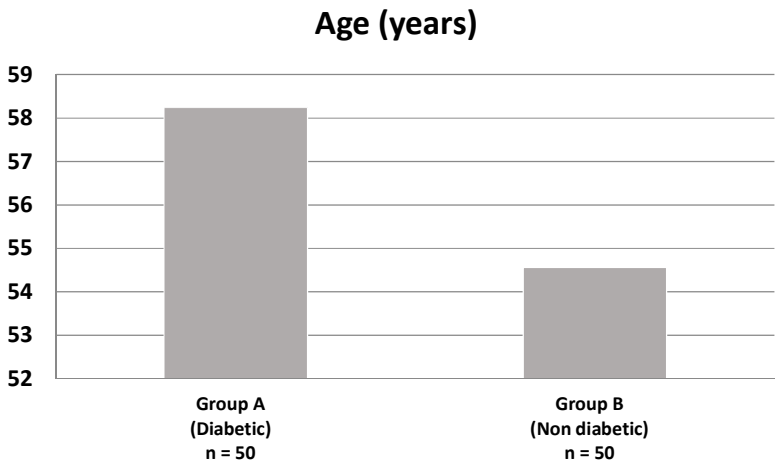
Scatter plots show the correlation of HbA1c with serum testosterone and duration of diabetes mellitus. (**Scatter plot I**) shows the positive correlation (Association) of HbA1c and duration of DM, as the duration of DM increases so an increase in % of HbA1c noted (**Scatter plot I & II**). Interpolation curve shows the negative correlation (association) of serum testosterone duration of disease in diabetic population.

TABLE I: AGE DISTRITBUTION OF THE DIABETIC AND NONDIABETIC GROUPS (n=100)

Variable	Group A (Diabetic) (n=50)	Group B (Non-diabetic) (n=50)	P value
Age (in years)	58.24±11.13	54.56±10.44	0.0001

Student t-test was applied for statistical analysis of age and SPSS output was presented as mean SD. P value 0.09 shows that the two study groups are age matched.

GRAPH I: COMPARISON OF AGE (YEARS) BETWEEN DIABETIC AND NON DIABETIC GROUPS (n = 100)



GRAPH II: COMPARISON OF SERUM HbA1C LEVEL BETWEEN DIABETIC AND NONDIABETIC GROUPS (n = 100)

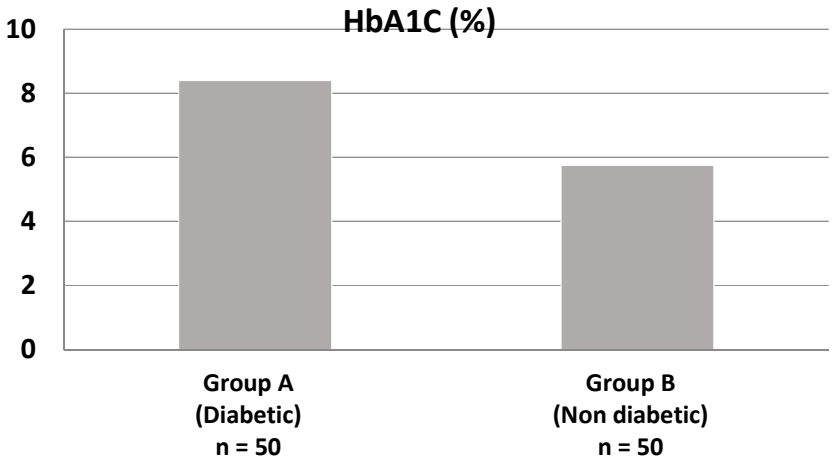
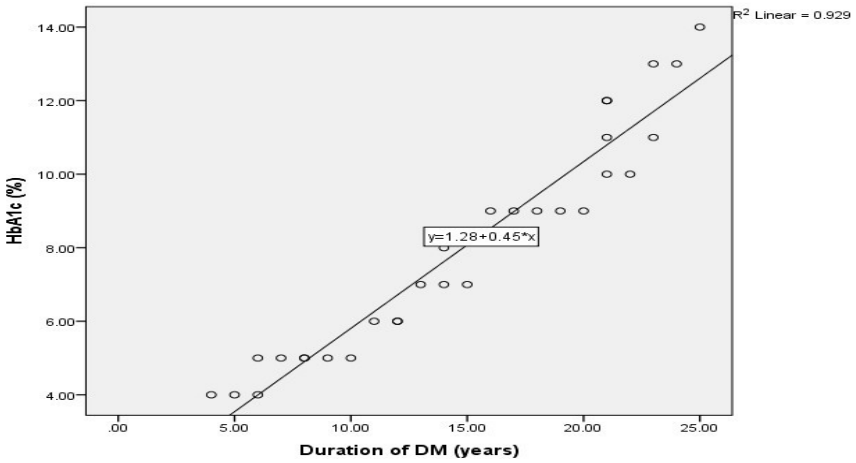


TABLE II: COMPARISON OF SERUM TESTOSTERONE AND HBA1C LEVELS BETWEEN DIABETIC AND NON DIABETIC GROUPS (n = 100)

Variable	Group A (Diabetic) n=50	Group B (Non diabetic) n=50	P value
Serum Testosterone(mg/dL)	2.53 ± 1.13	3.53 ± 0.74	<0.05
HbA1c (%)	8.402 ±1.27	5.752 ± 0.24	<0.05

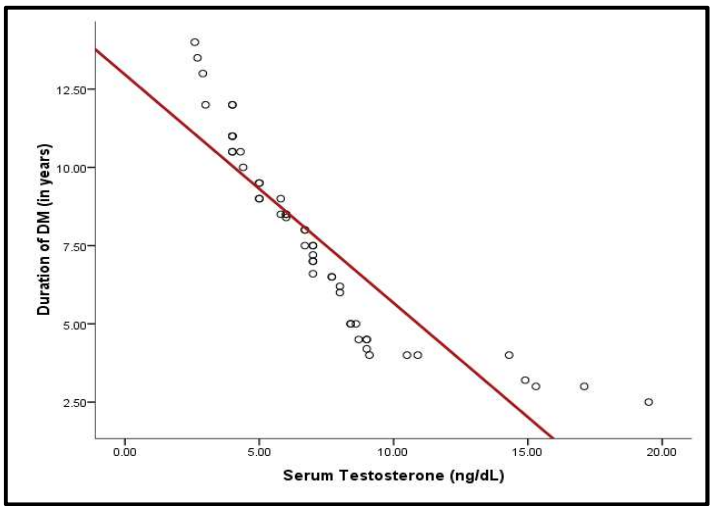
-Results are presented as Mean ± SD
-P value calculated by Student "t" test is statistically significant

SCATTER PLOT- I



HbA1c shows positive correlation with Duration of DM type- 2. As Duration of Diabetes mellitus type 2 increases, the Levels of HbA1c also increase (r= 0.67, P=0.001).

SCATTER PLOT-II



Duration of DM shows inverse correlation with Serum Testosterone. As there is rise in duration of DM, the Serum Testosterone concentrations decrease in patients with DM type- 2(r= - 0.79, P=0.0001).

DISCUSSION

Characteristics hyperglycaemia of type 2 diabetes mellitus damages the various organ systems of human body. In addition to chronic microvascular complications, such as diabetic retinopathy, neuropathy, nephropathy and microangiopathy. Type 2DM is reported to be associated with dysfunction of gonads resulting in hypogonadism, osteoporosis, cancer, non-alcoholic fatty liver disease and so forth. Male hypogonadism seriously affects the quality of life in diabetic patients[12,13]. However, so far, the association between low testosterone levels and T2DM needs more clarification[14]. One clinical study found that patients with T2DM are prone to hypogonadism[10].

The control of blood glucose in diabetic patient is monitored by the HbA1c. Several studies have been done and they showed that HbA1c positively associated with the duration and complications of DM and its elevation is a high risk factor for cardiovascular diseases[15,16].

Retrograde ejaculation, orgasmic dysfunction, erectile dysfunction and reduced libido are recognized complications established with changeable occurrence in men with diabetes. Many researchers have observed that prevalence of hypogonadism in T2DM patients due to low plasma concentration of free testosterone[17], another study associated the low serum testosterone with high aortic atheroma[18].

In a study of obese subjects with T2DM, 20(76.9%) had low serum free testosterone while 16 (61.51%) had low level of total testosterone. Mean serum total testosterone was 300.72±33.14 and mean serum free testosterone was 7.65±2.04[13]. Whereas another study reported occurrence of low testosterone in obese diabetics to be 50%[19]. Thoma C 2016[20] showed that 34% patients of diabetes mellitus had low level of serum total testosterone and Shigehara K et al[21] showed 25% of low level of serum total testosterone in diabetic patient which is consistent to present and previously reported studies. In present study 20-40% patients were having low serum total testosterone levels while in non-diabetic group 5(10%) had normal serum testosterone levels.

In a study, multivariate analysis did not find a correlation between the serum testosterone concentration and HbA1clevel, which was consistent with the results obtained by studies by Soriguer F et al[22] and Fukui M 2008[23], while it was in contrast with those by Kapoor D 2007[10] and the present study. Findings from the study by Reinstatler L 2012[24] also contradicted from those of the study by Fukui M 2008[23] in which TT levels positively correlated with HbA1c levels. The finding of how serum testosterone decreases is beyond the scope of present study that needs further research.

CONCLUSION

Current study revealed that type 2 diabetic patients shows low serum testosterone levels, while glycated HbA_1 shows positive correlation with duration of type 2 DM. Significant negative correlation of Serum Testosterone was observed with glycated haemoglobin A1. Thus it is important to evaluate diabetic patients for testosterone levels that will ultimately help in early detection and prevention of future complications associated with diabetes mellitus.

Ethical permission: Liaquat University of Medical & Health Sciences ERC approval letter No. LUMHS/REC/-453, dated 18-12-2015.
Conflict of interest: There is no conflict of interest in authors.
Funding: There was no funding from any agency or institution.

AUTHOR CONTRIBUTIONS

Laghari M: Drafting and final approval, editing, literature review
Shaikh F: Data collection
Murtaza S: Analysis and interpretation of data
Meraj M: Edition & critical revision of article
Shaikh F: Study survey & literature review

REFERENCE

1. Grandi C, Tapia JL, Cardoso VC. Impact of maternal diabetes mellitus on mortality and morbidity of very low birth weight infants: a multicenter Latin America study. J Pediatr (Rio J). 2015; 91(3): 234-41. doi: 10.1016/j.jped.2014.08.007.
2. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res ClinPract 2010; 87(1): 4-14. doi: 10.1016/ j.diabres.2009.10.007.
3. Zafar J, Nadeem D, Khan SA, Abbasi MM, Aziz F, Saeed S. Prevalence of diabetes and its correlates in urban population of Pakistan: A Cross-sectional survey. J Pak Med Assoc. 2016; 66(8): 922-7.
4. Memon IA, Almani SA, Shaikh TZ, Ujjan I, Kazi N, Khoharo HK. Berberine mitigates insulin resistance in newly diagnosed type 2 diabetics. Int J Med Sci Clin Invent. 2017; 4(1): 2566-2572. doi:10.18535/ijmsci/ v4i1.09
5. Chan JC, Malik V, Jia W, Kadowaki T, Yajnik CS, Yoon KH, et al. Diabetes in Asia: Epidemiology, risk factors, and pathophysiology. JAMA. 2009; 301(20): 2129-40. doi: 10.1001/jama.2009.726.
6. Ajlouni K, Khader YS, Batieha A, Ajlouni H, El-Khateeb M. An increase in prevalence of diabetes mellitus in Jordan over 10 years. J Diabetes Complications. 2008; 22(5): 317-24. doi: 10.1016/j.jdiacomp.2007.01.004.
7. George JT, Veldhuis JD, Tena-Sempere M, Millar RP, Anderson RA. Exploring the pathophysiology of hypogonadism in men with type 2 diabetes: Kisspeptin-10 stimulates serum testosterone and LH secretion in men with type 2 diabetes and mild biochemical hypogonadism. Clin Endocrinol (Oxf). 2013; 79(1): 100-4. doi: 10.1111/cen.12103.
8. Reid K, Toben C, Fakler P. Effects of garlic on serum lipids: an updated meta-analysis. Nutr Rev. 2013; 71(5): 282-99. doi: 10.1111/nure.12012.
9. Saboor Aftab SA, Kumar S, Barber TM. The role of obesity and type 2 diabetes mellitus in the development of male obesity-associated secondary hypogonadism. Clin Endocrinol (Oxf). 2013; 78(3): 330-7. doi: 10.1111/cen.12092.
10. Kapoor D, Aldred H, Clark S, Channer KS, Jones TH. Clinical and biochemical assessment of hypogonadism in men with type 2 diabetes: Correlations with bioavailable testosterone and visceral adiposity. Diabetes Care. 2007; 30(4): 911-7. doi: 10.2337/dc06-1426.
11. Jones TH, Arver S, Behre HM, Buvat J, Meuleman E, Moncada I, et al. Testosterone replacement in hypogonadal men with type 2 diabetes and/or metabolic syndrome (The TIMES2 study). Diabetes Care. 2011; 34(4): 828-37. doi: 10.2337/dc10-1233.
12. Portillo-Sanchez P, Bril F, Maximos M, Lomonaco R, Orsak B, Subbarayan S, et al. High prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus and normal plasma aminotransferase levels. J Clin Endocrinol Metabol. 2015; 100(6): 2231-8.
13. Yaturu S. Diabetes and skeletal health. J Diabet. 2009; 1(4): 246-54. doi:10.1111/j.1753-0407.2009.00049.x.
14. Laaksonen DE, Niskanen L, Punnonen K, Nyyssonen K, Tuomainen T, Valkonen V, et al. Testosterone and sex hormone-binding globulin predict the metabolic syndrome and diabetes in middle-aged men. Diabetes Care. 2004; 27(5): 1036-41. doi: 10.2337/diacare.27.5.1036.

15. Tomar R, Dhandsa S, Chaudhuri A, Mohanty P, Garg R, Dandona P. Contrasting testosterone concentration in type 1 and type 2 diabetes. Diabetes Care; 2006; 29(5):1120-2. doi: 10.2337/diacare.2951120.
16. English KM, Mandour O, Steeds RP, Diver MJ, Jones TH, Channer KS. Men with coronary artery disease have lower levels of androgens than men with normal coronary angiograms. Eur Heart J. 2000; 21(11): 890-4. doi: 10.1053/euhj.1999.1873.
17. Hak AE, Witteman J, De Jong FH, Geerlings MI, Hofman A, Pols HAP. Low levels of endogenous androgens increase the risk of atherosclerosis in elderly men: The Rotterdam study. J Clin Endocrinol Metab. 2002; 87(8): 3632-9. doi: 10.1210/jcem.87.8.8762.
18. Boulton AJ, Vinik AI, Arezzo JC, Bril V, Feldman EL, Freeman R, et al. Diabetic neuropathies: a statement by the American Diabetes Association. Diabetes Care. 2005; 28(4): 956-62. doi: 10.2337/diacare.28.4.956.
19. Bhasin S, Pencina M, Jasuja GK, Travison TG, Coviello A, Orwoll E, et al. Reference ranges for testosterone in men generated using liquid chromatography tandem mass spectrometry in a community-based sample of healthy nonobese young men in the Framingham Heart Study and applied to three geographically distinct cohorts. J Clin Endocrinol Metab. 2011; 96(8): 2430-9. doi: 10.1210/jc.2010-3012.
20. Thoma C. Vardenafil in men with T2DM. Nature Reviews Urology. 2016 Mar;13(3):126-.doi.org/10.1530/EJE-15-1100 (2016.
21. Shigehara K, Konaka H, Kato Y, Iijima M, Nakashima K, Kawaguchi S, et al. Effect of testosterone replacement therapy on sexual function and glycemic control among hypogonadal men with type 2 diabetes mellitus. Int J Impotence Res. 2019; 31(1):25-30. doi.org/10.1038/s41443-018-0065-z.
22. Soriguer F, Rubio-Martin E, Fernandez D, Valdes S, Garcia-Escobar E, Martin-Nunez GM, et al. Testosterone, SHBG and risk of type 2 diabetes in the second evaluation of the Pizarra cohort study. Eur J Clin Invest. 2012; 42(1): 79-85. doi: 10.1111/j.1365-2362.2011.02559.x.
23. Fukui M, Tanaka M, Hasegawa G, Yoshikawa T, Nakamura N. Association between serum bioavailable testosterone concentration and the ratio of glycated albumin to glycated hemoglobin in men with type 2 diabetes. Diabetes Care. 2008; 31(3): 397-401.
24. Reinstatler L, Qi YP, Williamson RS, Garn JV, Oakley Jr. GP. Association of biochemical B_{12} deficiency with metformin therapy and vitamin B_{12} supplements: the National Health and Nutrition Examination Survey, 1999-2006. Diabetes Care. 2012; 35(2): 327-33. doi: 10.2337/dc11-1582.