Evaluation of serum vitamin C, malondialdehyde & lipid profile levels in type 2 diabetic patient in Hawler city

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Abstract

Background and objectives: Diabetes mellitus is a metabolic disorder, characterized by hyperglycemia. The oxidative stress in diabetes was greatly increased due to prolonged exposure to hyperglycemia and impairment of oxidant /antioxidant equilibrium. Proteins and lipids are among the prime target for oxidative stress. The objective of this study was to find out correlation between lipid peroxidation in terms of malondialdehyde (MDA) and impairment of antioxidant in terms of vitamin C (Vit C) to severity and complication of type 2 diabetes mellitus.

Methods: We study a total of 163 subjects among them 109 patients were type 2 diabetic patients and remaining 54 subjects were healthy control subjects. Fasting blood samples were collected in Layla Qasm center for diabetes /Hawler city and analyzed for plasma vitamin C, serum MDA, serum lipid profile and serum glucose in the research lab of Clinical Biochemistry College of Medicine/ Hawler.

Results: Plasma vitamin C levels were significantly decreased in diabetes mellitus (0.48 ± 0.026) mg/dl compared to non- diabetic patients (1.18 ± 0.057) mg/dl. Patients with type 2 diabetes showed a significant increase in serum MDA (1.52 ± 0.04) mmol/L in comparison to non- diabetic patients (0.73 ± 0.026) mml/L..

Conclusion: In this study group, there is significant correlation between lipid peroxide concentration and lipid fractions except vitamin C and HDL-cholesterol.

Key words: Type 2 diabetes, plasma vitamin C, serum (MDA) lipid profiles.

Introduction

Diabetes is associated with a number of metabolic alterations and principal among these is hyperglycemia. Hyperglycemia in diabetic patients can increase the oxidative stress by several mechanisms, including glucose auto oxidation, nonenzymatic protein glycation and activation of polyol pathway. In the pathological events, the increased free radical activity is suggested to play an important role in the lipid peroxidation and protein oxidation of cellular structures causing cell injury and is implicated in the pathogenesis of vascular disease in type I and type II diabetes [1, 2, 3]. In diabetes, impaired lipid metabolism involves both quantitative and qualitative changes often referred to as diabetic dyslipidemia. The major lipid disorders detected in type 2 diabetes are an increased triglycerides, total cholesterol, LDL-C and HDL-C concentrations [4]. Lipid peroxidation and protein carbonyl group content was used as a marker of oxidative stress. Therefore, the evaluation of protein carbonyl group content in plasma is respected marker of free radical activity [5, 6].

Membrane lipids present in sub cellular organelles are highly susceptible to free radical damage. Lipids when reacted with free radicals can undergo the highly damaging chain reaction of lipid peroxidation (LP) leading to both direct and indirect effects, reactive oxygen species–mediated oxidation of membrane lipids results in the

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formation of lipid peroxidation products such as malondialdehyde [7]. A variety of natural antioxidants exists to scavenge oxygen free radicals and prevent oxidative damage to biological membranes. One group of these antioxidants is enzymatic (intracellular), which include superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase. In addition to enzymatic antioxidants, others are natural antioxidants; these are derived from natural sources by dietary intake such as vitamins A, C & E and carotenoids. [3]. Several studies have been carried out to evaluate the free radical induced lipid peroxidation and the antioxidants in diabetic patients. Many of these studies assessed only individual antioxidants. Controversial reports have been reported concerning the antioxidant status in diabetic patients [8, 9, 10]. Hence, the present study has been undertaken to evaluate oxidative stress in NIDDM diabetic patients by measuring the levels of oxidative products lipid peroxidation such as (MDA) and antioxidant such as vitamin C.

The aims of the study was to evaluate the possibility of the involvement of malondialdehyde and vitamin C in the pathogenesis of non-insulin dependent diabetes mellitus through estimation of serum MDA levels as oxidative product and plasma vitamin C levels as an antioxidant in type 2 diabetes mellitus.

Methods

One hundred nine diabetic patients were selected randomly in Liyla Qasm center for diabetes patients in Erbil city (65 females and 44males). Fifty-four apparently healthy individuals were selected randomly (32 females and 22 males), mean and range of age were 50.5±1.1 (Mean± S.E.M), (30 - 67) years respectively.Venous fasting blood samples (8-10 ml) were obtained from all patients and controls after overnight fast. About (2-3 ml) of the sample was collected in heparinized tubes and obtained plasma which was used for determination of ascorbic acid (Vit C), that analyzed immediately. Plasma vitamin C is oxidized by Cu (II) to form dehydroascorbic acid, which reacts with acidic 2, 4-dinitrophenylhydrazine to form a red bis-hydrazone, which is measured at absorbance 520 nm [11]. The remaining volume about (6-8 ml) was collected in plastic tubes (non-heparinized tube), serum was used with in the same day for estimation of MDA, serum glucose and lipid profile. Malondialdehyde (MDA) the product of lipid peroxidation reacts with thiobarbituric acid in acidic condition at 95°C to form a pink colored complex with an absorbance maximum at 532 nm [12].

Statistical Analysis:

An expert statistical advice was sought and statistical analyses were done using SPSS (Statistical Package for Social Science) version 17 and Microsoft Excel, 2003. Results are expressed as (means ± S.E.M). Means were compared by using students t-test. Statistical significance was set at a P value < 0.01.

Results

Table (1) provides the mean plasma Vit.C levels with fasting serum glucose levels in the diabetic and non-diabetic groups, plasma levels of vitamin C were significantly decreased in diabetic (0.48 ± 0.026) mg/dl when compared to non-diabetes groups (1.18 ± 0.057) mg/dl. Diabetic patients had fasting serum glucose levels nearly more than 2.5 fold higher than non diabetic patients. In (Table 2) the mean serum were significantly increased in same diabetic groups (Table 1) (1.52 ± 0.04) mmol/L in comparison to non- diabetic patient (0.73 ± 0.026) mmol/L. Results in Table (3) indicated that the mean value of serum total cholesterol, triglyceride, and LDL-cholesterol in diabetic groups increased (male and female) when compared to control group (P<0.01). Mean value of serum HDL- cholesterol was decreased in diabetic groups when compared to control groups (P<0.01). Table (4) reveals the Relationship between the mean value of serum MDA (mmol/L) according to duration of diabetes (years). Increased

levels of serum MDA were found with increasing the duration of diabetes in (years). Table (5) provides the Relationship between the mean values of plasma Vit. C (mg/dl) according to duration of diabetes (years). Decreased levels of plasma Vit. C were found with increasing the duration of diabetes in (years).

Table 1: Plasma Vit. C and S. glucose levels in mg/dl (Mean ± S.E.M) in non-diabetic and diabetic groups

Groups	No.	Non-diabetic groups		No.	Diabetic groups	
		S. glucose mg/dl	Vit. C mg/dl		S. glucose mg/dl	Vit. C mg/dl
Males	22	74 ± 1.53	1.2 ± 0.096	44	209 ± 8.2	0.486 ± 0.05
Females	32	74.22 ± 1.41	1.16 ± 0.07	65	217 ± 7	0.49 ± 0.03
Both	54	74 ± 1.03	1.18 ± 0.057	109	214 ± 5.3	0.48 ± 0.026

Diabetic Vs Non-diabetic **(P<0.01)**
Diabetic (*females*) Vs Diabetic (*males*) **(P>0.01)**

Table 2: Serum MDA in (mmol/L) and serum glucose (mg/dl) (Mean ± S.E.M) in non-diabetic and diabetic groups with test of comparison.
Diabetic Vs Non-diabetic

Groups	No.	Non-diabetic groups		No.	Diabetic groups	
		S. glucose (mg/dl)	MDA (mmol/L)		S. glucose (mg/dl)	MDA (mmol/ L)
Males	22	74 ± 1.53	0.834 ± 0.0456	44	209 ± 8.2	1.5 ± 0.07
Females	32	74.22 ± 1.41	0.66 ± 0.025	65	217 ± 7	1.53 ± 0.05
Both	54	74 ± 1.03	0.73 ± 0.026	109	214 ± 5.3	1.52 ± 0.04

Diabetic Vs Non-Diabetic **(P<0.01)**
Diabetic (*females*) Vs Diabetic (*males*) **(P>0.01)**

Table 3: Lipid profile in type 2 diabetic patients and control group.

Data	Male		Female		Both	
	Controls	Diabetic	Controls	Diabetic	Controls	Diabetic
S. total cholesterol (mg/dl)	163.7 ± 3.82	191.9 ± 6.59	166.8 ± 5.2	205 ± 5.57	165.7 ± 4	199.9 ± 4.3
S.triglyceride (mg/dl)	102.6 ± 7.8	165.9 ± 7.99	104.8 ± 5.9	161.8 ± 6.44	104 ± 4.7	163 ± 5
S. HDL (mg/dl)	42.9 ± 1.46	36 ± 1.7	48 ± 1.5	38 ± 1.45	46 ± 1.11	37.68 ± 1.11
S. LDL (mg/dl)	100 ± 3.53	122.6 ± 5.58	97.8 ± 4.7	134.6 ± 5.1	97.3 ± 2.9	129 ± 3.8

Table 4: Relationship between the mean values of MDA (mmol/L) and duration of diabetes mellitus

Duration of diabetes in (years)	Case No.	MDA (m mol/L)	P. value
1-7	46	1.44	P<0.01
8-14	36	1.52	P<0.01
15-21	22	1.65	P<0.01
22 and above	5	1.72	P<0.01
Control group	54	0.73	P<0.01

Table (5): Relationship between the mean values of Vit.C (mg/dl) and duration of diabetes mellitus

Duration of diabetes in (years)	Case No.	Vit.C (mg/dl)	P. value
1-7	46	0.52	P<0.01
8-14	36	0.45	P<0.01
15-21	22	0.41	P<0.01
22 and above	5	0.28	P<0.01
Control group	54	1.18	P<0.01

Discussion

Free radicals are formed disproportionately in diabetes mellitus by glucose degradation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation, which may play an important role in the development of complications in diabetic patients. The generation of free radicals may lead to lipid peroxidation resulting in several damage in diabetes mellitus. In the present study, we have observed that (MDA) levels as a lipid peroxidation product and a marker of oxidative stress, were elevated significantly in males and as well as in females in type 2 diabetic patients P<0.01, (Table 2). This clearly shows that diabetic patients, irrespective of the sex, were exposed to an increased oxidative stress via lipid peroxidation. The other researchers have also reported elevated lipid peroxidation products in blood samples of type 2 diabetic patients [13, 14]. The most probable causes for the increased MDA, lipids (cholesterol and TG), lipoprotein (LDL-C) and lipid peroxides (MDA) levels in serum of diabetic groups may be due to the abnormal lipid metabolism. Elevated levels of lipid peroxide in diabetes mellitus may be due to the alteration of function of erythrocytes membrane, and a deficiency of the antioxidant activity of vitamins (C and E) has been related to higher concentration of peroxide [15]. There may be imbalance between production and scavenging of free radicals produced due to the lack of antioxidant system [16]. The mean plasma Vit.C in type 2 diabetes mellitus was 0.48mg/dl which was significantly lower than that of control group (1.18mg/dl), (Tables 1). This result was in agreement with other observations (the biochemical evidence of ascorbate deficiency in the presence of diabetes could be due to impaired tubular reapportion or increased oxidation) [17]. On the other hand, other studies reported no significant difference in vitamin C level between patients with diabetes and controls [18], this may be due to many factors such as duration of diabetes, dietary intake of vitamin C, physical activity, number of cigarettes smoked, Data obtained conducted in the group of patients with type 2 diabetes revealed a significant increases in triglycerides (TG) and a significant decrease in HDL-C in comparison to the control group. Total cholesterol levels

were moderately increased and levels of LDL cholesterol was slightly increased (Table 3). Altered lipid profile is characteristic and typical of type 2 diabetes, which is described by other researchers too [19, 20].

Conclusion

A high level of glucose decreases the levels of antioxidants among these vitamin C and impairment of lipid metabolism with the consequence of increased levels of (MDA). Further studies shout be undertaken to establish other vitamins such as vitamin A and vitamin E, which have a potential role in boosting antioxidant related defenses. Enzymatic antioxidant (ex: glutathione peroxidase, superoxide dismutase, and catalase), also should be studied to find out the effect of hyperglycemia on their activities.

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