Study the association of testosterone levels with insulin resistance in Iraqi men with *Diabetes mellitus* type 2

دراسة علاقة مستويات التيستوستيرون مع مقاومة الأنسولين عند الرجال العراقيين المصابين بداء السكري نمط 2

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Abstract

This study was set to evaluate the testosterone serum levels and their association with insulin resistance to uncover if any related possible negative consequences in Iraqi patients with *Diabetes mellitus* (DM). Sixty six men with type 2 DM were randomly selected and compared to 18 healthy volunteers, demographic data were collected in addition to 10 ml of venous blood was drawn to evaluate serum levels of total Testosterone (T), free testosterone (FT), c-peptide, Sex Hormone Binding Globulin (SHBG), lipid profile, and blood sugar. The mean serum level of total testosterone was 2.434 ± 0.12 ng/ml in diabetic patients and 3.62 ± 0.32 ng/ml in non-diabetic subjects which was significantly different (p=0.003). The mean value of free serum level of testosterone was significantly higher in non-diabetic group (14.71 ± 1.4 pg/ml) in comparison with diabetic group (10.71 ± 0.54 pg/ml; p=0.017). Total testosterone correlates positively with FT and SHBG, while negatively with c-peptide, age and Body mass index (BMI). It's concluded that total testosterone, free testosterone, and SHBG were significantly lower in diabetic group which strongly associated with insulin resistance.

Key words: Testosterone, Type 2 Diabetes, SHBG, Insulin Resistance

الملخص

أعدت هذه الدراسة لتقييم مستويات هرمون التيستوستيرون المصلية و لتسليط الضوء على التأثيرات السلبية المحتملة المرتبطة بالتيستوستيرون و مقاومة الأنسولين في مجموعة من المرضى العراقيين المصابين بداء السكري نمط 2. ست و ستون رجلا مصابا بالسكري نمط 2 تم اختيارهم عشوائيا و قورنوا بـ 18 رجلا بصحة جيدة غير مصابين بالسكري, جمعت المعلومات المتعلقة بالتاريخ الطبي لكل شخص و كذلك تم أخذ عينة دم (10 مل) لغرض إجراء اختبارات هرمونات التيستوستيرون, و التيستوستيرون الحر, الببتيد C, بروتين SHBG, فحوصات انواع الدهون بالدم, و السكر بالدم. وجد أن متوسط مستوى التيستوستيرون المصلي الكلي لمرضى السكري كان (2.434 ± 0.12 ng/ml)و (3.62 ± 0.32 ng/ml) لدى مجموعة السيطرة و كان الاختلاف معنويا (p=0.003). متوسط قيم التيستوستيرون الحر سجلت ارتفاعا معنويا لدى مجموعة السيطرة (14.71 ± 1.4 pg/ml) مقارنة مع مجموعة السكري (10.71 ± 0.54 pg/ml; p=0.017) . وجد أيضا أن التيستوستيرون يتناسب طرديا مع التيستوستيرون الحر و SHBG بينما يتناسب عكسيا مع هرمون الببتيد C و العمر و معامل كتلة الجسم. يستنتج من هذه الدراسة بان كلا من التيستوستيرون, التيستوستيرون الحر, بروتين SHBG عانت مستوياتها انخفاضا معنويا بمجموعة السكري و ذلك يتناسب بشدة مع مقاومة الأنسولين.

الكلمات المفتاحية: التيستوستيرون، بداء السكري نمط 2,بروتين SHBG , مقاومة الأنسولين

Introduction

Diabetes mellitus (DM) is a syndrome of abnormal metabolism with inappropriate hyperglycemia due to either an absolute deficiency of insulin secretion or a reduction in the biologic effectiveness of insulin [1,2]. Over the last few years, there have been several reports demonstrating that men with type 2 *Diabetes mellitus* have a higher prevalence of low circulating testosterone levels compared with matched, healthy controls [3,4]. It is well documented that testosterone is the principal sex hormone in men. It is important not only for normal sexual function but also for maintaining bone and muscle strength, mental and physical energy [5]. Insulin resistance and visceral obesity are important features of type 2 diabetes. Studies have shown that free testosterone levels are low in obese men [6]. Type 2 *Diabetes* mellitus (T2DM) comprises an array of dysfunctions resulting from the combination of resistance to insulin action and inadequate insulin secretion; there is evidence that testosterone levels are inversely associated with insulin resistance [7]. There is further evidence suggesting that a low testosterone is a risk factor for development of metabolic syndrome and diabetes [8]. However, low levels of sex hormone binding globulin (SHBG), the main carrier protein of testosterone in the circulation, may be independently associated with the risk of type 2 diabetes and is strongly associated with insulin resistance [9,10]. In men, however, the low level of plasma testosterone has

been observed to be associated with obesity, upper body fat distribution, and increased level of glucose and insulin [11]. Men with chronic conditions, such as diabetes, obesity, hypertension, hyper lipidemia, and asthma are more likely to have low testosterone level compared to normal population [12]. The purpose of the present study, was (i) to evaluate the testosterone levels and some other hormones and parameters in Iraqi patients with *Diabetes mellitus* type 2, (ii) figure out to how extension of insulin resistance could affect testosterone level, (iii) uncover if any other related possible negative consequence.

Research design and methods

This research was designed as a case-control study. Population-based samples of (66) men with type 2 *Diabetes mellitus* (T2DM) aged (24-65) years were randomly selected in the national center for diabetes researches, a referral center affiliated with Al-Mustansirya University, Baghdad. The study was done with male patients referred to the center for management of diabetes. Eighteen apparently healthy volunteers with no medical history of diabetes aged (27-59 yr.) were included in this study as a control group. Patients with known history of hypogonadism, hyperpituitarism, or other chronic diseases such as renal or hepatic failure, cirrhosis or those who used exogenous hormones or medications which might affect sex hormone level were excluded from the study. Demographic data were collected from participants; height (m), weight (kg), Body mass index (BMI) (kg/m^2), and waist circumference (cm) were measured. A venous blood sample (10 ml) was taken at the early morning (8:00-11:00 am), serum samples were obtained by centrifugation and immediately frozen at (-20 C°) pending for analysis later in the general and immunology labs. of the biotechnology research center/Al-Nahrain University, Baghdad. Enzyme-Linked Immunosorbent Assay technique (ELISA) was used to measure Total testosterone (Human, Germany), SHBG, free testosterone and C-Peptide (Demeditec Diagnostics, Germant). Fasting blood sugar, lipid profile (LDL-cholesterol, HDL-cholesterol, Triglycerides, Cholesterol) were measured using biochemical methods (Human, Germany).

Statistical Analysis:

All analyses were performed using Minitab program for windows OS. Data were presented as mean ± SEM. For the assessment of correlation between variables, Pearson correlations were used; correlation coefficient (r) was calculated. Statistical difference was set at $p \leq 0.05$. T-student test (t) was used to compare variables.

Results:

Our results should that 62 % (44) of diabetics were hypertensive, vs. 12 % (2) of control group figure (1), it has been observed that 20 men 31% with T2DM were smokers, vs. 3 (19%) smokers in non-diabetic subjects. As for ABO system, in diabetic group, (A=25%; B=15%; AB=13%; O=47%), while in control group was (A=38%; B=15%; AB=8%; O=39%). Ninety six percent of diabetic subjects were Rh^+, whereas 69% of control group were Rh^+ figure (1).

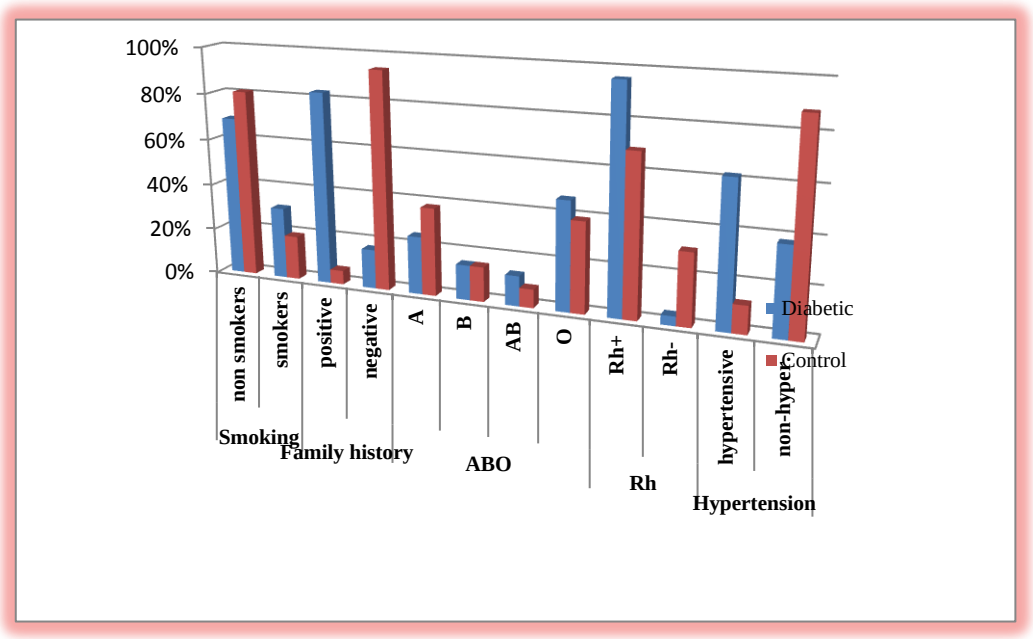

Fig. (1): Sociodemographic data of the study groups.

Regarding family history of diabetes, it has been figured out that there were a dramatic clear difference for those who have positive family history of diabetes in different degree of relatives whether first, second, or third degree. 83% of diabetic men recorded a positive family history, while only 6 % of non-diabetic subjects had a positive family history figure (1). Of the patients with T2DM, 26.5% received Sulphonylureas, 23 % received Metformin, and 40.6 % were on both Sulphonylureas and Metformin. About 10% of men also received insulin in combination with oral hypoglycemic therapy figure (2).

In this study 66 of type 2 *Diabetes mellitus* patients were randomly selected and compared to 18 control group. Table (1) shows the results of all individual T2DM and non-diabetic (control group) represented as a mean value ± standard error of the mean (mean ± SEM). Regarding the age, the mean age of patients was 46.47 ± 1.1 yr, vs. 41.94 ± 2.3 yr, no significant difference between the two groups ($p \geq 0.05$). Both BMI and waist circumference had significantly higher results in T2DM group than control group (30.34 ± 0.5 vs. 27.5 ± 0.93 kg/m^2; p=0.013); (105.9 ± 1.8 vs. 95.6 ± 3.0 cm; p=0.006) respectively.

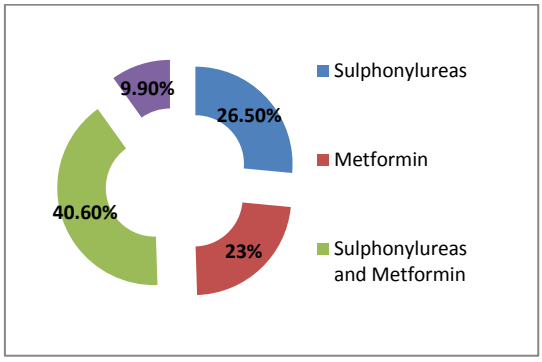

Fig. (2): Percentages of Hypoglycemic treatments taken by diabetic patients.

Table (1): Comparison of variables studied in T2DM population and non-diabetic control group.

Parameter	Diabetic group (mean ±SE)	Non-Diabetic group (mean ±SE)	p-value	Significancy
Age (year)	46.47 ± 1.1	41.94 ± 2.3	0.09	NS
BMI (kg/m2)	30.34 ±0.5 ↑	27.5 ± 0.93	0.013	HS
Waist Circumference (cm)	105.9 ± 1.8 ↑↑	95.6 ± 3.0	0.006	VHS
FBS (mg/dl)	119.2 ± 1.5 ↑↑	90.16 ± 2.3	0.00001	VHS
C-peptide (ng/ml)	5.06 ± 0.35 ↑↑	3.62 ± 0.31	0.003	VHS
SHBG (nmol/l)	65.8 ± 3.4 ↓	85.0 ± 7.6	0.034	HS
Total Testo. (ng/ml)	2.434 ± 0.12 ↓↓	3.62 ± 0.32	0.003	VHS
Free testosterone (pg/ml)	10.71 ± 0.54 ↓	14.71 ± 1.4	0.017	HS
Cholesterol (mg/dl)	174.1 ± 5.8 ↑↑	142.0 ± 6.0	0.00001	VHS
Triglyceride (mg/dl)	218.8 ± 9.5 ↑↑	133.7 ± 8.4	0.00002	VHS
LDL-Cholesterol (mg/dl)	92.6 ± 5.1 ↑	75.7 ± 4.8	0.021	HS
HDL-Cholesterol (mg/dl)	44.5 ± 6.8	37.73 ± 1.4	0.333	NS

NS: Non-Significant; HS: Highly significant; VHS: Very highly significant

The mean of FBS was 119.2 ± 1.5 mg/dl in T2DM group and 90.16 ± 2.3 mg/dl in control group, which reached very high significant level ($p \leq 0.00001$). The mean of c-peptide was also significant between the two groups (5.06 ± 0.35 vs. 3.62 ± 0.31 ng/ml; p=0.003). The mean of sex hormone binding globulin (SHBG) in T2DM group was 65.8 ± 3.4 nmol/l and in control group was 85.0 ± 7.6 (p=0.034). Both total testosterone and free testosterone showed a significant decrease in diabetic patients (2.434 ± 0.12 ng/ml and 10.71 ± 0.54 pg/ml) than non-diabetic subjects (3.62 ± 0.32 ng/ml and 14.71 ± 1.4 pg/ml) p=0.003, p=0.034 respectively Table (1). In terms of lipid profile, it has been found that both total Cholesterol and triglycerides were increased very high significantly in T2DM group comparing to control group (cholesterol: 174.1 ± 5.8 vs. 142.0 ± 6.0 mg/dl; $p<0.0001$) and (triglycerides: 218.8 ± 9.5 vs. 133.7 ± 8.4 mg/dl; $p \leq 0.0002$) respectively. LDL-Cholesterol level was also increased significantly (p=0.012), while HDL-Cholesterol did not Table (1).

Correlations

Total testosterone correlated very strongly and directly with free testosterone (r=0.633; $p<0.00001$) as well as with SHBG (r=0.414; $p \leq 0.00001$), while it correlates negatively with both BMI (r=-0.364; p=0.002) and body weight (r=-0.278; p=0.043) but not with waist circumference Table (2). In addition we have found that testosterone correlates inversely with age (r=-0.281; p=0.015) and c-peptide (r=-0.366; p=0.002).

Table (2): Correlation of Total Testosterone with other parameters in male diabetic group.

Variable	r	*p*
Free testosterone	0.633	< 0.00001
SHBG	0.414	< 0.00001
C-Peptide	-0.366	0.002
Age	-0.281	0.015
BMI	-0.364	0.002
Body weight	-0.278	0.043
Waist circumference	-0.272	0.114

SHBG correlates positively with age (r=0.43; p=0.002) and directly with free testosterone (r=0.324; p=0.011) see figure (3). Free testosterone correlated strongly but inversely with age (r=-0.523; $p \leq 0.0001$) and also with BMI (r=-0.311; p=0.045) figure (4).

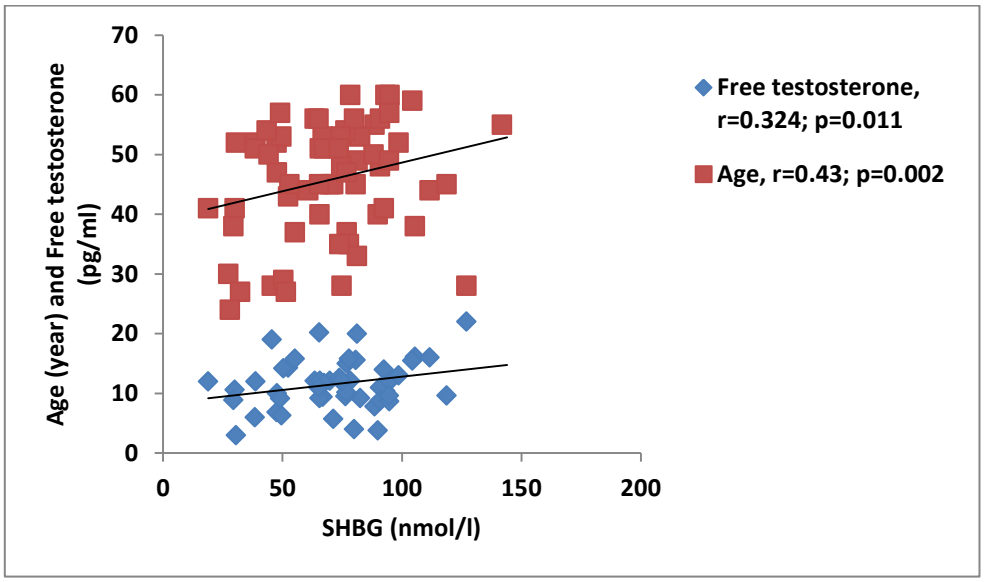

Fig. (3): Correlation of SHBG (nmol/l) with Age (year) and Free testosterone (pg/ml).

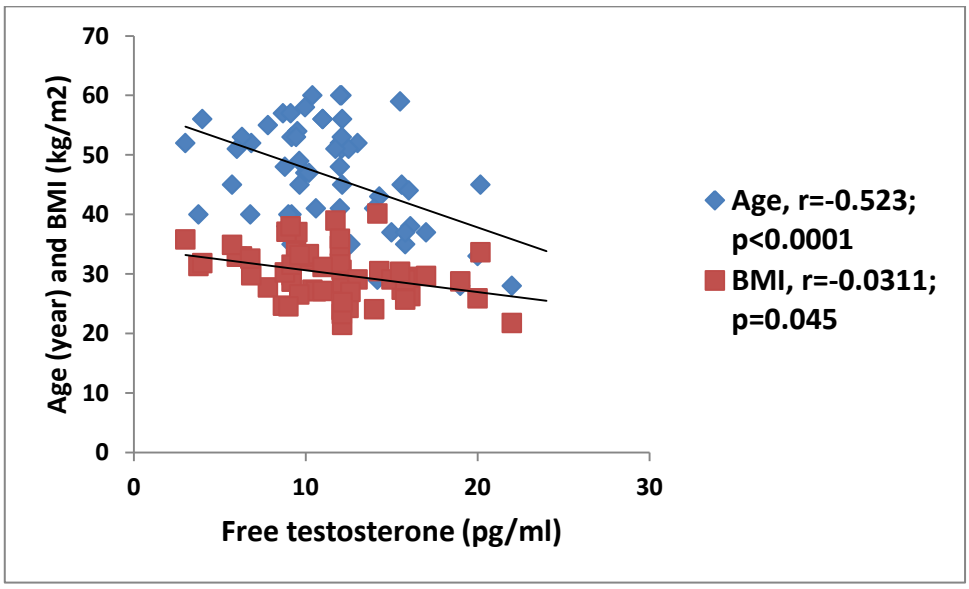

Fig.(4): Correlation of free testosterone (pg/ml) with Age (year) and BMI (kg/m2).

Discussion

Testosterone is a key-player in glucose homeostasis and lipid metabolism [13], serum triglycerides, total cholesterol, LDL-cholesterol were higher in diabetic men, the fact that testosterone is a significant factor in this difference in risk profile is demonstrated by observations in men receiving androgen ablation treatment for prostate carcinoma, resulted in a rather acute and profound decline of serum testosterone. In these men, there is an increased serum levels of cholesterol, LDL-cholesterol, and triglycerides and a decrease in HDL-cholesterol [14], these results agree with Vikan [15]. Our results registered a clearly visible higher positive family history of diabetes frequency among diabetic patients versus non-diabetic subjects, family history is relatively easy to obtain and conveniently conveys information on genes and environment shared by close relatives. The association between family history of diabetes and risk for the disease has been well documented [16,17].

In the current study, we have found a significant decrease of SHBG conc. Table (1) in diabetic group comparing to non-diabetic group, these results resembles other results obtained by Afkhami-Adrekani [18]

who found a significant difference in SHBG in T2DM group and control group. Onat [19] found that low SHBG level is associated with an increasing incidence in male only in absence of obesity [19], the same results coincident with the findings of Lakshman [20], we found also that SHBG is negatively correlated with age, this agrees with findings of Salem [21]. Insulin is known to inhibit hepatic production of SHBG [22], and SHBG levels fall acutely during hyperglycemic-euglycemic clamp studies [23]. Jayagopal [24] suggested that a low SHBG concentration is a stable integrated marker of insulin resistance and therefore has the characteristics to be potentially used as a surrogate measure of insulin resistance, perhaps in monitoring the response of an individual to insulin sanitizers.

It has been found that the mean of total and free testosterone in men with diabetes was lower than control group. Total testosterone and SHBG were also lower in the diabetic men than in control group. In cross-sectional studies, low concentrations of testosterone and SHBG have been associated with visceral obesity, insulin resistance or hyperinsulinaemia and dyslipidaemia [25-29]. It's well documented that visceral obesity is an important cause of insulin resistance [3], in addition, our findings support the hypothesis that circulating testosterone levels in men with diabetes may be influenced by insulin sensitivity and vice versa. In our work we measured c-peptide "a 31 amino acid residue peptide, it connects A and B chains of insulin [30]"; since c-peptide and insulin are secreted in equimolar amounts, the immunoassay of it permits the quantitation of insulin secretion [31]. Reports showed that male mice with a targeted deletion of androgen receptor have increased blood glucose levels due to insulin resistance [32]. Men with Klinefelter syndrome have increased insulin resistance [33]. Particularly, visceral adiposity is an important cause of insulin resistance and also decreases testosterone levels through conversion to estradiol by aromatase [7]. Hyperinsulinaemia, as encountered in insulin resistance, might impair testosterone secretion by the leydig cell may be directly since there are insulin receptors on the Leydig cells [34]. It has also been found in obese men that there is an attenuated pulse amplitude of Luteinizing hormone (LH), thus producing a less strong stimulation of testicular testosterone production [29]. In our study as well as others [25,35,36], testosterone levels in men with type 2 diabetes were correlated with BMI Table (2), although BMI and weight are suboptimal markers of visceral adiposity, previous studies have reported an association of loss of weight in obese insulin-resistant men with increased testosterone levels [37]. Dhindsa [26], found in a multiple linear regression model using Testosterone, BMI, and SHBG. Both BMI and SHBG were independent predictors of testosterone, thus, it seems that in diabetics, BMI has an effect on Testosterone independently of SHBG concentrations. In the present study, men with T2DM had lower mean levels of free testosterone (FT) Table (1) than non-diabetic men, the usual explanation stated in review articles [38,39] is the presumption that low testosterone is a consequence of the visceral obesity and aging or that the fall in SHBG associated with obesity in diabetics will result in an inadequate level of FT [40]. It has been found that FT correlates inversely with body mass index and a very high significant inverse correlation figure (4) with age. It is commonly accepted that the fat mass increases with age, resulted into the higher BMI noted during aging [41], due to the presence of aromatase in the fatty tissue which lead to decreased testosterone levels (3) and essentially decreased free testosterone levels as we had a very high positive correlation between testosterone and FT Table (2). In a Massachusetts Male Aging Study [9], a cohort population-based study of men aged 40-70 years, they observed that mean testosterone level was significantly lower among men who later developed diabetes in comparison with mean levels of free testosterone and SHBG. But after controlling for all other factors in the model, lower levels of free testosterone and SHBG jointly and independently predicted incident diabetes. Haffner [42] reported a retrospective association of diabetes with SHBG and free testosterone but not with total testosterone. Conversely, Tibblin et al. found an independent association for testosterone but only a marginally significant association for SHBG [43].

Conclusion

In our study, total testosterone, free testosterone and SHBG were significantly lower in diabetic group which strongly associated with insulin resistance (type 2 diabetic men), whereas, hyperlipidaemia, BMI, and waist circumference were registered in a sample of Iraqi diabetic patients, hypogonadism (low T and FT levels) is a common defect in type 2 diabetes that requires further assessment in terms of the etiology of the defect and the possible consequences, complications and treatment.

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