Original Article

Effect Of Vitamin E On Oxidative Stress Indicated By Serum Malondialdehyde And Paroxonase Level In Type 2 Diabetes Mellitus With Retinopathy

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Abstract

Background In type 2 diabetes mellitus, the body gradually loses its ability to produce adequate insulin from the pancreas and/or becomes resistant to the typical effects of insulin.**Objectives** This case-control study was carried out to evaluate the impact of vitamin E on oxidative stress in T2DM patients with retinopathy as measured by malondialdehyde and paronxanase 1 levels.**Subjects and Methods** This study was conducted over a period of 11 months on thirty diabetic patients divided into two equal groups; the first group had T2DM without retinopathy (control), while the second group had T2DM with retinopathy (case). Ophthalmological evaluation, Serum paraoxonase-1 and malondialdehyde levels, after 12-hour fasting, blood samples were taken to estimate all laboratory investigation. **Results** In the group of participants with T2DM and retinopathy, there was a statistically significant positive correlation between the paroxanase 1 level and HDL. Additionally, the T2DM with retinopathy group's paroxanase 1 level increased statistically significantly after receiving antioxidant vitamin E treatment for three months. **Conclusion** Serum MDA levels were related to DR stage, indicating that oxidative stress is a major factor in the development of DR. However, proliferative DR patients' MDA levels dropped after taking vitamin E for three months.

Keywords Vitamin E, Oxidative Stress, Serum Malondialdehyde, Paroxonase Level, Type 2 Diabetes Mellitus, Retinopathy.

INTRODUCTION

Increased vascular permeability, ocular haemorrhages, and lipid exudate are all symptoms of diabetic retinopathy (DR), a microangiopathy that affects all of the small retinal vessels, including arterioles, capillaries, and venules. These symptoms are caused by the development of new vessels on the retina and the choroid. It is the most typical microvascular problem associated with diabetes. The likelihood of developing DR increases with the length and quality of diabetes control, and it is linked to hyperglycemia, hypertension, hyperlipidemia, pregnancy, nephropathy, and anaemia [1].
The molecule having the formula CH2(CHO)2 is known as malondialdehyde (MDA). As a biomarker of oxidative stress, it is a byproduct of lipid peroxidation. It has been used mostly in the assessment of oxidative stress induced on by exposure to air pollution. The oxidation of polyunsaturated fatty acids with more than two double bonds disrupted by methylene is the primary endogenous source of MDA [2].

Human blood plasma contains a significant amount of naturally occurring vitamin E, which is primarily concentrated within cell membranes and blood proteins. It plays a role in a variety of mechanisms that have been researched, one of which is lipid peroxidation by preventing the production of malondialdehyde; it has been demonstrated to lower fasting plasma glucose in diabetes at doses as high as 2000 mg/day [3].

Measurement of serum malondialdehyde by **Chatziralli et al.** [4] revealed the effect of oxidative stress on the development of diabetic retinopathy (DR) in insulin-dependent type 2 DM patients (MDA).

They concluded that while vitamin E appears to lower MDA levels and subsequent oxidative stress, indicating that it might have a protective role in the pathogenesis and progression of DR. Oxidative stress has been found to play a significant role in the pathogenesis and progression of DR.Paraoxonase 1 (PON1), an antioxidizing enzyme that aids in the breakdown of lipid peroxides into oxidised lipoproteins, has been linked to conditions including diabetes and cardiovascular disease that exhibit high levels of oxidative stress. It performs a number of different biochemical pathways' multifunctional functions, including preventing oxidative damage and lipid peroxidation, enhancing innate immunity, detoxifying reactive molecules, bioactivating drugs, reducing endoplasmic reticulum stress, and controlling cell growth and apoptosis [5].

AIM OF THE WORK

to assess the relationships between paraoxanase 1 and oxidative stress in diabetic retinopathy. to verify the levels of paraoxanase-1 and malondialdehyde in diabetic retinopathy. to study the effects of vitamin E on oxidative stress in type 2 diabetes mellitus with retinopathy as determined by serum malondialdehyde and paroxonase levels.

SUBJECTS AND METHODS

This case-control study was conducted over a period of 11 months among individuals who recruited from the Ophthalmology and Internal Medicine Outpatient Clinic of Al-Zahraa University hospital. 60 individuals with type 2 diabetes were included in the study, and they were divided into two groups as follows: **Group I:** 30 individuals diagnosed with type 2 diabetes who don't have diabetic retinopathy, with the mean age (54.6±10.4). **Group 2:** An ophthalmologist diagnosed and staged 30 patients with type 2 diabetes mellitus and diabetic retinopathy, with the mean age (56.8±11.1). The American Diabetes Association's Standards of Medical Care were used to diagnose type 2 diabetes mellitus. and all of the patients' diabetes was largely under control. Based on the history, physical examination, and laboratory tests, type 2 diabetes was identified. With a HbA1c of less than 7.5% (58 mmol/l), all patients had relatively under control diabetes. Ethical approval was obtained from by the Research Ethics Committee of the Faculty of Medicine, Al-Azhar University in accordance with the Declaration of Helsinki, 2013. Written informed consent was obtained from all participating patients.

Exclusion criteria

Patients with uncontrolled hypertension, liver or renal disease, smoking, a history of alcohol use, cancer, coronary heart disease, cerebrovascular disease, chronic obstructive pulmonary disease, end-stage renal disease or diabetic nephropathy, use of antioxidant supplements, ocular surgery, intraocular inflammation, and obesity (BMI > 30).

Methodology

The following procedures were applied to all subjects:

A thorough medical history is taken, including the following information: name, age, sex, the length of diabetes, the use of anti-diabetics, cardiovascular disease, signs of diabetic nephropathy, retinopathy, and neuropathy, hypertension and how it is treated, drug use history, and smoking.

clinical examination including

Ophthalmological evaluation, including best corrected visual acuity (BCVA), slit-lamp examination, tonometry, fundus examination.

estimation of blood pressure, **measurement** of BMI (less than 30, without taking any special diet), and detection of BMI. All patients were informed about the study and given the opportunity to give their consent.

Analyzing anthropometric data Using defined methods, anthropometric measurements such as height, weight, and waist measurements were taken. A tape measure was used to measure height to the closest centimeter. The subjects were instructed to stand with their backs to the wall, heels together, and eyes forward. They were not to wear shoes.Weight was measured with a traditional balance; it was kept flat and stable. One layer of clothing was allowed, and the individuals were instructed to stand straight, relaxed, and with their feet together on a flat surface. Weight was reported to the nearest 0.5 kg and subjects were instructed to wear light clothing. Weight (Kg) / height (m2) was the formula used to determine body mass index (BMI).

Blood pressure determination

After 10 minutes of resting, the subject's blood pressure was checked with a standard mercury sphygmomanometer on the right arm while they were supine. The beginning of the tapping Korotkoff sounds was used to calculate systolic blood pressure. Diastolic blood pressure was identified as the cause of the disappearance of Korotkff noises. Two independent measurements performed at 5-minute intervals were used to calculate the means. The conventional definition of hypertension is systolic blood pressure (SBP) greater than or equal to 140 mmHg and/or diastolic blood pressure about 90 mmHg (DBP).

Laboratory investigations

Using the Bio-Rad D-10 HbA1c Testing System, fasting blood sugar, postprandial blood sugar, and HbA1c were measured using high performance liquid chromatography (HPLC) in accordance with the method of glycated haemoglobin analysis. serum lipid profile, which was calculated using a colorimetric method and included serum triglycerides (TG), high density lipoproteins (HDL), low density lipoproteins (LDL), and total cholesterol levels; serum SGOT and SGPT, which are liver enzymes; Complete Blood Picture (CBC): red blood cells (RBC) count, white blood cells total and differential (WBC) count, hemoglobin concentration (Hb), platelet count; Blood urea and serum creatinine, which were estimated by colorimetric technique; urine analysis; Serum paraoxonase-1 levels serve as an antioxidant and serum malondialdehyde levels serve as a measure of lipid peroxidation residue in diabetic retinopathy, respectively. After a 12-hour fasting, blood samples were taken in the morning to estimate all parameters. In order to separate the serum, one blood sample was aspirated and contains k-EDTA for measuring HbA1c. Within a reasonable amount of time, measurements of the lipid profile, SGOT, SGPT, urea, and creatinine were made.

elements of oxidative stress in the blood:

1. Serum malondialdehyde level (MDA) (μmol/L)
2. Serum paroxonase level

Statistical analysis

SPSS version 23 was used for data processing, checking, entering, and analysis of the data. The results of this research study were analysed using the following statistical techniques. For quantitative variables, the data were reported as mean + standard deviation (SD), and for qualitative variables as number and percentage. The level of significance (p-value) for all of the aforementioned statistical tests was set at 5%; a p value of > 0.05 denotes non-significant results, and a p-value of ≤0.05 denotes significant results. The results are more significant the lower the p value that was achieved.

Results

This case-control study was carried out to evaluate the impact of vitamin E on oxidative stress as assessed by levels of paronxanase 1 and malondialdehyde on T2DM patients with retinopathy which included two equal groups each consisted of thirty patients; the 1st group was T2DM without retinopathy (control) and the 2nd one was T2DM with retinopathy (case).

Table 1: Socio-demographic characteristics and BMI of the two studied groups

Variables	Control No=30	Case No=30	t-test	p-value
	mean ± SD	mean ± SD		
Age (years)	54.6±10.4	56.8±11.1	0.6	0.5
BMI	25.47 ± 1.85	26.59±2.76	1.85	0.07
Sex Male Female	 14 (46.7%) 16 (53.3%)	 12 (40.0%) 18 (60.0%)	 0.2	 0.6

This table shows that there was no statistically significant difference between the two studied groups regarding age, BMI and sex (matched case-control)

Table 2: Laboratory investigations among the two studied groups

Variables	Control No=30	Case No=30	t-test	p-value
	mean ± SD	mean ± SD		
HbA1c *(%mmol/ml)*	5.79 ±1.14	6.18 ± 1.22	1.28	0.206
Total cholesterol (*mg/dl)*	213.5±34.1	225.4±42.3	1.5	0.2
HDL (*mg/dl)*	56.1±10.6	50.2±5.6	3.6	**0.001****
LDL (*mg/dl)*	124.5±37.1	131.2±30.5	1.4	0.2
Triglycerides (*mg/dl)*	131.5±46.2	156.1±50.4	1.19	0.6

**Highly statistically significant different

This table demonstrates that among T2DM patients without retinopathy compared to those with, there was a highly statistically significant rise in HDL levels. HbA1c, total cholesterol, LDL, and triglycerides, on the other hand, showed no statistically significant difference.

Table 3: Malondialdehyde and paronxanase 1 levels among the two studied groups

Variables	Control No=30	Case No=30	t-test	p-value
	mean ± SD	mean ± SD		
Malondialdehde *(mmol/ml)*	3.71±0.45	4.28±0.13	6.6	<0.001**
Paroxanase 1 *(mmol/min/ml)* *Median (IQR)*	158.4 (101-443.5)	114.3 (40.5-419.6)	4.7	<0.001**

**Highly statistically significant different

This table demonstrates that the levels of malondialdehyde were statistically significantly higher in T2DM patients with retinopathy than in those without. On the other hand, T2DM with retinopathy had statistically significantly lower paroxanase 1 activity than T2DM without retinopathy.

Table 4: Stages of retinopathy among the T2DM with retinopathy group

Stages of retinopathy	***The T2DM with retinopathy group* (n=30)**	
	No	**%**
Stage I	8	(26.7%)
Stage II	6	(20.0%)
Stage III	6	(20.0%)
Stage IV	10	(33.3%)

This table shows that third of the case group (33.3%) had Stage IV retinopathy followed by Stage I (26.7%) then Stage II and Stage III (20.0%) for each stage.

Table 5: Correlation between paroxanase 1 level with HDL among the T2DM with retinopathy group

Variables	Serum paroxanase 1		
	r	p-value	SIG
HDL	0.64	0.001**	HS

** Highly statistically significant different

HS= highly significant

This table shows that there was statistically significant positive correlation between serum paroxanase 1 level and HDL among the T2DM with retinopathy group as there was decrease in paroxanase 1 activity along with decrease on HDL.

Table 6: Malondialdehyde level before and after 3 months administration of vitamin E as antioxidants among the T2DM with retinopathy group with different diabetic stages

Malondialdehyde *(mmol/ml)* ***Stages of retinopathy***	**Before Vit. E**	**After Vit. E**	**p-value**
	mean ± SD	**mean ± SD**	
Stage I ***No=8***	3.7±0.19	3.46±0.15	0.001**
Stage II ***No=6***	4.02±0.09	3.75±0.11	0.001**
Stage III ***No=6***	4.17±0.12	4.04±0.86	0.001**
Stage IV ***No=10***	4.65±0.21	4.31±0.1	0.001**

**Highly statistically significant different

This table shows that there was statistically significant decrease on malondialdehyde level after 3 months vitamin E administration as antioxidants among the T2DM with retinopathy group on all diabetic stages.

Table 7: Paroxanase 1 level before and after 3 months administration of vitamin E as antioxidants among the T2DM with retinopathy group

Variable	**Before VIT E**	**After VIT E**	**p-value ^**
	Median (IQR)	**Median (IQR)**	
Paroxanase 1 level No=30 (mmol/min/ml) Median (IQR)	114.3(40.5-419.6)	157.1(101-487.5)	0.001**

^ Mann-Witenny U test

This table shows that there was statistically significant increase on Paroxanase 1 level after 3 months vitamin E administration as antioxidants among the T2DM with retinopathy group.

DISCUSSION

Diabetes mellitus (DM) is strongly associated to both macro- and micro-vascular problems. such as persistent hyperglycemia and the ensuing hypoxia, which cause diseases like neuropathy, nephropathy, and retinopathy. In developed societies, diabetic retinopathy is a substantial contributor to vision loss in people of working age. Thus, it is important to put into practice DR prevention techniques and to pinpoint precise early predictors. Although the causes of DR are multifaceted, recognized risk factors include long-term diabetes, severe hypertension, and high blood sugar [6].

Nearly all individuals with type 1 diabetes and 60% of type 2 diabetes have diabetic retinopathy 20 years after the onset of the condition. High glucose levels are thought to play a significant role in the pathogenesis of DR by causing vascular endothelial cells to undergo apoptosis and a wide range of hemodynamic changes, including increased blood viscosity, increased erythrocyte aggregation, altered erythrocyte permeability, and increased erythrocyte adhesion to endothelial cells [7].

Subsequently, a number of candidate genes and gene polymorphisms have been linked to the pathogenesis of DR, and DR is also thought to be caused by epigenetic mechanisms [8]. In particular, DNA methylation was discovered to be a prospective marker of proliferative DR, predicting the development of DR, in a study by Agardh et al.[9] A significant risk factor for atherosclerosis and associated conditions such ischemic heart disease, cerebrovascular disorders, and retinal atherosclerosis is hyperlipidemia [10].

Our findings diverged from **Price et al. [11]** study as obesity with a BMI of > 30 kg/m^2 is major risk factor for DR**.** We found that there was no statistically significant difference regarding HbA1c.

Fong et al.,[12] discovered that a higher HbA1c is linked to both an increase in the incidence and development of diabetic retinopathy. The retinal vasculature becomes dysfunctional as a result of high blood sugar levels' effects on the shape and physiology of retinal vascular cells, including endothelial cells, pericytes, and astrocytes. According to **Sharma et al. [13]** the HbA1c levels were higher in the DR group (mean 11.23.26) compared to the control healthy group (4.72.24), and the findings were statistically significant (P value 0.001). An established marker of time-integrated hyperglycemia is glycated haemoglobin (HbA1c).

When comparing T2DM patients without and with retinopathy, we discovered a very statistically significant increase in HDL levels. Other than that, we discovered no statistically significant difference between the patients and control group in terms of total cholesterol, LDL, or triglycerides. According to **Gnaneswaran et al. [10]** there is no correlation between serum lipids and diabetic retinopathy. Additionally, **Tomic et al. [14]** found no connection between DR, triglycerides, HDL, and total cholesterol in the Danish diabetic community.

Sharma et al. [13] discovered that individuals with diabetic retinopathy had greater levels of lipids (serum total cholesterol, triglycerides, and LDL), whereas HDL levels were lower than those of healthy controls. The findings were statistically significant (P value <0.001).

However, **Yau et al**. (2012) observed that greater total serum cholesterol was associated with a higher frequency of diabetic retinopathy and diabetic macular edema. Additionally, diabetic individuals with DR had significantly different levels of total cholesterol and LDL-C, according to **Gnaneswaran et al.** [10]. Higher overall triglyceride levels were shown to be related to more severe retinopathy.

The risk of retinal hard exudates is significantly associated with increasing serum lipid levels, according to **Lyons et al. [15]** Triglycerides, as well as VLDL and HDL cholesterol, were favourably correlated with the severity of retinopathy and inversely correlated with HDL cholesterol**. Sharma et al. [13]** compared subjects with type-2 diabetes to healthy non-diabetic subjects to assess the state of antioxidant markers and oxidative stress in diabetic retinopathy. In comparison to healthy subjects, diabetic retinopathy patients had higher values for all of these biochemical parameters, with the exception of HDL, antioxidant-SOD, GPx, and vitamin E. These differences were found to be statistically significant. Our research found that T2DM with retinopathy had significantly higher levels of malondialdehyde than T2DM without retinopathy. On the other hand, paroxanase was statistically significantly lower in T2DM with retinopathy than without. Our findings are in line with **Ramakrishna and Jailkhani [16]** They have also discovered elevated levels of oxidative stress markers in patients with insulin-dependent diabetes and a link between them and vascular problems. Increased LPO in DM has been linked to a number of metabolic changes, the most notable of which is hyperglycemia, which causes the formation of thiose phosphate, whose oxidation results in an excess of free radicals, damaging blood cells through oxidative stress, cross-linking of membrane lipids and proteins, speeding up cell ageing, and vasoconstriction. Thus, oxidative stress-mediated retinal neurodegeneration and the formation of free radicals that fuel DR progression may share a similar route with DM itself and its consequences [17].

In the T2DM with retinopathy group, our study found a statistically significant positive connection between serum paraoxanase 1 level and HDL because there was a concomitant decline in paraoxanase 1 activity and HDL. According to our research, stage IV retinopathy was present in one-third (33.3%) of the case group, followed by stages I (26.7%), stage II, and stage III (20.0%) for each stage. We discovered that there was a statistically significant decrease on malondialdehyde level after 3-month vitamin E application as an antioxidant among the T2DM with retinopathy group on all diabetic stages.

Vitamin E is a crucial antioxidant that acts as a chain-breaker to stop the propagation of free radical reactions in all cell membranes in the human body. The correlation between serum malondialdehyde levels and diabetic retinopathy has only been found in a small number of investigations. According to **Mancino et al.** [18] elevated MDA is linked to oxidative stress and inadequate antioxidant defence, which encourages the development of DR into its proliferative stage. This finding suggested that the degree of oxidative stress, as reflected by an elevated level of MDA among PDR patients, is directly connected to retinal microvascular problems. Our study showed that there was statistically significant increase on paroxanase 1 level after 3-month vitamin E administration as an antioxidant among the T2DM with retinopathy group.

However, all biochemical changes brought on by DM cause anatomical and functional impairment in the retinal microvascular network, including changes in blood flow in the retina, disruption of the blood-retina barrier, and ultimately capillary occlusion and ischemia. The precise mechanism by which oxidative stress contributes to diabetic complications is still unknown.

Conclusion

Our work contributed us to the conclusion that serum MDA levels were related to DR stage, indicating that oxidative stress is a major factor in the development of DR. However, proliferative DR patients' MDA levels dropped after taking vitamin E for three months. Antioxidant supplementation, such as vitamin E, may therefore be utilised as an adjuvant therapy in DR patients to lessen oxidative stress and maybe safeguard against later complications of DM; a high frequency of and a significant correlation between the severity of diabetic retinopathy and poor glycemic control; A longer history of diabetes is linked to DR advancement.

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