Study the Relationship Between Vitamin E and Some Biochemical Changes in Patients with Type 2 Diabetes Mellitus

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الخلاصة

تضمنت الدراسة الحالية مقارنة مستويات فيتامين E وأنزيم كاما- كلوتامايل ترانس ببتيديز ومستوى البروتين المتفاعل من نوع سي والكوليسترول الكلي والسكر الصائم في امصال المرضى المصابين بداء السكري من النوع الثاني ومقارنة النتائج مع اشخاص سويين كمجموعة سيطرة . وايجاد العلاقة بين فيتامين E كمانع للتأكسد وبعض المتغيرات البايوكيميائية الاخرى.

تضمنت الدراسة ٣٠ شخصا مصابا بداء السكري من النوع الثاني (١٨ ذكوراً و١٢ اناثاً) اللذين يراجعون مركز الكندي للغدد الصم والسكري في بغداد . وتضمنت مجموعة السيطرة ٣٠ شخصاً سوياً (١٥ ذكوراً و١٥ اناثاً) كمجموعة سيطرة . تم قياس مستوى السكر الصائم (7.39 ±3.095 ملي مول / لتر) في امصال المرضى T2DM.

اشارت النتائج الى عدم وجود فروقات معنوية في العمر في المرضى المصابين T2DM مقارنة بمجموعة السيطرة بينت الدراسة الحالية بان هناك فروقات معنوية في مؤشر كتلة الجسم (20.13±1.79 كغم/م ٢) وأنزيم كاما- كلوتامايل ترانس ببتيديز(41.95 ± 6.012 وحدة دولية/لتر) والكوليسترول الكلي (201.36±38.17 ملي غرام / ديسيليتر) و فيتامين E (0.565 ±0.178 ملي غرام/ ديسيليتر) ونسبة فيتامين E الى الكولستيرول الكلي (0.003±0.001) في مرضى T2DM مقارنة بمجموعة السيطرة . واظهر البروتين المتفاعل من نوع سي مستوى سالبا (< من 0.6 ملغم /دسيلتر) في ١٢ مريضاً ومستوى موجبا (> من 0.6 ملغم /دسيلتر) في ١٨ مريضا (2.07 ± 0.066 ملغم /دسيلتر) . علاوة على ذلك اظهرت الدراسة علاقة ايجابية معنوية بين فيتامين E ونسبة فيتامين E الى الكولستيرول الكلي وعلاقة سلبية معنوية بين فيتامين E مع كل من مؤشر كتلة الجسم والسكر الصائم وأنزيم كاما- كلوتامايل ترانس ببتيديز، بينما علاقة موجبة غير معنوية مع كل من العمر والكولستيرول الكلي و البروتين المتفاعل من نوع سي.

ABSTRACT

The present study is an attempt to assess the serum levels of vitamin E, γ- GGT.CRP, total cholesterol, and fasting glucose in patients with type 2 Diabetes Mellitus and compare the results obtained with healthy controls group, and to ascertain the relationship between the antioxidant vitamin E with some associated parameters.

Type 2 diabetes mellitus group include 30 patients (18 male and 12 female) who were selected from patients attending Specialist Center for Endocrine and Diabetes in AL-Kindy Hospital in Baghdad. The control group included 30 healthy subjects (15 male and 15 female). Fasting glucose level in patients with T2DM was (**7**.39$\pm$3.095 mmol/L).The results reveal there were no significant differences between T2DM and control in age.The present study shows that the means for BMI (20.13$\pm$1.79 kg/m^2), γ- GGT (41.95$\pm$6.012 U/L), cholesterol (201.36$\pm$38.17mg/dL), vitamin E (0.565$\pm$0.178 mg/dL), and vit.E/TC (0.003$\pm$0.001) were significantly differences in T2DM as compared to control group. CRP levels were negative (<0.6 mg/dL) in 12 patients and positive (>0.6 mg/dL) was (2.07$\pm$2.066 mg/dL)) in 18 patients.Furthermore, serum vitamin E concentrations showed significantly positive relationship with the ratio of vit.E/TC, and negative significant relationship found between vitamin E and BMI, fasting glucose, and γ- GGT. While non significant positive relationship found between vitamin E and both of cholesterol, age and CRP.

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) or non insulin-Dependent Diabetes Mellitus, is the most common form of diabetes, due to dietary habits and increasing obesity and sedentariness in both Western and developing countries, the prevalence of T2DM is growing at an exponential rate (1).It is a heterogeneous group of disorders usually characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production. Insulin resistance develops from obesity and physical inactivity, acting on a substrate of genetic susceptibility (2, 3). Although the precise mechanism responsible for insulin resistance remains unclear, it would appear that a number of adipocyte-derived factors impair insulin activity and that the secretion of these factors is altered in the obese individual (4).

There has been currently great interest in the potential contribution of increased oxidative stress to the development of diabetes mellitus .An increase in oxidative stress may occur due to an increase in the production of free radicals. These reactive oxygen species (ROS) are capable of chemically altering all major classes of biomolecules (e.g. lipids, proteins and nucleic acid) by changing their structure and function, thus leading to cell damage in diabetes (5). These ROS are potentially harmful to cellular functions. To prevent these harmful effects, the cell has developed a complex antioxidant system to dispose of ROS. However, antioxidant concentrations are reduced in obese individuals, and the resulting imbalance between the production of ROS and antioxidant defenses results in oxidative stress (6).The biological effects of free radicals are normally controlled in vivo by a wide range of antioxidants such as vitamin A ,C ,E ,glutathione and antioxidant enzymes (7). Vitamin E is a generic term for a group of a compound known as tocopherols and tocotrienols, of which α-tocopherol has been shown to have the greatest biological activity (8). The antioxidant property of vitamin E is well established in the literature (9).

Vitamin E, blocks the chain reaction of lipid peroxidation by scavenging intermediate peroxy radicals. The α- tocopherol radical is much less reactive in attacking adjacent acid side chain and can be converted to α-tocopherol by vitamin C (5).

In longitudinal studies, gamma-glutamyl transferase (γ-GGT) predicts future risk of developing diabetes (10). γ-GGT is a cell- surface protein contributing to the extracellular catabolism of glutathione (11). In the serum, γ-GGT is carried primarily with lipoproteins and albumin (12). Serum levels of γ-GGT are determined by several factors: alcohol intake, body fat content, plasma lipid/lipoproteins, glucose levels, and various medications (11, 13). Systemic concentrations of hepatic enzymes reflect hepatocellular health. Raised levels in obese individuals probably reflect nonalcoholic fatty liver disease, which is itself a marker of insulin resistance (14).C-reactive protein (CRP) plays a key role in the host's defense against infection. CRP is predominantly made in the

liver and is secreted in increased amounts within six hours of an acute inflammatory stimulus. Some workers demonstrated later that diabetic individuals with stigmata of insulin resistance ,for example ,central obesity and hypertension had higher serum CRP levels .They regarded this as evidence that inflammation was related to insulin resistance (15).Cholesterols play key roles in controlling molecular fluidity in a biological membrane (16).Cholesterol is one of the major components of biological membranes, and it is known to influence various membrane properties such as elasticity, mechanical strength, and molecular fluidity. Diabetes mellitus is a common secondary cause of hyperlipidaemia, particularly, if glycaemic control is poor(17), which in-turn is an important risk factor for atherosclerosis and coronary heart disease(18).

MATERIALS AND METHODS

Sample Collection

A total of 30 (T2DM) patients were recruited from the diabetic center, in AL-Kindy Hospital (specialized center of endocrinology center and diabetes). Medical records were screened by specialist physicians. The mean age of the patients was 53.3±13 years with a range of 35-60 years. Body mass index of the corresponding patients was 29.76±7.5 Kg/m^2 .

Healthy control group consisted of 30 individuals. The mean age was 40.7±11 years and body mass index was 20.13 + 1.79 Kg/m^2 . All individuals were non smokers and none had taken vitamin supplements.

Preparation of Blood Samples

Six milliliters of blood samples were taken from patients and normal controls in the morning after 12 hours fasting .Blood sample were left for 20 minutes at room temperature, after blood coagulation, the sera were separated by centrifugation at 3000 xg for 15 minutes. Hemolyzed samples were discarded.

The concentration of serum glucose and cholesterol were measured by enzymatic colorimetric assay using kit supplied by Biomegrab. The concentration of vitamin E in the collected serum samples was determined according to the method of Hashim & Schuttriger (19) .Serum GGT activity was also determined using kit supplied by Szasz G.c . C-reactive protein was measured by rapid test for the qualitative and semiquantitative determination of CRP in serum by agglutination of latex particles on slide using a kit supplied by linear chemicals-Spain.

Statistical analysis

The data was analyzed on the computer statistical programme SPSS version10. The mean ±SD was also computed for the comparison of results. The comparison of mean between two groups was tested by Student's't' test. Results were considered statistically significant if P value is less than 0.05.

RESULTS AND DISCUSSION

The incidence of T2DM as well as its related morbidity and mortality has been well correlated with generalized Obesity measured by BMI. The body mass index was calculated as weight (in Kilograms) / height (in meter) squared shows significant changes in T2DM as compared to controls group Table (1). This different in the obesity might be explained the differences in the operation of the risk factors or the causal pathways leading to the disorder (20).

The mean age of the T2DM was 53. ±13 and the mean age of the control group were 40.7 ±11 years Table (1).The groups were not statistically different with respect to age ($p > 0.05$).

Table-1: Comparison of serum glucose, cholesterol, CRP ,GGT, vitamin E and BMI.

Parameters	**Controls(Mean+SD) N=20**	**Diabetic patients (Mean+SD) N=20**
Age (years)	40.7±11	53.3±13
BMI (kg/m^2)	20.13 ± 1.79	31.33±6.86**
FBG (mmol/L)	5.35±0.532	7.39±3.095*
Cholesterol (mg./dL)	169.45± 20.91	201.36±38.17*
GGT (U/L)	20.6±3.95	41.95±6.012**
CRP (mg/dL)	< 0.6	< 0.6 (N=12)
		2.07±2.06 (N=18)
Vitamin E (mg/dL)	0.847 ± 0.206	0.565±0.178**
Vit.E/cholesterol	0.005 ± 0.001	0.003±0.001**

Values significantly different from the controls *p<0.01, **p<0.001

In T2DM, fasting blood glucose (FBG) is the main parameter of glucose metabolism that is used to monitor and control hyperglycaemia (21). In this study, there is also a significant difference ($p<0.01$) in the mean FBG of the T2DM was 7.39 ±3.095 and the mean FBG of the control group were 5.35 ±0.532 mmol/L as shown in Table (1). Because sugar is not getting into the tissues, abnormally high levels of sugar build up in the blood. This is called hyperglycemia. Many people with insulin resistance have hyperglycemia and high blood insulin levels at the same time. People who are overweight have a higher risk of insulin resistance, because fat interferes with the body's ability to use insulin(22).

Cholesterols play key roles in controlling molecular fluidity in a biological membrane. There were, however, significant differences ($p<0.01$) in mean serum total cholesterol in T2DM 201.36±38.17 when compared to control group 169.45± 20.91 mg/dL as shown in Table (1).

In this study, we observed an increase in CRP levels in some diabetic patients table (1), and this study graded increment in CRP may predict for future diabetes but the cut-off point of CRP can not been determined .Pradhan *et al* (23)
showed in a retrospective study that there was a strong and graded association of CRP level with incident diabetes mellitus independent of established risk factors. The above findings support the hypothesis that diabetes mellitus has inflammatory aspects, but the causal pathway between diabetes mellitus and inflammation was not clear. It was possible that inflammation could be the primary disorder that leads to insulin resistance .Also it is possible that inflammation and diabetes mellitus arise from another yet unidentified common genetic antecedent.

Serum γ-GGT, a marker of oxidative stress, has been shown to be associated with diabetes mellitus in some population (24). We examined the association between serum γ-GGT and diabetes mellitus, we observed a highly significant increase ($p<0.001$) in the levels of γ-GGT in T2DM (41.95±6.012 U/L) as compared to control group (20.6±3.95 U/L) Table (1). γ-GGT retains a large part of its predictive power after adjusting for correlated risk factors such as obesity, fasting glucose, and insulin resistance. Our results are in good agreement with Sabanayagam *et al* who found higher serum γ-GGT levels were positively associated with diabetes mellitus, independent of, alcohol consumption, body mass index, hypertension and other confounders (24). The risk of T2DM increased with increasing levels of serum γ-GGT, and the γ-GGT is an important predictor for incident T2DM in men and women from the general population.(25, 26).

The reduction in fasting plasma insulin and glucose concentrations, during vitamin E supplementation suggests improved insulin sensitivity. Furthermore, the magnitude of this improvement in insulin sensitivity, as indicated by fasting insulin levels, depends on the magnitude of the increase in plasma vitamin E (10). In addition, increased vitamin E may enhance the endogenous cellular antioxidant defense system and reduce levels of ROS that are produced by mitochondria. In the present study, vitamin E was significantly reduced ($p<0.001$) in the levels in T2DM (0.565+0.178 mg/dL) as compared to control group (0.847±0.206 mg/dL) Table (1). This lower mean serum vitamin E value may reflect reduced dietary intake or increased consumption associated with increased oxidative stress in patients with diabetes mellitus (27).

Manning *et al* show in thier study that vitamin E improves insulin sensitivity and several of its associated parameters in overweight individuals, but the effect of treatment is not sustained. In addition, vitamin E decreased circulating levels of ALT, a risk factor for the development of T2DM, during entire study period. These results suggest that vitamin E could have a role to play in delaying the onset of diabetes in at-risk individuals (10). Other small

studies have shown improvements in insulin sensitivity in elderly subjects (28) and diabetic individuals receiving high-dose vitamin E therapy (29).

We observed significant decrease in the vitamin E/Total cholesterol (vit.E/TC) ratio in T2DM as compared to control groups (Table 1). Ziegler D. *et al* (30) concluded a significance decrease in vit. E/ TC and non significant change in the level of cholesterol in T2DM patients without polyneuropathy and cardiovascular autonomic neuropathy disease compared to control. In this study we observed non significance positive correlation between vitamin E and age in T2DM patients table (2).

Table-2: Correlation coefficients and the significance levels of different Chemical components in T2DM.

Component vs vitamin E	Slope	R_2	r
Age	0.008	0.146	0.383
BMI	-0.013	0.264	-0.514*
glucose	-0.0264	0.202	-0.449*
GGT	-0.016	0.281	-0.530*
Cholesterol	0.0022	0.145	0.381
CRP	0.077	0.2851	0.534
Vit.E/cholesterol	158.39	0.624	0.790**

* Correlation is significant at the level 0.05
**Correlation is significant at the level 0.01

Mobarhan M.G. et al (31), observed non significance correlation between age and either serum vitamin E or serum (vit.E/TC) ratio. Ford and Sowell (32) in the third National Health and Nutrition examination survey found that age directly related to serum vitamin E.

In this study significant negative correlation between vitamin E and BMI were observed table(2), These results consistent with other study done by Kimmons J E *et al* (33), who reported that low vitamin E levels among overweight and obese persons may result from the increased systemic and adipose tissue specific oxidative stress found in over weight persons ,which may leads to increased oxidative catabolism of these lipid soluble nutrients .Ford and Sowell(32) reported that mean serum vitamin E concentration was not related to BMI . Mobarhan M.G. etal(31), found non significante negative correlation between BMI and vitamin E in T2DM patients associated with dyslipidemia .Other studies done by Kardinaal etal, (34) which revealed a significant positive relationship between plasma vitamin E and BMI.

This study revealed a significant negative correlation between glucose and vitamin E table(2) and this result consistent with experimental studies suggested that oxidative stress impaired pancreatic B-cell insulin secretion

(35,36), interfered with glucose disposal in peripheral tissues ,and elicited systemic inflammation (37) ,there by accelerating the development and progression of T2DM (38) .In vitro studies indicated that vitamin E may improve insulin action and insulin secretion by protecting peripheral tissues and B- cells from free radical –mediated damage , leading to hypothesis that vitamin E may help delay development of T2DM (39).However ,in a large cohort of Finnish men and women those who self -selected for higher intake of dietary vitamin E experienced a significantly decreased incidence of T2DM(40).

In addition to these results ,this study found non significance positive correlation between cholesterol and vitamin E and a highly significant positive correlation between vitamin E and vit.E/TC ratio table(2). our results in agreement with Bouwstra R.J.(41), Mubarhan M.G. *et al* (31), and Ford and Sowell (32) who stated that the strongest positive unadjusted correlation was between serum vitamin E concentration and serum cholesterol.

Since vitamin E is mainly transported by plasma lipoproteins, this strong correlation suggested that changes in plasma vitamin E should be considered as epiphenomenon of altered plasma transport capacity.

In the present study, we observed a significant negative relationship between γ-GGT and vitamin E (Table 2).The decreases in vitamin E due to blocks the chain reaction of lipid peroxidation by scavenging intermediate peroxy radicals. Cellular γ-GGT is an ectoplasmic enzyme responsible for the extracellular catabolism of glutathione and is widely distributed in various cell with secretary or absorptive activities (42).So, the increase in γ-GGT levels with the decrease of vitamin E levels may be reflect the excess of ROS in T2DM . Our study were in a agreement with Mobarhan M.G. etal(31) who found non significance positive correlation between vitamin E and CRP table(2).

These results suggest that vitamin E can work as endogenous antioxidants to protect cells from oxidative stress and could have a role to play in delaying the onset of diabetes in at-risk individuals

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