

Lipid Peroxidation , Lipid profile and Vitamins A, E in Type II Diabetes Mellitus

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Abstract

The study was designed to find out the relation between lipid peroxidation, lipoprotein levels to severity and complication of diabetes mellitus. Degree of lipid peroxidation was measured in term of malondialdehyde (MDA) along with antioxidants, lipid profile and blood glucose in type 2 diabetes mellitus. Total 400 human subjects, out of which 200 healthy individuals of age group (40 - 80 years) were taken as control and 200 diabetic subjects of age group (40 - 80 years) were taken as cases.

The level of lipid peroxidation (MDA) increased as per the increase in concentration of blood glucose as well as. There was significant increase in the lipid profile except HDL cholesterol, which is decreased, also significant decrease in antioxidant vitamin E when the major lipid soluble antioxidant vitamin presents in cell membrane and lipoproteins, which may be due to their increased consumption during the process of combating excessive free radicals generated in diabetes.

Key words : Vitamins A, E, Type II Diabetes Mellitus

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Introduction

Type 2 diabetes is the commonest form of diabetes and associated with multiple metabolic derangements that result in the excessive production of reactive oxygen species (ROS) and oxidative stress.[1]

In diabetes, there are more mechanisms that induce oxidative stress than in normal individuals; glucose auto-oxidation, non-enzymatic glycation of protein, and polyol pathway. These pathways enhance generation of reactive oxygen species (ROS) that leading to the tissue damage and cause several complex syndromes in diabetic patients such as cataracts, renal dysfunction,

nerve damage, and atherosclerosis. Especially, atherosclerosis leading to the coronary heart disease (CHD) is the major cause of death among diabetics.[2] Atherosclerosis is the thickening and rigidity of the artery and arterioles . Its pathogenesis begins with oxidation of low density lipoproteins (LDL-c) by ROS. Increased lipid peroxidation (measured as levels of malondialdehyde or MDA) caused crosslink formation between single molecules of proteins and oxidation of LDL particles and led to the oxidized LDL formation. The oxidized LDLs cannot be recognized by LDL receptors and be scavenged by macrophages

to generate foam cells. After these foam cells accumulation , fatty streak will form and progress to a fibrous plaque and finally to the atherosclerosis. Restriction of blood supply in the coronary arteries causes myocardial infarction and sudden death.[3]

Type 2 diabetes mellitus is associated with multiple metabolic derangements that result in the excessive production of reactive oxygen species and oxidative stress.

Oxidative stress and resultant tissue damage are hallmarks of chronic disease and cell death. There is increasing evidence that, in certain pathological states, the increase production and / or ineffective scavenging of such reactive oxygen species may play a crucial role in determining tissue injury.

Endothelial dysfunction is considered an intrinsic element in the pathogenesis of diabetic angiopathies. A variety of potential mechanisms diabetes for the initiation of endothelial dysfunction of type 2 diabetes have been described including the effects of hyperglycemia, advanced glycation end products (AGE) and dyslipidemia[4]. In addition, hyperglycemia has been showed to induce free radical release and reduce anti-oxidant defense, both which are associated with endothelial dysfunction[5].

Vitamins A,C and E are diet – derived and detoxify free radicals directly. They also interact in recycling processes to generate reduced forms of the vitamins , α -Tocopherol is reconstituted when ascorbic acid recycles the tocopherol radical; dihydroascorbic acid, which is generated and recycled by glutathione . These vitamins also foster toxicity by producing prooxidants under some conditions. Vitamin E, a component of the total peroxy radical-trapping antioxidant system[6], reacts directly with peroxy and superoxide radicals and singlet oxygen and protects membranes from lipid peroxidation. The deficiency of vitamin E is concurrent with increasing peroxides and aldehydes in

many tissues. There have been conflicting reports about vitamin E levels in diabetic animals and human subjects . plasma and / or tissue levels of vitamin E are reported to be unaltered [7], Increased [8], or decreased by diabetes. Discrepancies among studies in terms of preventive or deleterious effects of vitamin E on diabetes induced vascular aberrations may arise from the variety of examined blood vessels or the administered dose of vitamin E [9].

Subjects and Methods

1. Subjects:

a) Control subjects

200 healthy subject were control group with mean FBS = 5.173 m mol/L, they were 100 males and 100 females. The age ranged from 40 to 80 years old. The mean age average was 53.17 years.

b) Type 2 diabetic patients

Type 2 diabetic patients were 200, 100 males and 100 females. The ages ranged from 20 to 80 years old. The mean age average was 57.91 years.

All samples were in a state of fasting for 12 hours before drawing blood were obtained on these samples Baquba Teaching Hospital in Diyala for the period between November 2011 until February 2012.

2. MDA assay in serum

MDA in serum performed as described by Muslih et al.[10] In brief, serum was mixed with 20% TCA and allowed to stand for 10 minutes. After that 0.05m H₂So₄ and TBA were added. The mixture was mixed and place in 70 c° water bath for 30 min. The resulting chromogen was extracted with n-butanol and centrifuged at 2000 rpm / min, and measured against butonol blank at 532 nm excitation and 553 nm emission by spectrophotometer.

3. Vitamin A, E assay

These antioxidant vitamins were assay by HPLC. In brief, serum, α -tocopheryl acetate

as internal standard and ethanol was mixed for 15 sec. The hexane was added and mixed vigorously for 2 min. The tube was centrifuged at 5198xg, 4c° for 5 min. The hexane layer was transferred and evaporated under a stream of nitrogen gas. The lipid residue was dissolved in ethanol and injected into the sphere clone 5 μ ODS, 250 × 4.60 mm of HPLC. The mobile phase was methanol: acetonitrile: chloroform (25:60:15) at a flow rate 1.5 ml/min.

Vitamin A and E were detected at 290 nm[11].

4. Statistical analysis

Result are expressed as mean ± SD. Data were compared by one- way analysis of variance (one- way ANOVA) using LSD test person rank correlation test was used for testing correlation between variables. Statistical analysis was performed using SPSS 14 software (SPSS. Inc., Chicago. IL,USA) [12].

Results

Table 1 showed the date of age, fasting serum glucose, MDA, lipid profile between the normal healthy group and the type 2 diabetic patients. The table showed that type 2 DM were significantly higher of FSG than healthy subjects (P< 0.001). The study of serum lipid profile showed that the total cholesterol, LDL-cholesterol, triglycerides, and VLDL- cholesterol were significantly increased from the healthy subjects (P<0.001).

While HDL- cholesterol was decreased, the lower level of HDL-c may exacerbate the cardiovascular complication in this group of diabetes.

Table 2, the serum vitamin A level of type 2 DM showed no significant difference when compared with healthy subjects, while vitamin E in type 2 DM showed significant decrease as compared with healthy subjects.

Discussion

The present study showed that MDA was increased significantly in serum of all diabetic patients comparing to healthy subjects. The rise in the MDA indicated that any oxidative stress incurred sufficiently cause of free radical – mediated peroxidation of lipid components in cell membrane[13].

Therefore, MDA is a good indicator for evaluating oxidative stress in degenerative diseases like diabetes mellitus. MDA levels were not different among all patients , these may be due to the enhancement of the serum lipid peroxide removal by aldehyde dehydrogenase enzyme in liver mitochondria, This enzyme has function to destroy toxic aldehyde and protects tissue aldehyde accumulation[14]. In addition serum MDA can be moderated by enhancement of the degradation of excretion[15] .These results demonstrated that diabetic patients were prone to accumulation of potentially harmful oxidative stress .These finding are consistent with the reports of the others[16] .All patients of diabetes are showed hyperglycemia which can directly cause increased reactive oxygen species (ROS) generation. Glucose can undergo autooxidation and generate hydroxyl .OH-radicalicals [17] .In addition, glucose reacts with proteins in a non-enzymatic manner leading to the formation of advanced glycation end products (AGEs) .ROS is generated at multiple steps during this process. In hyperglycemia , there is enhanced metabolism of glucose through the polyol (sorbitol) pathway, which also result in enhanced production of superoxides O•-2 . Type 2 diabetes mellitus we found that HDL cholesterol levels were significantly low and other parameters of lipoproteins were significantly high as compare to healthy subjects. The low levels of HDL cholesterol

which exerts anti-atherogenic and anti-oxidative effects when present in sufficient amounts is key feature for oxidative stress status[18].

Protein identified as a key component of the VLDL assembly process leads to increase level of TG and reduce levels HDL-c in addition the elevation of free fatty acid and glucose in diabetes mellitus can decrease activity of lipoprotein lipase a pivotal enzyme in the removal of these lipoproteins from circulation that control the TG rich lipoproteins and HDL protein[19].

The determination of antioxidant vitamins to prevent lipid peroxidation were also performed in this study. Many researchers reported the role of antioxidant vitamins including vitamin C, E, A, and β -carotene to defense damage by ROS in human disease such as cancer, inflammation, and arthritis. Diabetes mellitus is another interesting one and it is currently under study.

Horwitt et al [20] suggested that total lipid content has an influence on the plasma vitamin E level since vitamin E is mainly found in LDL particles. There is evidence that vitamin E: cholesterol ratio is a more

reliable criterion for vitamin E status then plasma vitamin E alone [21].

This is because the use of this ratio can correct for conditions that result in increased plasma lipid levels [22].

In our study the lower levels of vitamin E status and high levels of serum MDA in type 2 patients may be supported by these evidence.

In the present study antioxidant vitamin A in type 2 diabetic patients showed reduced significantly in males but did not reach to the significant level in females when compared to healthy subjects. This indicate that oxidative stress induced by high level of glucose may increase superoxide radical production with diabetic patients.

Conclusion

The present study shows that significant lipoprotein abnormalities in type 2 diabetic patients when compared with healthy subjects. The increased level of serum MDA and lower level of serum vitamin E clearly shows that diabetic patients was exposed to an increased oxidative stress via lipid peroxidation.

Table (1): Lipid peroxidation, serum glucose and lipid profile in type 2 diabetes compared with normal.

Subject	N	Age (year)	FBS (mmol/L)	MDA (mmol/L)	TC (mmol/L)	TG (mmol/L)	HDL-c (mmol/L)	LDL-c (mmol/L)	VLDL-c (mmol/L)
			Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
Control									
Male	100	55.180 \pm 9.431	5.142 \pm 0.828	1.752 \pm 0.557	3.484 \pm 0.764	1.810 \pm 0.604	1.226 \pm 0.354	1.435 \pm 0.737	0.882 \pm 0.274
Female	100	51.160 \pm 8.867	5.204 \pm 0.857	1.248 \pm 0.468	3.742 \pm 0.693	1.548 \pm 0.589	1.294 \pm 0.421	1.744 \pm 0.751	0.703 \pm 0.267
Total	200	53.160\pm9.350	5.173\pm0.841	1.500\pm0.572	3.613\pm0.739	1.679\pm0.609	1.260\pm0.390	1.589\pm0.758	0.763\pm0.276
NIDDM									
Male	100	59.710 \pm 10.942* *	15.808 \pm 4.433 ***	3.091 \pm 1.566 ***	4.427 \pm 1.684* **	2.000 \pm 1.152	0.991 \pm 0.380* **	2.526 \pm 1.583* **	0.909 \pm 0.524
Female	100	56.100 \pm 10.645	13.222 \pm 4.712 ***	3.060 \pm 1.276 ***	4.359 \pm 0.885* **	2.868 \pm 1.430* **	0.999 \pm 0.551* **	2.156 \pm 1.215* **	1.303 \pm 0.649* **
Total	200	57.905\pm10.981	14.515\pm4.743 ***	3.075\pm1.425 ***	4.393\pm1.342* **	2.434\pm1.366* **	0.995\pm0.472* **	2.291\pm1.427* **	1.106\pm0.621* **

P* < 0.05

P** < 0.01

P*** < 0.001

Table (2): Serum antioxidant vitamin A and E in type 2 DM compared with control.

Subject	N	Vitamin A (mmol/L) Mean±SD	Vitamin E (mmol/L) Mean±SD
Control			
Male	100	1.394±0.406	22.785±7.400
Female	100	1.011±0.366	19.038±6.633
Total	200	1.202±0.431	20.911±7.256
NIDDM			
Male	100	0.988±0.488***	314.223±7.322***
Female	100	0.959±0.366	16.407±8.424*
Total	200	1.052±1.237	15.315±7.948***

P* < 0.05

P** < 0.01

P*** < 0.001

Table (3): Serum lipid peroxidation, lipid profile and glucose levels in type 2 DM males compared with females.

Subject	N	Age (year)	FBS (mmol/L) Mean±SD	MDA (mmol/L) Mean±SD	TC (mmol/L) Mean±SD	TG (mmol/L) Mean±SD	HDL-c (mmol/L) Mean±SD	LDL-c (mmol/L) Mean±SD	VLDL-c (mmol/L) Mean±SD
NIDDM									
Male	200	59.710±10.942	15.808±4.433	3.091±1.566	4.427±1.684	2.000±1.152	0.991±0.380	2.526±1.583	0.909±0.524
Female	200	56.100±10.645*	13.222±4.712 ***	3.060±1.276	4.359±0.885	2.868±1.430* **	0.999±0.551	2.056±1.215* *	1.303±0.649 **

P* < 0.05

P** < 0.01

P*** < 0.001

Table (4): Serum antioxidant vitamin A and E in type 2 DM males compared with females.

Subject	N	Vitamin A (mmol/L) Mean±SD	Vitamin E (mmol/L) Mean±SD
NIDDM			
Male	200	0.988±0.488	14.223±7.322
Female	200	0.959±0.366	16.407±8.424*

P* < 0.05

P** < 0.01

P*** < 0.001

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