Primary Hypogonadism In Ghanaian Men With Type 2 Diabetes Mellitus

H. Asare-Anane, E.K. Ofori, F.A.Yeboah, E.A. Tagoe, S.B. Bani, A.T. Bawah, R.O Ateko

Abstract-Emerging evidence links insulin resistance, a key feature in type 2 diabetes with decreased leydig cell secretion of testosterone. Low gonadal steroids, which include testosterone, dihydrotestosterone and estradiol have also been implicated with metabolic abnormalities such as hyperglycemia, hypertension and cardiovascular risk. The aim of this study was to investigate hypogonadism and its risk factors in Ghanaian men with type 2 diabetes. Two hundred and ten volunteers were used for this study. Total testosterone, Sex hormone binding globulin, prolactin, fasting blood glucose, glycated hemoglobin, total cholesterol, triglyceride, luteinizing hormone, follicle stimulating hormone, estradiol, blood pressure and body mass index were assessed. A questionnaire to assess androgen deficiency was administered to each consenting participant. Total testosterone levels were lower in diabetic men compared with non-diabetic men (10.8 ± 5.0 vs 15.6 ± 3.9, p=0.0001). There was no significant change in follicle stimulating hormone, prolactin, sex hormone binding globulin and estradiol between controls and type 2 diabetics. However, luteinizing hormone was significantly higher in diabetics than non-diabetics (5.4±2.0 vs 4.5±1.5 p≤0.0003). Total testosterone levels were inversely related to BMI (R^2=3.94, p=0.04), FBG (R^2=6.492, p=0.0008) and TG (R^2=31.41, p=0.025). The low testosterone observed in this study was a case of primary hypogonadism.

Keywords- Diabetes, Estradiol, Glycated hemoglobin, Hypogonadism, Insulin, Obesity, Prolactin,

INTRODUCTION

Male hypogonadism is a clinical condition resulting from testicular failure to produce adequate testosterone levels (1, 2). Testosterone is the primary male sex hormone that is vital for sustaining proper erectile function and libido. It is also critically involved in building muscle, burning fat, and supporting endothelial function, energy level, mood, immune function, red blood cell production and bone density (3). The hypothalamic-pituitary-gonadal axis is responsible for the regulation and maintenance of testosterone levels.

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The hypothalamus releases gonadotropin-releasing hormone, which stimulates the pituitary gland to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH). In the testes, LH stimulates Leydig cells to release testosterone and Total testosterone comprises free testosterone (2-3%) and testosterone bound to either sex hormone binding globulin (SHBG) (60-80%) or albumin (20-40%) (6,11). Low testosterone levels have been associated with diabetes, dyslipidaemia and hypertension (7-9). This study was aimed at investigating the cause of hypogonadism in Ghanaian men with type 2 diabetes. There is no established data on Ghanaian men with type 2 diabetes who are hypogonal. Data on this research would be a useful tool for the management of hypogonadism in male diabetics.

POPULATION, STUDY DESIGN AND METHODS

This was a case control study involving a total of 105 type 2 diabetic male and 105 healthy controls between 30 and 60 years. The study was carried out at the National Diabetes Management and Research Center, Korle-Bu Teaching Hospital Accra, Ghana. The hospital serves as a referral hospital for the country. Participants gave their consent and also answered a questionnaire which provided information on their family history of diabetes, reproductive, socio-demographic, anthropomorphic and other medical conditions. The University of Ghana Medical School Ethical and Protocol Review Committee reviewed the consenting process. Participants fasting blood (10mls) samples were drawn between 08.00am and 10.00 a.m. for all assays. Three milliliters (3mls) of whole blood was transferred into ethylene diamine tetra-acetic acid (EDTA) and 2ml of blood into sodium fluoride containing tubes for the estimation of glycated hemoglobin and glucose respectively. The remaining five milliliters (5mls) of whole blood was then put into serum separator tubes and centrifuged after which serum was aliquoted in 1ml portions into a sterile plain tube and stored at -20 ˚C until required for use. Serum total testosterone (TT), Sex hormone binding globulin (SHBG), estradiol (E_2), luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin (PRL) were determined by

chemiluminescent immunoassay using VITROS ECi immunodiagnostic system. The system uses Enhanced Chemiluminescence detection technology for heterogeneous assays. All reactions necessary took place in a coated well and are specific for one type of assay. Lipid profile, albumin and fasting blood glucose were analyzed using VITROS system auto analyzer (version 950). Glycated hemoglobin (HbA_{1c}) was measured using Randox Daytona auto analyzer. Data was entered unto a spreadsheet and analyzed using Microsoft Office Excel 2007 and the values were expressed as mean plus/minus standard deviations (mean ± SD). GraphPad Prism 3.02 program was used for independent sample *t*-test (student *t*-test) with a level of statistical significance set at $p<0.05$ for all tests and at 95% confidence interval (CI). Student *t*-test was used for comparison of means of variables between case and control subjects. Variables with significant associations were assessed through linear and multiple regressions to determine their independent contributions to the variance of total testosterone.

RESULTS

The total number of volunteers who participated in the research was two hundred and ten (210). The subjects comprised one hundred and five (105) type 2 diabetic men who were not on insulin. These were matched for age with one hundred and five (105) apparently healthy non-diabetics.

Table 1 : Clinical and biochemical parameters of the study population

Parameters	Diabetics (N = 105)	Non- Diabetics (N = 105)	95 % CI of mean difference	p-value
Age (yrs)	48.9 ± 9.4	42.8 ± 5.6	-8.193 – (-4.007)	0.0001**
SBP (mmHg)	131.1 ± 17.5	122.9 ± 9.1	-11.970 – (-4.427)	0.0001**
DBP (mmHg)	76.8 ± 10.4	74.7 ± 5.9	-4.387 – 0.1871	0.0734
BMI (Kg/m^2)	25.4 ± 3.7	24.0 ± 2.6	-2.265 – (-0.535)	0.0017*
FBG (mmol/L)	9.5 ± 4.5	4.5 ± 0.4	-5.864 – (-4.136)	0.0001**
HbA1C (%)	9.4 ± 2.4	6.1 ± 0.6	-3.773 – (-2.827)	0.0001**
T. Chol (mmol/L)	5.1 ± 1.2	4.8 ± 0.6	-0.556 – (-0.043)	0.0229*
TG (mmol/L)	1.2 ± 0.4	1.1 ± 0.2	-0.221 - 0.021	0.0049*
HDL-C (mmol/L)	1.3 ± 0.2	1.3 ± 0.1	-0.078 - 0.078	1.0000
LDL–C (mmol/L)	3.2 ± 1.0	3.0 ± 0.5	-0.413 - 0.013	0.0682
Albumin (mmol/L)	45.3 ± 4.1	41.9 ± 4.2	-4.523 – (-2.277)	0.0001**
Creatinine (mmol/mL)	103.1 ± 82.6	96.8 ± 14.5	-22.340 - 9.741	0.4423
Uric Acid (mmol/L)	343.8 ± 90.8	294.8 ± 63.3	29.830 - 72.170	0.0001**
Period of diabetes (yrs)	6.8 ± 5.7	-	-	-

Values are given as mean ± standard deviation. SBP = systolic blood pressure, DBP = diastolic blood pressure, BMI = body mass index, $HbA1_C$ is glycated hemoglobin, LDL-C is low density cholesterol. *mean difference is significant *(p<0.05).* **mean difference is highly significant *(p<0.0001).*

The mean age for the diabetic men (study group) and the non-diabetic men (control group) were 48.9 and 42.8 years respectively (Table 1). The systolic blood pressure (SBP), fasting blood glucose (FBG), glycated hemoglobin (HbA1c), albumin and uric acid were highly significant ($p=0.0001$) between the study and control groups. Significant difference ($p<0.05$) between means of both groups were also observed for total cholesterol (T.chol), triglyceride (TG) and body mass index (BMI). No significant difference ($p=0.07$) was observed in the diastolic blood pressures (DBP) from both groups (Table 1). The difference in means between the diabetic and non-diabetics groups for total testosterone were highly significant ($p<0.0001$, Table 2). Significant difference ($p = 0.0003$) was also observed for mean concentrations levels of luteinizing hormone (LH) in diabetic and non-diabetic subjects (Table 2). No significant difference was however observed between means of diabetic and non-diabetics for follicle stimulating hormone ($p=0.59$), estradiol ($p=0.25$), sex hormone binding globulin ($p=0.19$) and prolactin ($p=0.16$) respectively (Table 2)

Table 2: Hormonal levels in diabetic and non-diabetic subjects

Parameters	Diabetics (N=105)	Non-diabetics (N=105)	95% CI of mean difference	*p*-value
Testosterone (nmol/L)	10.8 ± 5.0	15.6 ± 3.9	3.587 – 6.013	0.0001**
FSH(IU/L)	5.8 ± 3.0	5.6 ± 2.3	-0.9231 – 0.5231	0.5883
LH (IU/L)	5.4 ± 2.0	4.5 ± 1.5	-1.374 – (-0.22)	0.0003*
Estradiol (pmol/L)	76.4 ± 20.1	79.3 ± 15.8	-1.990 - 7.790	0.246
Prolactin (nmo/L)	12.8 ± 2.75	12.2 ± 2.61	-1.831 – 6.251	0.16
SHBG (nmol/L)	27.6 ± 2.0	27.9 ± 1.3	-0.563 – 0.7563	0.198

Table 2 shows the hormonal levels in diabetic subjects compared with non-diabetic subjects.
Values are given as mean ± standard deviation. FSH = Follicle stimulating hormone. LH = Luteinizing hormone.
*mean difference is significant (p<0.05). **mean difference is highly significant (p<0.0001)

The percentage distribution of total testosterone levels of diabetic and non-diabetic subjects were investigated in this study. Subjects were categorized into three groups. These were hypogonadal group (< 8 nmol/L), borderline group (8-12 nmol/L) and eugonadal (> 12 nmol/L). Incidence of hypogonadism was five times more in the diabetic subjects than non-diabetic controls (Figure 1). A total of 37 (35.2%) of the diabetic men and 7 (6.7%) of the control subjects were hypogonadal at less than 8 nmol/L. Also, 21 (20%) and 10 (9.5%) had testosterone levels between 8 -12 nmol/L for the diabetic men and non-diabetic men respectively. In addition, 47 (44.8%) and 88 (83.8%) were eugonadal for diabetic and non-diabetic men respectively (Figure 1).

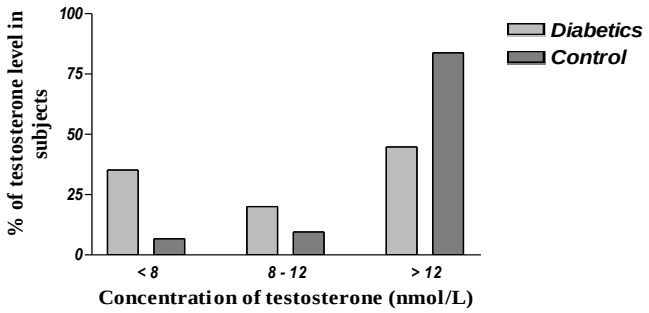

Figure 1: Percentage distribution of categorized testosterone levels in the study

Figure 1 shows total testosterone levels of diabetic and non-diabetic men that have been categorized into hypogonadal (< 8nmol/L) borderline (8-12nmol/L) and eugonadal (>12nmol/L). Percentage distribution of categorized testosterone levels for the diabetic men was 35.2, 20.0 and 44.8 for hypogonadal, borderline and eugonadal respectively. Percentage distribution of categorized testosterone levels for the control group was 6.7, 9.5 and 83.8 for hypogonadal, borderline and eugonadal respectively. Total testosterone levels from diabetic subjects were stratified into duration (years) of diabetes. Total testosterone levels were found to decrease with increase in diabetic years. Decline in total testosterone levels was greater after eleven (11) years with type 2 diabetes mellitus (Figure 2). A statistically significance (p<0.01) difference were observed between mean testosterone levels for 1-5 vs 12-17 and 1-5 vs >18 year duration of diabetes. Again, statistically significance (p<0.01) difference were observed between mean testosterone levels for 6-11 vs 12-17 and 6-11 vs >18 year duration of diabetes. No statistically significance (p>0.05) difference were however observed between mean testosterone levels for 1-5 vs 6-11 and 12-17 vs >18 year duration of diabetes (Figure 2).

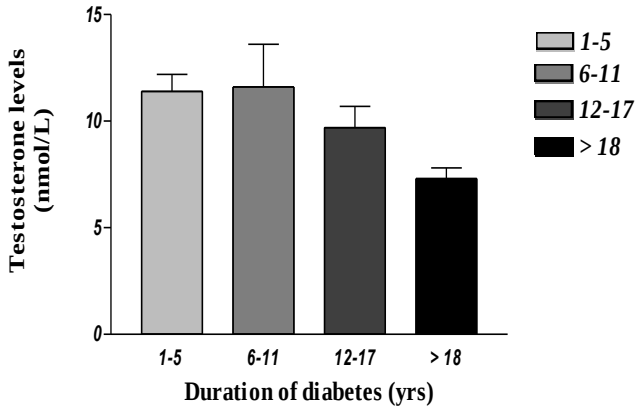

Figure 2: Levels of total testosterone with duration of diabetes in type 2 diabetic men

Figure 2 showed a decline in total testosterone levels with increase in duration of diabetes in years. Decline in total testosterone was observed to be steeper after 11 years of diabetes. Data was presented as mean ± SD. Comparison of body mass index (BMI) distribution within the study population was investigated (Figure 3). The proportional difference observed for overweight and obesity between diabetic and non-diabetic subjects were both significant (Z=2.795, Z=2.601). Underweight however, did not show any proportional significance (Z=0.555) between diabetics and non-diabetics in the study population (Figure 3).

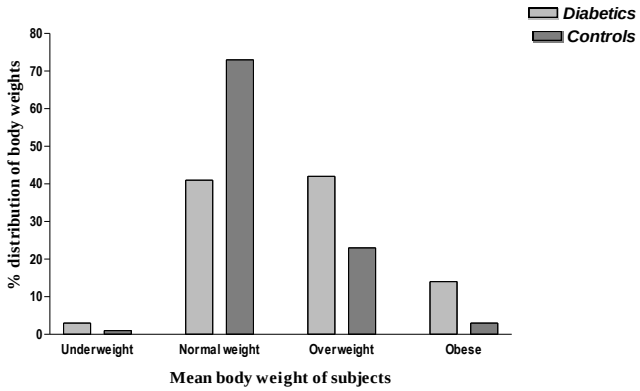

Figure 3: Percentage distribution of body weight within the study population. There was proportional significant difference between diabetic and non-diabetic subjects for overweight and obesity (Z=2.795, Z=2.601) respectively. No proportional significance difference (Z=0.555) existed for underweight between diabetics and non-diabetics.

Association between several correlates (age, body mass index, triglyceride, glycated haemoglobin, fasting blood glucose and high density lipoprotein cholesterol) with total testosterone was determined among diabetic subjects as shown in Table 3. Significant association with total testosterone was found for body mass index (p = 0.04), triglyceride (p = 0.025) and fasting blood glucose (p < 0.001). Thus elevated fasting blood glucose, raised body mass index and elevated triglyceride emerged as risk factors for low total testosterone levels in type 2 diabetic Ghanaian men. Table 3 shows the association of correlates (age, body mass index, triglyceride, glycated haemoglobin, fasting blood glucose and high density lipoprotein) with total testosterone levels of diabetic subjects. Body mass index (BMI), triglyceride (TG) and fasting blood glucose (FBS) were selected as independent explanatory variables for serum total testosterone. HbA1C: glycated haemoglobin, HDL-C: high density lipoprotein cholesterol.

Table 3: Multiple regression analysis of correlates with testosterone of diabetic subjects

Variables	Coefficients	Standard error	p-value	95% CI	
Age (years)	1.3928	0.7407	0.0630	-0.0770	2.8627
BMI (Kg/m^2)	-3.9429	1.9868	0.0400	-7.8857	-0.0002
TG (mmol/L)	31.4095	13.7968	0.0250	4.0302	58.7888
HbA1C (%)	2.9443	3.2353	0.3650	-3.4760	9.3647
FBS (mmol/L)	6.4923	1.8674	0.0008	2.7865	10.1982
HDL-C (mmol/L)	12.6551	19.2061	0.5115	-25.4588	50.7689

Table 3 shows the association of correlates (age, body mass index, triglyceride, glycated haemoglobin, fasting blood glucose and high density lipoprotein) with total testosterone levels of diabetic subjects. Body mass index (BMI), triglyceride (TG) and fasting blood glucose (FBS) were selected as independent explanatory variables for serum total testosterone. HbA1C: glycated haemoglobin, HDL-C: high density lipoprotein cholesterol.

DISCUSSION

Hypogonadism has been implicated in many disorders including diabetes mellitus (10, 12). Hypogonadism in this study was described as total testosterone levels < 8 nmol/L, with or without signs and symptoms. The prevalence of hypogonadism in type 2 diabetic men in this study was 35.2%. Others have shown prevalence ranging from 30 to 80% in men with type 2 diabetes (10, 13, 14). Thus type 2 diabetics were highly at risk of being hypogonadal, as indicated by lower total testosterone levels than non-diabetics. The differences observed in prevalence were because of the different populations studied and the definition used for the diagnosis of hypogonadism. It has been reported that lower total testosterone levels found in the diabetics could be as a results of increased conversion of the testosterone to estradiol (E_2) in the presence of insulin resistance and obesity (10, 12, 15). This study did not however reveal such trend (Table 2) but was in agreement with an earlier finding (16). An inverse association of fasting blood glucose, triglyceride and body mass index with total testosterone was observed in type 2 diabetic men (Table 3). A multiple regression analysis also revealed that fasting blood glucose, together with triglyceride and body mass index, synergistically played a role in lowering total testosterone levels in type 2 diabetic men (Table 3). This finding was supported by prior studies (10, 16). The association between glycemia and reduced total testosterone concentration may be an effect of glycemia on the testicular microvasculature. Glycemia alters Leydig cell function directly causing primary hypogonadism. This may account for why no association was observed between total testosterone levels and the gonadotropin hormones (FSH and LH) and prolactin in type 2 diabetic men. Prolactin attenuates LH secretion in males, leading to low testosterone levels. LH acts on Leydig cells of the testes stimulating them to synthesize and secrete the male sex hormone, testosterone. The insignificant difference in LH, FSH and prolactin between diabetics and non-diabetics indicate that the hypogonadism observed in this study was primary rather than secondary. In addition, low testosterone levels is as a result of glucose not reaching the cells due to insulin insensitivity, to provide enough energy for the various metabolic processes involved in maintaining testosterone levels. Testosterone levels correlated negatively with BMI (Table 3). This was in agreement with work done elsewhere (16-18). It has been suggested that increased abdominal fat leads to the liver being exposed to higher concentrations of free fatty acids.

The free fatty acids increase hepatic glucose production and decrease hepatic insulin uptake. This results in systemic hyperinsulinaemia and skeletal muscle insulin resistance, which in turn causes further release of insulin by the islet cells (19, 20). Triglyceride levels being significantly higher in the diabetic men than the non-diabetic men in this study (Table 3), was consistent with earlier work done (19, 21). Increased abdominal fat leads to increased aromatase activity (15, 16, 22). The resulting low testosterone increases lipoprotein lipase activity, the main enzymatic regulator of triglyceride uptake in adipose tissue. This results in inhibition of triglyceride uptake, increased lipid mobilization leading to increased visceral adiposity and insulin resistance. This in turn causes further hypogonadism and abdominal fat deposition (21, 23). An inverse association was found between total testosterone levels and the duration of diabetes in the present study (Figure 2). Thus, increase in years diagnosed for type 2 diabetes is strongly linked with decrease in total testosterone levels. This observation was supported by an earlier report (1).

CONCLUSION

Serum total testosterone concentration was certainly lower in a relatively large number of Ghanaian males with type 2 diabetes compared with healthy men. This study demonstrated that fasting blood glucose, triglyceride and body mass index were risk factors for hypogonadism in type 2 Ghanaian diabetic. This study also showed that these risk factors affected testicular microvasculature accounting for primary hypogonadism. The implications of the reduced serum total testosterone concentration in type 2 diabetic men however merit further investigation.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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