Serum High-Sensitivity C-Reactive Protein and Endogenous Sex Hormones in Diabetic Men.

المستويات المصلية لبروتين C التفاعلي عالي الحساسية والهرمونات الجنسية الداخلية في الرجال المصابين بداء السكري

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Abstract

The objective of this cross sectional study was to assess the effect of diabetes mellitus (DM) type 2 in men on endogenous sex hormones: estradiol (E2) and total testosterone (TT); pituitary gland hormones: follicle-stimulating hormone (FSH) and luteinizing hormone (LH) as well as high sensitive C-Reactive protein (hs-CRP) in men. This study comprised a total of (80) subjects out of which (40) were normal and (40) were diabetic males. The results obtained indicated a significant increasing ($p \leq 0.05$) of serum hs-CRP and E2 in men with DM type 2 comparison to non-diabetics, while a significant reduction ($p \leq 0.05$) of serum testosterone in men with DM type 2 comparison to non-diabetics, and non-significant reduction of serum LH, FSH in men with DM type 2 comparison to non-diabetics were observed. The data from this study show the role of endogenous sex hormones and hs-CRP in diabetes risk. Testosterone levels are partly influenced by insulin resistance, which may represent an important avenue for intervention.

Key words: diabetes mellitus type 2, hs-CRP, FSH, testosterone

الملخص

تهدف الدراسة الحالية لتقييم تأثير مرض السكري من النوع الثاني في الرجال على الهرمونات الجنسية (T. testosterone) وهرمون (estradiol) ولهرموني الفص الامامي للغدة النخامية (follicle-stimulating hormone) و (luteinizing hormone) بالإضافة الى البروتين التفاعلي نوع C عالي الحساسية (hs.CRP). أجريت الدراسة على 40 رجل عراقي مصاب بداء السكري النمط الثاني، ولغرض المقارنة اعتمد 40 رجل من الأصحاء (السيطرة). اظهر المستوى المصلي للبروتين التفاعلي نوع C عالي الحساسية و لهرمون الـ (estradiol) زيادة معنوية ($p \leq 0.05$) في المرضى مقارنة بالاصحاء، بينما أنخفض معنويا المستوى المصلي لهرمون الشحمون الخصوي ($p \leq 0.05$) في المرضى بالمقارنة مع الاصحاء، كما واظهر المستوى المصلي لهرموني الفص الامامي للغدة النخامية (FSH وLH) انخفاضا غير مهم من الناحية المعنوية في المرضى بالمقارنة مع الاصحاء. نستنتج من ذلك ان انخفاض مستوى هرمون الشحمون الخصوي وزيادة مستوى هرمون ال E2 والبروتين التفاعلي نوع C عالي الحساسية يتزامن مع زيادة خطورة داء السكري النمط الثاني. ان مستويات هرمون الشحمون الخصوي تتأثر جزئيا بمقاومة الانسولين والتي تمثل اهم الية تقود للإصابة بمرض السكري من النوع الثاني.

الكلمات المفتاحية: مرض السكري النوع الثاني، البروتين التفاعلي نوع C عالي الحساسية، testosterone , FSH

Introduction

Most elderly men presenting with diabetes type 2 suffer from the metabolic syndrome. The metabolic syndrome is an insulin resistance (IR) syndrome with simultaneous occurrence of abdominal obesity, impaired fasting glucose, impaired glucose tolerance or overt type 2 diabetes, dyslipidemia and hypertension [1].

Testosterone deficiency is common in men with type 2 diabetes [2], in whom it may contribute to impaired performance, mood, and libido [3]. Recently, the association between late-onset hypogonadism and type 2 diabetes mellitus (T2DM) has been demonstrated in numerous studies, indicating that up to 40% of men with T2DM have testosterone deficiency, and up to 75% of them have sexual dysfunction, particularly erectile dysfunction [4].

Low serum testosterone level may be accompanied by normal or elevated LH, FSH and sexual function may be preserved in some patients. In addition to aging, T2DM has also been associated with hypothalamic-pituitary-gonadal (HPG) axis suppression [5,6]. Furthermore, a study suggest that low concentrations of endogenous androgens such as testosterone had been related to the development of

insulin resistance, which is an important cause for inducing type 2 diabetes and other abnormalities including hyperglycemia, hypertension, dyslipidemia, or carotid atherosclerosis [7].

C-reactive protein (CRP) is an acute phase reactant and a sensitive marker of inflammation. Several studies support the concept that hs-CRP, even when within the clinical normal range, is an important precursor of the metabolic syndrome (MetS) and type 2 diabetes [8-10].

In addition to that many epidemiologic studies have shown that body mass index (BMI), a general measure of obesity, is a powerful predictor of type 2 diabetes [11,12].

The age-related changes in neuroendocrine functioning that lead to a diminished efficacy of LH stimulation of the Leydig cell and impairments of the steroidogenic process of testosterone synthesis are probably inherent factors in the age-related decline of circulating testosterone levels [11].

Material and Methods

The present study was carried out in the National Diabetes Center for Treatment and Research at Al-Mustansiriya University between July 2012- March 2013. A total of 40 patients of type2 diabetes mellitus (males of age group37-66 years). The diagnosis of T2DM was made on the basis of the recommended criteria by WHO [13]. Forty age and sex matched (males) healthy individuals served as controls who attended for routine health check up at the center. None of the healthy control was taking any medicine or dietary supplement; they were selected after detailed physical examination and laboratory tests.

Samples collection: After 12 hrs fasting, 5 ml venous blood sample was collected in plain tubes, the samples were allowed to clot for half an hour following which a samples were centrifuged for 15 minutes at 4000 rpm. Then serum was stored immediately at -20C until use.

Serum glucose was determined by a glucose oxidase method [14].

Serum concentrations of hs-CRP was determined by ELISA using a commercial kit manufactured by Biosource Europe S. A, Belgium. Estradiol (E2), TT, LH and FSH were determined by ELISA using commercial kits (DRG diagnostics Company, Germany and USA). Micro ELISA system (washer & reader) and Incubator were used in ELISA determination.

Weight was measured using standardized beam weight scales without footwear and with only light clothes. Height was measured with the subjects barefoot and standing with the feet together. BMI is defined as weight in kilogram per height in meters squared and is independent of gender and age.

Statistical Analysis

All Data are presented as mean ± standard deviation (S.D.), and differences between means were assessed by the Student t test. Cohen's d was used to compare the effect size for variables. Differences were considered significant at $P \leq 0.05$ [15]. All statistical analysis was performed using SPSS statistical software (version 19).

Results

Table (1) shows the average ages of the control and diabetic subjects were (46.39±7.85) and (48.12±6.26) years, respectively, ($P = 0.274_{[NS]}$). The mean BMI values of both groups were in the range of obesity and the mean value in diabetic group was higher (29.72 ± 4.75) than in healthy group (27.00 ± 3.47 $kg/m^{2)}$ although the difference was statistically significant ($P \leq 0.001$). Also there was a significant ($P \leq 0.001$) elevated of FSG level in type 2 diabetic patients as compared to control group.

Serum hs.CRP was significantly ($P \leq 0.001$) higher in type 2 diabetes mellitus males when compared to control. The level of serum testosterone was significantly ($P \leq 0.001$) lower in type 2 diabetic patients compared to control. The level of serum hs.CRP was significantly ($P \leq 0.01$) higher in type 2 diabetic patients compared to control. The normal levels of hs.CRP (2.21 ± 0.61ng/ml) elevated in the diabetic patient who recorded (3.05±1.45 ng/ml).

The normal levels of testosterone (9.65±1.71 ng/ml) recorded for the non-diabetic males were significantly ($p \leq 0.001$) depleted in the diabetic patient who recorded (5.50±1.07 ng/ml) for testosterone Serum E2 was significantly ($p \leq 0.001_{[HS]}$) higher in type 2 diabetes mellitus patients when compared to control group. The normal level of E2 (6.09±1.63 pg/ml) elevated in the diabetic patients who recorded (18.03±5.53 pg/m).

Serum LH was lower in type 2 diabetes mellitus patients when compared to control but the difference was statistically non-significant ($P=0.239_{[NS]}$).The normal level of LH (4.79±1.38 mIU/ml) decreased in the diabetic patients who recorded (4.46±1.09 mIU/ml).

Serum FSH was lower in type 2 diabetes mellitus patients when compared to control but the difference was statistically non-significant ($P=0.171_{[NS]}$).

Table (1): Comparison of various parameters between controls and T2DM cases

Parameters			Mean±S.D	cohen's d	t	P-value
AGE	Years	cases	48.12±6.26	0.244	-1.101	0.274
		controls	46.39±7.85			
BMI	Kg/m^2	cases	29.72±4.75	0.653	-2.763	0.007
		controls	27.00±3.47			
F.S.G	mg/dl	cases	182.4±45.63	2.354	-9.978	0.000
		controls	95.27±6.11			
hc-CRP	ng/ml	cases	3.05±1.45	0.760	-3.080	0.003
		controls	2.21±0.61			
Testosterone	ng/ml	cases	5.50±1.07	2.907	13.526	0.000
		controls	9.65±1.71			
Estradiol	pg/ml	cases	18.03±5.53	2.623	12.723	0.000
		controls	6.09±1.63			
FSH	mIU/ml	cases	9.04±1.11	0.293	1.381	0.171
		controls	9.51±2.00			
LH	mIU/ml	cases	4.46±1.09	0.262	1.186	0.239
		controls	4.79±1.38			

*** BMI (body mass index) * F.S.G (fasting serum glucose) * hc-CRP (high sensitive-C reactive protein) * FSH (follicle-stimulating hormone) * LH (luteinizing hormone)**

Discussion

Researches in recent years have provoked the thought whether hypogonadism is a risk factor for development of T2DM or not [16-18]. Most elderly men presenting with diabetes type 2 suffer from the metabolic syndrome. The metabolic syndrome is an insulin resistance syndrome with simultaneous occurrence of abdominal obesity, impaired fasting glucose, impaired glucose tolerance or overt type 2 diabetes, dyslipidemia and hypertension [1].

Our results showed that, BMI, FSG, hs-CRP, E2 were significantly increased in diabetic patients than control group, but TT was significantly lower in the diabetic than control group the LH and FSH were non significantly lower in the diabetic patients than control group, these results were in agreement with many other studies [19,20].

In testosterone deficient men suffering from diabetes type 2, baseline testosterone levels inversely correlate with CRP levels. This is also the case in nondiabetic men [6].

The presence of low free testosterone levels in our patients suggests that testosterone insufficiency may be a risk factor for type 2 diabetes. This is found to be comparable with other studies [21,22]. Men with diabetes have significantly lower serum concentrations of T- testosterone than no diabetic men. This is can be explained by the conversion of testosterone to estradiol by the actions of aromatase in adipose tissue. Therefore, a reduction of testosterone is inevitable with increased expression of aromatase, which is a result of an increased number of adipocytes in diabetic men. In addition, the normal negative feedback regulation of testosterone depends mainly on its aromatization to estradiol. Thus, a high level of aromatization results in the suppression of testosterone secretion. Falling testosterone promotes increasing adipocyte number and fat deposition which gradually leads to a further lowering effect on testosterone levels. In addition, the majority of the normal negative feedback of testosterone on the hypothalamo-pituitary axis occurs via its aromatization to oestradiol [4,21]. Thus, the increased risk for CVD in diabetic men could be partially mediated through low concentrations of testosterone.

Testosterone plays a crucial role in maintaining metabolic homeostasis; thus, this hormone may play a vital role in maintaining glycemic control. The exact mechanism by which diabetes and/or IR impair T

biosynthesis and how reduced T levels increase insulin resistance and development of T2DM remain poorly understood [23].

Type 2 diabetes is associated with low testosterone and high estradiol levels in men. Again, the high estradiol/testosterone ratio is an effect of Type 2 diabetes, not the cause. Type 2 diabetes is strongly associated with increased abdominal and subcutaneous fat, and this causes more aromatization of testosterone to estradiol.

Interestingly, while testosterone levels decline with age, estradiol E2 levels remain relatively stable, resulting in a decreased testosterone: E2 ratio. Because E2 levels tend to be elevated in morbid obesity, insulin resistance, and diabetes, it is reasonable to hypothesize that high E2 levels are associated with metabolic syndrome in older men.

Although the mechanism of the relationship between oestrogen and inflammatory markers is still poorly understood, it has been speculated that the effect of oestrogen on hs-CRP is most probably an effect on gene expression in the liver [24].

Some studies have shown that adipose tissue is not only the main source of peripheral conversion of testosterone to E2 but also itself has endocrine capacities and produces adipokines such as adiponectin, and interleukin-6 (IL-6) [25,26] inducing chronic low-grade inflammation through increased CRP production by the liver.

The present study also showed the patients with Type 2 diabetes had revealed defect in hypothalamic than pituitary axis. In study by [27] indicated that insulin-like factor 3 (INSL3), a member of the insulin–relaxin super family of peptide hormones, has been used as a specific marker of Leydig cell differentiation and function, since it appears more sensitive than testosterone itself in evaluating function . The global impairment of Leydig cell function in T2DM is confirmed by the finding of reduced circulating levels of INSL3, a novel peptide hormone mainly derived from Leydig cells, which have been indicated as an absolute measure of either quality or number of the Leydig cells, independently from gonadotropin stimulation. The higher LH level in diabetic patients than in controls might suggest that when few or poor-quality Leydig cells are present, more LH is required to achieve normal circulating testosterone levels. Since the presence of the INSL3 receptor has been demonstrated at pituitary level, a possible negative feedback of INSL3 has also been hypothesized. Also may be because that leydig cells and follicle cells become resistant to gonadotropin hormone as aresult higher levels of FSH and LH with low normal range of testosterone in diabetic patients.

References

1. Yaturu, S. (2011). Obesity and type 2 diabetes. Journal of Diabetes Mellitus. 1(4): 79-95.

2. Dhindsa, S., Prabhakar, S., Sethi, M., Bandyopadhyay, A., Chaudhuri, A., Dandona, P. (2004). Frequent occurrence of hypogonadotropic hypogonadism in type 2 diabetes. J Clin Endocrinol Metab. 89(11):5462-5468.

3. Kapoor, D., Aldred, H., Clark, S., Kevin, S., Channer, K,S., Jones, T,H. (2007). Clinical and Biochemical Assessment of Hypogonadism in Men with Type 2 Diabetes: Correlations with bioavailable testosterone and visceral adiposity. Diabetes Care. 30(4):911-917.

4. Kelly, D, M., Jones, T, H. (2013). Testosterone: a metabolic hormone in health and disease. J Endocrinol. 217(3): 25-45.

5. Grossmann , M., Thomas M, C., Panagiotopoulos, S., Sharpe, K., Macisaac, R, J., Clarke, S., Zajac, J, D., Jerums G. (2008). Low Testosterone Levels Are Common and Associated with Insulin Resistance in Men with Diabetes. J Clin Endocrinol Metab. 93(5):1834–1840.

6. Kapoor, D., Clarke, S., Stanworth, R., Channer, K, S., Jones, T, H. (2007). The effect of testosterone replacement therapy on adipocytokines and C-reactive protein in hypogonadal men with type 2 diabetes. Eur J Endocrinol. 156(5):595-602.

7. Kapoor, D., Clarke, S., Stanworth, R., Channer, K, S., Jones, T, H. (2010). Low testosterone and sex hormone-binding globulin levels and high estradiol levels are independent predictors of type 2 diabetes in men. Eur J Endocrinol.162:747-754.

8. Al-Hamodi, Z., AL-Habori, M., Al-Meeri, A and Saif, A, A. (2014). Association of adipokines, leptin/adiponectin ratio and C-reactive protein with obesity and type 2 diabetes mellitus. Diabetology & Metabolic Syndrome. 6(1):99.

9. Sigdel, M., Kumar, A., Gyawali, P., Shrestha, R., Tuladhar, E, T., Jha, B. (2014). Association of High Sensitivity C-Reactive Protein with the Components of Metabolic Syndrome in Diabetic and Non-Diabetic Individuals. Journal of Clinical and Diagnostic Research. 8(6):11-13

10. Sabanayagam, C., Shankar, A., Lim, S, C., Lee, J., Tai, E, S., Wong, T, Y. (2011). Serum C reactive protein level and prediabetes in two Asian populations. Diabetologia. 54(5):767-775.
11. Farid, S. (2009). The role of testosterone in type 2 diabetes and metabolic syndrome in men. Arq Bras Endocrinol Metab. 53(8):901-907.
12. Bagatell, C.J., Bremner, W,J. (1996). Androgens in men–uses and abuses. N Engl J Med. 334:707-714.
13. World Health Organization (1999).World Health Organization Consultation. Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. Part I: Diagnosis and Classification of Diabetes Mellitus. Report of WHO Consultation. Geneva.
14. Huggett, A, S, T., Nixon, D, A. (1957). Use of glucose oxidase peroxidase and O-dianisine in the determination of blood and urine glucose. Lancet. 2:368–370.
15. Casson, R, J. (2011). "The pesty P value". Clinical & Experimental Ophthalmology. 39 (9): 849–850.
16. Khan, I., Kant, C., Samaria, A. (2012). Assessment of Hypogonadism with Reference to Clinical Features and Serum Testosterone Levels in Asian-Indian Male Type 2 Diabetics Indian. Journal of Clinical Practice. 23 (2):92-99
17. Chen, M., Dou, J. (2014). Diabetes and gonadal disorders. Journal of Translational Internal Medicine. 2(3):119-123
18. Afkhamizadeh, M., Ghaderian, S,B., Rajabian, R., Aleali, A, M. (2015)**.** Prevalence of Hypogonadotropic Hypogonadism in Type 2 Diabetes Male Patients. Open Journal of Endocrine and Metabolic Diseases. 5(3): 29-36
19. Grossmann M., Thomas, M, C., Panagiotopoulos, S., Sharpe, K., Macisaac, R, J., Clarke, S., Zajac, J, D., Jerums, G. (2008). Low Testosterone Levels Are Common and Associated with Insulin Resistance in Men with Diabetes. J Clin Endocrinol Metab. 93(5):1834–1840.
20. Cui, R., Su, B., Sheng, C., Cheng, X., Yang, P., Bu, L., Li, H., Wang, J., Sheng, H., Qu, S. (2014). Total osteocalcin in serum predicts testosterone level in male type 2 diabetes mellitus. Int J Clin Exp Med. 7(4):1145-1149.
21. Cai, X., Tian, Y., Wu, T., Cao, C, X., Li, H., Wang, K,J. (2014). Metabolic effects of testosterone replacement therapy on hypogonadal men with type 2 diabetes mellitus: a systematic review and meta-analysis of randomized controlled trials. Asian Journal of Andrology. 16(1): 146-152.
22. Shaheen, A,D., Shahin, A, D., Habib, A, A., El Amin, A, A, M. (2014). The effect of serum free testosterone level on glycemic control and atherosclerosis in type 2 diabetic men. Advances in Biochemistry. 2(2): 34-39.
23. Abdulmajed, M. T., Farid, S., Andre, G. (2009). The Dark Side of Testosterone Deficiency: II. Type 2 Diabetes and Insulin Resistance. Journal of Andrology. 30(1): 23-32.
24. Vongpatanasin, W., Tunce, M., Wang, Z., Arbique, D., Mehrad, B., Jialal, I. (2003). Differential effects of oral versus transdermal estrogen replacement therapy on C-reactive protein in postmenopausal women. Journal of the American College of Cardiology. 41:1358–1363.
25. Trayhurn, P., Wood, I, S. (2004). Adipokines: inflammation and the pleiotropic role of white adipose tissue. British Journal of Nutrition. 92(3): 347–355.
26. Bruun, J,M., Lihn, A,S., Verdich, C., Pedersen, S,B., Toubro, S., Astrup, A., Richelsen, B. (2003). Regulation of adiponectin by adipose tissue-derived cytokines: *in vivo* and *in vitro* investigations in humans. American Journal of Physiology. Endocrinology and Metabolism. 285(3): 527–533.
27. Ermetici, F., Donadio, F., Iorio, L., Malavazos, A, E., Dolci, A., Peverelli, E., Barbieri, A, M., Morricone L, Chiodini I, Arosio M, Lania A, Beck-Peccoz P, Ambrosi B, Corbetta S. (2009). Peripheral insulin-like factor 3concentrations are reduced in men with type 2 diabetes mellitus: effect of glycemic control and visceral adiposity on leydig cell function. European Journal of Endocrinology. 161(9):853–859.