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Relation of Antioxidant Vitamins (A, E, C) with Insulin Level and Selenium in Diabetic Patients Type 2

علاقة الفيتامينات المضادة للأكسدة(A,E,C) مع مستوى الأنسولين وعنصر السيلينيوم في مرضى السكرمن النوع 2

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Abstract

Free radicals have important role in the pathogenesis of diabetes mellitus. In diabetes, free radical production is increased whereas capacity of antioxidant system is reduced. In the present study we determined and evaluated changes in levels of selenium and antioxidant vitamins (A, E, C) in serum of patients with Diabetes Mellitus type 2. Total of 200 diabetic patients (110 male, 90 female) with mean age of 47.52±8.46 years were recruited into the study. Control group was composed of 150 healthy volunteers (102 male, 48 female) with mean age of 48.31±11.99 years. In addition to aforementioned parameters, levels of fasting blood glucose, hemoglobin A1c (HbA1C) levels were determined in diabetic patients and controls. There was a significant increase in insulin level, highly significant decrease in antioxidant vitamin level (A, E, C) and highly significant decrease in selenium level. On the other hand negative correlations ($p<0.0001$) were found between fasting serum insulin level and vitamin A and positive correlation between serum insulin level and vitamins C and E while there is highly positive correlation between serum selenium and vitamin A($p<0.01$) and negative correlation between serum selenium level and antioxidant vitamins C and E ($p<0.05$).

In conclusion supplementation of antioxidant vitamins into the daily diets of diabetic patients with type 2 will enhance power of non-enzymatic antioxidant defense systems.

Key words: *Insulin - Antioxidant vitamins – Selenium*

الخلاصة:

للجذور الحرة دور في الاصابة بمرض السكر , حيث ان انتاج الجذور الحرة يزداد عند مرضى السكر بينما تقل قابلية النظام المضاد للتاكسد . في هذه الدراسة تم حساب التغيرت في مستويات عنصر السيلينيوم والفيتامينات المضادة للتاكسد (A,B and C) في مصل

دم مرضى السكر من النوع 2 . وشملت الدراسة 200 مريضا مصاب بالسكرمن النوع 2 (110 ذكور , 48 اناث) بمعدل عمر 47,52±8,64 سنة و 150 خصا كمجموعة سيطرة (102 ذكو و 48 اناث) بمعدل عمر 48,31±11,99 سنة.

وبألاضافة للمتغيرا ت السابقة تم قياس مستويات السكر الصائم وهيموكلوبين السكر الصائم في مصل الدم عند المرضى ومجموعة السيطرة . وقد كان هناك ارتفاع ملحوظ في مستوى هرمون الانسولين ,انخاض ملحوظ ي مستوى الفيامينات المضادة للاكسدة (A,E and C)

وانخفاض في ملحوظ في مستوى عنصر السيلينيوم عند مرضى السكر . ومن ناحية أخرى وجدت هناك علاق خطية مع ارتباط سالب بين مستوى الانسولين وفيتامين A ,علاقة خطية موجبة بين عنصر السيلينيوم وفيتامين A وعلاقة خطية سالبة بين عنص لسينيوم وفيتامين C,E.

وبألنتيجة أضافة الفيتامينات المضادة للاكسدة للغذاء اليومي لمضى السكر من النوع 2 يحسن من قوة الانظمة الدفاعية الضادة للاكسدة غير الانزيمية .

Introduction

Diabetes Mellitus, a chronic disease characterized by high levels of blood glucose resulting from defects in insulin secretion, insulin action, or both.[1] Oxidative stress is produced under diabetic conditions and possibly causes various forms of tissue damage in patients with diabetes [2]. Under diabetic conditions, reactive oxygen species (ROS) are produced mainly through the glycation reaction (which occurs in various tissues and may play a role in the development of complications in diabetes [3,4]. Vitamin C is an important antioxidant in human [5]. Capable of scavenging oxygen-derived free radicals[6]. Vitamin C is structurally similar to glucose and can replace it in many chemical reactions, and thus is effective in prevention of non-enzymatic glycosylation of proteins[7]. Several studies showed decreased basal vitamin C level in diabetic patients [8, 9]. Since effects of free radicals in diabetes are now documented, it has been proposed to use antioxidant vitamins to block formation of free radicals and hence prevent development of diabetes [10] . While superoxide radicals are cleaned by enzymatic dismutation, compounds known as antioxidants clean free radicals in organism. Glutathione is a very important non-enzymatic antioxidant together with antioxidant vitamins. Vitamins A, E and C are among these important nonenzymatic antioxidants [11, 12]. Vitamin A functions as catalyzer of removal of singlet oxygen and as a result vitamin A inhibits singlet oxygendependent reactions [13,14]. Vitamin C is also has a role in activating vitamin E when it loses its antioxidant capacity by turning into tocopherol [15] Selenium, an essential trace element, is involved in the complex system of defense against oxidative stress through selenium-dependent glutathione peroxidases and other selenoproteins. Because of its antioxidant properties, selenium might thus prevent the development of diabetes. In addition, selenate, an inorganic form of selenium, mimics insulin activity in experimental models[16].

Material and Methods

This study was performed at the National Diabetes Center, College of Medicine at Al-Mustansiryia University from 1 August 2008 to 25th of May 2010The subjects employed for this study were 200 diabetic patients of both sexes(male =110 ,females=90) Mean age of diabetic patient was(54.8±11.4 years) hundred and fifty serve as control(male=102,female=48). Age, sex, weight, height (and
calculated BMI), duration of diabetes, were examined and recorded. Fasting blood samples were obtained after an 8-12 hour overnight fast, from the subjects. The blood samples were centrifuged at 1000 rpm for 20 minutes using a desktop centrifuge. ***Serum glucose*** was measured at biochemistry Laboratory at College of Science/chemistry Department at Al-Nahrain university by enzymatic colorimetric method (GOD-PAP).***HbA1c*** was measured by column chromatography method (Varna-Biurat /HPLC). To measure ***selenium***, whole blood was collected in containers previously screened for selenium contamination. After clotting and centrifugation, serum was collected, frozen at -20 C, and shipped to the laboratory for measured with the use of atomic absorption spectrometry (shimadzu,modale 680-AA in Ibn Seena Company) .

. ***While serum insulin hormone and Antioxidant Vitamin(A,C,E)*** were measured using High Performance Liquid Chromatography (HPLC), Shimadzu (Kyoto, Japan) which consisted of a system controller model SCL-10 AVP, a degasser model DGU-12A, two liquid delivery pumps model LC-8AVP, UV-Visible detector model SPD-10AVP, and an injector model SIL-10A, equipped with 20 µl sample loop. The HPLC system was interfaced with a computer via a Shimadzu class-VP5 chromatography data system program supplied by the manufacturer; Epson LQ-300 printer model P852A (Japan).

All samples and standard solutions were chromatographically analyzed with ODS column using gradient mobile phase 30% acetonitrile, 70% water, flow rate 1ml/min and UV-VIS detection . For estimating serum insulin,[17] samples were analyzed using C18 column, gradient mobile phase

consisting of a mixture of 74% of 0.2 M Sodium Sulfate adjusted at pH 4.5 and 26 volume of acetonitrile, the flow rate was 1.2 ml/min at wavelength 214 nm. utilizing absorption spectra of 326 and 296 nm for vitamin A and E, respectively The quantification was made according to Miller and et al. (18) The precipitation of proteins for vitamin C analysis were made according to the method described by Cerhata et al. (19). The supernatant was filtered and the vitamin C levels were determined using the method of Tavazzi et al. (20).

Statistical analysis

All data were analyzed by SPSS version 17. The data obtained are expressed as mean values ± S.D. Student's t-test and pearson test was used to correlations determine whether differences between the means were significant, with $p<0.05$ taken as the significance level.

Results and Discussion

The basic characteristics of the subjects were shown in (Table 1). Two hundred diabetic patients with type 2,110 male and 90 female, mean age of male was 47.38±9.16 years [mean ± SD] mean age of female was 47.69±7.63, mean BMI of male was29.39±6.93, mean BMI of femalewas 30.23±11.29,mean of duration of male was 5.6±4.77, mean of duration of female was 3.6±2.46. The diabetic patients were generally heavier than the control subjects were. The result of the body mass index (BMI) indicated that the diabetic subjects were over weight.

Table 1: General Characteristics of Diabetes Patients and Control Subjects

Subjects	n*	Age (years)	BMI** (Kg/m2)	Duration of diabetes(years)
Patients				
Male	110	47.38±9.16	29.39±6.93	5.6±4.77
Female	90	47.69±7.63	30.23±11.29	3.6±2.46
Total	200	47.52±8.46	29.77±9.11	4.7±4.02
Control				
Subjects	102	45.68±8.22	24.15±1.89	
Male	48	53.88±16.5	25.74±2.95	
Female	100	48.31±11.99	24.67±2.38	
Total				

**n= number of subjects **BMI = Body mass index = weight/height$_2$*

In the present study there was highly significant increase in serum glucose ,insulin and HbA1c of patients than controls($p<0.0001$) On the other hand, levels of antioxidant vitamins (A, E, C) and selenium were observed to be highly significantly reduced in diabetic patients ($p<0.0001$) as show in table 2. The benefits and dangers of selenium have been debated in recent years because some studies show it might help protect people from cancer and heart disease. Selenium is an essential element and antioxidant, but medical experts say there is a fine line between the amount that the body needs and the amount that is harmful [21]. We determined a significant decrease in serum selenium level of diabetic patients compare to control.

The mean serum selenium concentration for the control group in the present study was 0.17±0.11 and for diabetic patients was0.133±0.12 ($p<0.0001$) similar observations were cited in the studies by Cunha Karatas F et al. [22] and Nsonwu AC et al. [23].

Table 2. Comparison of FBS, Insulin level,HbA1c, Se and antioxidant vitamins (A,C,E) of diabetes patients with type 2 and control

	Diabetic patients (n:200)	Control (n:100)
FBS (mg/dl)	159.29+13.49	85.06±3.65**
Fasting Insulin(µU/ml)	25.02±8.93	17.40±18.44**
HbA1C	7.36±0.61	6.49±0.38**
Serum Selenium(ppm)	0.133±0.12	0.17±0.11**
Vitamin A (mg/dl)	0.56±0.62	0.81±0.87**
Vitamin C (mg/dl)	0.66±0.13	1.11±0.23**
Vitamin E (mg/dl)	0.57±014	1.1±0.2**

*Values are means ± SD ** p<0.0001 when compared to control*

Free radicals have important roles in pathogenesis of diabetes mellitus. It has been well documented that there is a link between oxidative stress and secondary complications of diabetes [17]. Vitamins A, C, and E are diet-derived and detoxify free radicals directly[18].They also interact in recycling processes to generate reduced forms of the vitamins.Tocopherol is reconstituted when ascorbic acid recycles the tocopherol radical; dihydroascorbic acid, which is generated, is recycled by glutathione. These vitamins also foster toxicity by producing prooxidants under some conditions. Vitamin E, a component of the total peroxyl radical-trapping antioxidant system [19]. Havivi et al. [24] claimed that levels of vitamin A in type 2 diabetic patients were increased whereas several reports documented that there is reduction in vitamin A levels rather than increase compared to controls [25, 26]. In the present study, serum vitamin A levels of diabetic patients were determined to be lower than controls. Earlier research has shown that diabetics have low levels of vitamin-C and vitamin E and that vitamin-E supplementation can help prevent the development of glucose intolerance and diabetes [27] our result agree with these results.

Table 3: Correlation Coefficient (r) of Fasting Insulin level and Antioxidant Vitamins of Diabetic patients

	r	P value
Vitamin A (mg/dl)	-0.835*	0.021
Vitamin C (mg/dl)	0.702*	0.039
Vitamin E (mg/dl)	0.664*	0.044

** Significant correlation when p<0.05; ** highly significant correlation when p<0.01 and r= correlation*

In the current work a direct negative correlation between serum antioxidant vitamin A and positive correlation between serum antioxidant vitamins for vitamin E and C and insulin was observed (table 3)while there is highly positive correlation between serum selenium and vitamin A($p<0.01$) and negative correlation between serum selenium and antioxidant vitamins C and E ($p<0.05$) as show in table 4

4: Correlation Coefficient (r) of Fasting Serum Selenium and Antioxidant Vitamins (A, C, E) of Diabetic patients

	r	P value
Vitamin A (mg/dl)	0.988*	0.0001

Vitamin C (mg/dl)	-0.84*	0.02
Vitamin E (mg/dl)	-0.911*	0.011

** Significant correlation when p<0.05; ** highly significant correlation when p<0.01 and r= correlation*

In view of the significantly reduced antioxidants concentrations in diabetes patients obtained in this study, and significant correlations between serum antioxidant vitamins and serum insulin level of the subjects, it may not be out of place to recommend the inclusion of antioxidant vitamins in therapeutic diabetes mellitus in general in the study area. This will assist in reducing or delaying the risk of diabetic late complications.

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