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Correlation of Aging and Body Mass Index with the Hypothalamic-Pituitary-Gonadal Axis Hormones in Men, with Diabetes Mellitus

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Abstract: *Objective:* The objective of this retrospective and cross sectional study was to assess the correlation of aging and body mass index (BMI) with hormones from hypothalamic-pituitary-gonadal (HPG) in patients with and without diabetes mellitus (DM).

Research Design and Methods: Electronic medical records of 605 patients were selected using specific exclusion criteria. The subjects were systematically divided into two experimental groups, with and without DM.

Results: There was a significant negative correlation between age and serum free testosterone and significant positive correlations between age and serum LH and FSH in both groups. BMI was significantly higher in diabetics. In diabetics, BMI was negatively correlated with free testosterone, but no relationship with LH or FSH existed. In contrast, in non-diabetics, BMI was negatively correlated with LH and FSH only. In diabetics, a significant reverse correlation between HbA1c and free testosterone was present and they also had significantly lower FSH. Inappropriately normal LH with low free testosterone levels were seen in majority of patients from both groups. But more diabetics had inappropriately normal FSH with low free testosterone, 88% of diabetics and 70% of control group $p<0.01$.

Conclusion: The data from this cross sectional study showed aged men, in both cohorts, had lower free testosterone and higher LH and FSH. Patients with good blood sugar control had higher serum free testosterone. In addition, FSH was significantly lower and impaired FSH response to low free testosterone was more pronounced in subjects with diabetes.

Keywords: Hypothalamic-pituitary-gonadal function, aging, diabetes mellitus, obesity.

INTRODUCTION

Hypogonadism in elderly men, manifested by decreased libido and poor sexual performance, has often either not been expressed, denied by patients or even ignored by physicians. Several longitudinal and epidemiological studies reported the presence of hypogonadism in aged men [1-10]. Low serum testosterone level may be accompanied by normal or elevated luteinizing hormone (LH), and follicle-stimulating hormone (FSH) and sexual function may be preserved in some patients. In addition to aging, diabetes mellitus has also been associated with hypothalamic-pituitary-gonadal (HPG) axis suppression [11-15]. Whether the primary source of gonadal failure resides in the testicles, the pituitary, or the hypothalamus, the ultimate result is diminished serum testosterone concentration. Inadequate production of testosterone may cause various complications such as osteoporosis [16, 17] and diminished level of physical and emotional energy [18]. Testosterone deficiency in aging men can be treated with testosterone replacement and the benefit may be significant in some cases [19-21]. Furthermore, testosterone therapy may also improve glucose homeostasis [22, 23].

We have designed this cross sectional study to ascertain the correlation of aging, BMI, and DM on HPG axis function in relatively healthy, community dwelling male subjects with and without DM.

RESEARCH DESIGN AND METHODS

In this cross sectional study we have retrospectively assessed the laboratory results of 605 individuals out of total 700 subjects (age 29-89 years) who had HPG axis hormones measured over the time period 10/1/97-6/31/00. This data was retrieved through the electronic medical record system of Hines VA Hospital and the study was approved by the institutional Human Studies Committee. The subjects were attending various medical clinics and all the medical information was available in their electronic medical file. Diabetes mellitus was diagnosed by criteria from the American Diabetes Association. The majority of patients with diabetes were on one or more therapeutic agents. Those patients not taking any anti-diabetic medication with normal HbA1c and normal blood sugar assessed multiple times were assigned as subjects without diabetes. HPG function was assessed on 2-3 occasions over a one month period and all the blood samples were obtained 2-3 hrs after rising between

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1876-827X/10

8-11 AM. The mean value for hormonal levels and HbA_1C were used for this study.

First, we collected the data with regard to free testosterone, LH, and FSH, all assessed 2-3 times. Then, we applied the exclusion criteria to eliminate patients suffering from variety of diseases that could have influenced gonadal functions. We have measured serum free testosterone to bypass the ill effect of some medication and possible effect of some illness on sex hormone binding globulin [24]. All measures used in this study were available in the electronic data base.

The exclusion criteria were: 1) current active endocrine disorders ; 2) pituitary or testicular surgery or radiotherapy; 3) renal insufficiency (serum creatinine $\geq$ 2 mg/dl); 4) advanced cardiac disease, NYHA class III and IV; 5) gastrointestinal problems that caused malabsorption syndrome (low albumin level and anemia); 6) overt alcoholic liver disease or hepatitis with consistently elevated liver enzymes (>2 fold), bilirubin, prothrombin time or low albumin; 7) active malignancies; 8) autoimmune disorders ; 9) COPD; 10) use of medications that might influence the function of gonadal axis such as glucocorticoids, psychoactive drugs, and anti- androgens.

The serum free testosterone levels were measured by a combination of equilibrium dialysis, extraction, chromatography, and radioimmunoassay (Quest Diagnostics Laboratory, Nichols institute, California, 92690). This methodology is considered as the gold standard to assess serum testosterone level. The normal serum free testosterone ranged from 50-210 pg/ml (173-728nmol/L). The inter-assay variation was 4.7 pg/ml with CV=5.5%. The intra-assay variation was 4.6 pg/ml with CV=4.3%. Two-site sandwich immunoassay using direct chemiluminometric technology was used to measure the serum LH and FSH and the assays were performed at Hines VA Hospital, using kits purchased from Bio-Rad Diagnostics Group (Hercules, California). The sensitivity of assays was 1-200 miu/ml for LH and FSH. The inter-assay coefficient of variation was 10% for LH and 6.7% for FSH and intra-assay coefficient of variation was 3.8% for LH and 4.5% for FSH. HbA_1C assay was also performed in hospital main laboratory using HPLC methodology. All the other laboratory assessments (chemistries) were performed in the hospital main laboratory.

Statistical Analysis

Correlation analyses were conducted using Pearson's correlation coefficient. T-tests for independent groups were used to identify differences in continuous variables and chi-square analyses were used to determine relationships between categorical variables. All analyses were conducted using SAS® Version 6.12 and all analyses were considered significant for $p< 0.05$. Data are expressed as mean $\pm$ SEM.

RESULTS

General characteristics of the two experimental groups, with and without diabetes, are shown in Table **1**. The subjects with diabetes were younger, 65.4 $\pm$10.6 vs. 67.6$\pm$ 11.2 years of age, $p<0.01$; had significantly higher BMI, 30$\pm$5.7 vs. 27.3$\pm$5.1 Kg/m^2 , $p<0.001$; and lower serum FSH level, 7.9$\pm$0.52 vs. 12$\pm$ 1.05 IU/l, $p<0.0005$ (Table **1**) but there were no significant differences between serum free testosterone and LH levels for two experimental groups. Approximately 7% of the individuals were younger than 50 years of age and 48% were older than 70 years of age (data not shown)

There was a significant negative correlation between age and free testosterone in subjects with and without diabetes, r= - 0.14639, $p<0.02$ and r= - 0.14783, $p<0.004$, respectively. Significant positive correlations existed between age and LH, r=0.20812, $p<0.004$, and r= 0.18693, $p<0.003$ in subjects with without diabetes, respectively. The correlations between age and FSH were also positive r= 0.32417, $p<0.0001$ and r= 0.19124, $p<0.005$ in subjects with and without diabetics, respectively (Table **2**).

There was a significant negative correlation between BMI and free testosterone in diabetic men, r= -0.14100, $p<0.03$, but there was no relationship between BMI with LH and FSH in this group. In contrast, in non-diabetic individuals (with higher BMI), there was no relationship between BMI and free testosterone but there was a significant inverse correlation between BMI and LH, r= -0.18219, $p<0.005$ and between BMI and FSH, r= -0.20303, $p<0.004$ (Table **3**).

Significantly lower serum free testosterone levels were associated with higher age, FSH, LH, and BMI in both groups when multiple regression analysis was used. In diabetics, significantly lower levels of free testosterone were associated with higher HbA1C levels, r= -0.2247, $p<0.007$

Table 1. Variables for Both Non-Diabetics and Diabetics. LH = Luteinizing Hormone; FSH = Follicle-Stimulating Hormone; HbA_1C= Glycated Hemoglobin; BMI = Body Mass Index, n=Number of Patients

Variables	Mean Values $\pm$SEM(n)			Normal Values
	DM	NDM	p value	
Age (years)	65.4$\pm$10.6(238)	67.6$\pm$11.2(367)	**p<0.01**	
BMI(Kg/m^2)	30$\pm$5.7(233)	27.3$\pm$5.1(350)	**p<0.001**	20-25
Free T (pg/ml)	71.7$\pm$1.73(236)	71.8$\pm$2(369)	NS	50-210
LH(miu/ml)	6.7$\pm$0.45(188)	7.6$\pm$0.54(252)	NS	1-9
FSH(miu/ml)	7.9$\pm$0.52(139)	12$\pm$1.05(112)	**p<0.005**	1-18
HbA1c%	7.8%$\pm$1.7%(238)	NA(367)		4.1%-6.5%

Table 2. Correlation Analysis Between Age and Free Testosterone; Luteinizing Hormone (LH); Follicle-Stimulating Hormone (FSH); n = Number of Patients

	Free Testosterone	LH	FSH
Group with DM	r = - 0.14639	r = 0.20812	r = 0.32417
	$p \leq 0.02$	**$p \leq 0.004$**	**$p \leq 0.0001$**
	n = 236	n = 188	n = 139
	r = - 0.14783	r = 0.18693	r = 0.19124
Group without DM	**$p \leq 0.004$**	**$p \leq 0.003$**	**$p \leq 0.005$**
	n = 369	n = 252	n = 212

Table 3. Correlation Analysis Between Body Mass Index (BMI) and Free Testosterone; Luteinizing Hormone (LH); and Follicle-Stimulating Hormone (FSH); n = Number of Patients

	Free Testosterone	LH	FSH
	r = -0.14100	r = -0.11560	r = 0.02077
Group with DM	**$p \leq 0.03$**	$p \leq 0.1$	$p \leq 0.8$
	n =233	n =185	n =137
	r = -0.02790	r = -0.18219	r =-0.20303
Group without DM	$p \leq 0.6$	**$p \leq 0.005$**	**$p \leq 0.004$**
	n =350	n =241	n =196

(data not shown).

Of interest, the majority of patients from each experimental group with low free testosterone levels from each experimental group had inappropriately normal LH and FSH (Table **4**). Elevated serum LH was seen in only 32% of subjects with DM and 37% of those without DM. Interestingly, the number of subjects with diminished response of mainly FSH to low free testosterone was more prevalent in diabetics than non-diabetics, 88% vs. 70%, $p<0.01$. Surprisingly, elevated serum LH and FSH with normal serum free testosterone were equally noted in groups, 9%vs14% for serum LH and 7%vs 6% for serum FSH (Table **4**).

DISCUSSION

This cross sectional study was designed to assess the correlation of age and BMI with HPG function in patients with and without DM, using the available data in the local electronic records. The subjects with DM were significantly younger and had higher BMI and lower FSH level compared to those without DM (Table **1**). We found a significant inverse correlation between age and free testosterone level and a positive correlation between age and serum LH and FSH levels in both cohorts (Table **2**). The decline in the levels of serum free testosterone became more substantial from fifth decade of life and a constant rate of decline, 1-2% per year, were noted thereafter (data not shown). Overall, low free testosterone levels were seen in 17.7% of total cohort. Our data concurs with many cross sectional and longitudinal studies with various designs on this subject. In a cross-sectional study 40% of men younger than 70's had free testosterone levels below those from young adults; while mean serum total testosterone declined after age 70 [1]. But others demonstrated only a continuous decline in free testosterone after the age70 [10]. A gradual decline in the free testosterone index [25] and in total testosterone [4] was also noted. The gradual decline of free testosterone accompanied with high level of LH and FSH have been reported in some studies [3, 6]. But, a cross sectional study from United Kingdom reported a decreasing serum total testosterone, increasing sex hormone binding globulin accompanied with unchanged immuno-active LH coupled with diminished bioactive LH as men aged [5]. And "the Osteoporotic Fractures in Men Study", a prospective study, showed a great individual variation in serum free and total testosterone [7].

The relationships between BMI, insulin resistance, blood glucose, and HPG axis function are complex. It is commonly accepted that the volume of the fat mass increases with age results into the higher BMI noted during aging [26]. Both generalized obesity and/or centrally deposited fat affect insulin function and testosterone production [27-29]. Due to the presence of aromatase in the fatty tissue, the ability of the aged men to convert testosterone to estradiol results I increase on estradiol level which increases suppress serum LH and possibly FSH levels [30,31]. Fatty tissue also produces leptin which can influence HPG function at various

Table 4. Correlation Analysis Between Free Testosterone and Luteinizing Hormone (LH); and Follicle-Stimulating Hormone (FSH) in Diabetics and Non-Diabetics

		LH 1-9mIu/ml		**LH>9miu/ml**		**FSH1-18miu/ml**		**FSH>18miu/ml**	
		With DM	Non-DM	With DM	Non-DM	With DM	Non-DM	With DM	Non-DM
FT	<50	68%	63%	32%	37%	88%	70%	12%	30%
Pg/ml	50-200	91%	86%	9%	14%	93%	94%	7%	6%

levels [32-35]. To our surprise we found a significant negative correlation between BMI and free testosterone in diabetic subject (with higher BMI) but not in control group. But the negative correlation was present between BMI and LH and FSH in control group with significantly lower BMI (Table **3**). In diabetics, this data indicates the higher BMI does not influence free testosterone level via suppressed LH and FSH. But most probably the suppressing effect of high BMI, in diabetics, on testosterone production is implemented by factor(s) other than lowering LH and FSH may be estradiol or inhibin.

We found no significant differences in free testosterone levels between the two groups (Table **1**), not the same as other reports [14, 15, 36]. But, our data shows a significant inverse correlation between the level of free testosterone and HbA1c in diabetics. This discrepancy could be partially due to the lower level of HbA1c in our study subjects, 7.8 ± 1.7 vs. 8.4 ± 0.3 [13] and/ or us using a very sensitive free testosterone assay. Furthermore, while serum LH levels between the two groups were the same, FSH levels in diabetics were significantly lower than those from non-diabetics (Table **1**). A number of studies have shown reduced levels of either total or free testosterone with inappropriately normal LH or FSH in subjects with diabetes mellitus and have concluded that the main defect in diabetics may be located at hypothalamic or pituitary areas [9-13, 37]. But, another study found a normal GnRH stimulation test in patients with type 2 DM [38]. We have found that majority of individuals in both cohorts with low free testosterone levels had inappropriately normal levels of LH and FSH. However the correlation was different between the two treatment groups. We found 88% of diabetics with low free testosterone had inappropriately normal FSH compared to 70% in non diabetics, P=0.05 (Table **4**). In contrast 9% of diabetics with normal testosterone had elevated LH compared to 14% in non diabetics, p=0.05 (Table **2**). These findings possibly suggest presence of a defective response in hypothalamus and/or pituitary to low free testosterone in aged diabetics more than non-diabetics.

In summary, in this cross sectional study we found a negative correlation between age and free testosterone and positive correlation between age and LH and FSH in both cohorts. Although serum free testosterone levels were the same in both cohort, higher serum free testosterone levels were seen in subject with better glycemic control. BMI, as an independent factor, had a negative correlation with free testosterone in diabetic subjects and with serum LH and FSH in non-diabetic subjects. While hypothalamic-pituitary function decreased with age, the presence of diabetes had aggravated this defect, FSH production was more affected than LH in patients with DM.

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Received: November 19, 2009 Revised: December 18, 2009 Accepted: January 28, 2010