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: Type 2 diabetes mellitus is a progressive condition in which the body becomes resistant to the normal effects of insulin and/or gradually loses the capacity to produce enough insulin the pancreas. Objectives: This case-control study was conducted to assess the effect of vitamin E on oxidative stress indicated by malondialdehyde and paronxanase 1 level on T2DM patients with retinopathy. Patients and Methods: 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). Results: There was statistically significant positive correlation Oppositely, paroxanase 1 level was statistically significantly negatively correlated with hydroperoxidase. As. Also, there was statistically significant increase on paroxanase 1 level after 3-month vitamin E application as
an antioxidant among the T2DM with retinopathy group. Conclusion: Serum MDA levels were associated with DR stage, suggesting that oxidative stress play a significant role in DR progression. On the other hand, MDA levels were reduced and proliferative DR patients after vitamin E intake for 3 months.

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

Introduction

Diabetic retinopathy (DR), a microangiopathy affecting all of the small retinal vessels, such as arterioles, capillaries and venules, is characterized by increased vascular permeability, ocular hemorrhages, lipid exudate, mediated by the development of new vessels on the retina and the posterior vitreous surface. It is the most common microvascular complication of diabetes. The incidence of DR is increased with duration and poor control of diabetes and is related to hyperglycemia, hypertension, hyperlipidemia, pregnancy, nephropathy, and anemia (Sayin et al., 2015).

Malondialdehyde (MDA) is the organic compound with the formula CH₂(CHO)₂. It is a product of lipid peroxidation and has been used as a biomarker of oxidative stress. It has been widely used in the evaluation of oxidative stress as a response to air pollution exposure. The main endogenous production of MDA arises from the oxidation of polyunsaturated fatty acids with more than two methylene-interrupted double bonds (Ho et al., 2013).

Natural vitamin E is a major lipid soluble antioxidant in human blood plasma, which concentrates mainly in the interior of membranes and blood proteins. It has a role in many different explored mechanisms, one of them being on lipid peroxidation by inhibiting the formation of malondialdehyde; at concentrations as high as 2000mg/day, it has been shown to reduce fasting plasma glucose in diabetes (Ulker et al., 2016).

Chatziralli et al. (2017) investigated the impact of oxidative stress on progression of diabetic retinopathy (DR) in insulin-dependent type 2 DM patients, measuring serum malondialdehyde (MDA). They concluded that oxidative stress has been found to play significant role in the pathogenesis and progression of DR, while vitamin E seems to reduce MDA levels and subsequent oxidative stress, suggesting that it might have protective role in DR progression.

Paraoxonase 1 (PON1) is an antioxidizing enzyme that contributes to the hydrolysis of lipid peroxides into oxidized lipoproteins, and it has been associated with diseases characterized by high oxidative stress such as cardiovascular disease and diabetes. It has a multifunctional role in various biochemical pathways such as protection against oxidative damage and lipid peroxidation, contribution to innate immunity, detoxification of reactive molecules, bioactivation of drugs, modulation of endoplasmic reticulum stress and regulation of cell proliferation/apoptosis (Martinelli et al., 2013).
Aim of the work To evaluate the relationship between the oxidative stress and paraoxanase 1 in diabetic retinopathy. To correlate malondialdehyde and paraoxanase-1 levels in diabetic retinopathy. To evaluate the effect of vitamin E on oxidative stress indicated by serum malondialdehyde and paroxonase level in type 2 diabetes mellitus with retinopathy.

Methods

Patients and methods

The study included 60 patients with type 2 diabetes mellitus, and they were classified into 2 groups:

Group I: 30 patients with type 2 diabetes mellitus without diabetic retinopathy.

Group 2: 30 patients with type 2 diabetes mellitus with diabetic retinopathy diagnosed and staged by an ophthalmologist.

Type 2 diabetes mellitus was diagnosed according to American Diabetes Association Standards of Medical Care (2015) guidelines, and all patients had relatively controlled diabetes mellitus. Type 2 DM was diagnosed based on history, physical examination, laboratory investigations. All patients had relatively controlled DM with HbA1c <7.5% (58 mmol/ml).

Exclusion criteria:

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

Methodology

All patients were subjected to the following:

1- Full history taking including: Name, age, sex, duration of diabetes, antidiabetic treatment, cardiovascular disease, evidence of diabetic nephropathy, retinopathy and neuropathy, hypertension and its treatment, drug history, smoking.

2- Clinical examination including: Assessment of BMI (less than 30, without any specific diet), assessment of blood pressure, ophthalmological examination. All patients were informed about the study and consents were taken from all patients before taking the blood samples and fundus examination.

Assessment of anthropometric data:

Anthropometric measurements including weight, height and waist measurements were obtained using standardized techniques. Height was measured with a tape to the
nearest centimeter. Subjects were requested to stand upright without shoes with their back against the wall, heels together, and eyes directed forward.

Weignt was measured with a traditional spring balance that was kept on a firm horizontal surface. The subjects were asked to stand erect in a relaxed position with both feet together on a flat surface; one layer of clothing was accepted.

Subjects were asked to wear light clothing, and weight was recorded to the nearest 0.5-Kg. Body mass index (BMI) was calculated by using the formula: weight (Kg / height (m²)).

**Assessment of blood pressure:**

The blood pressure was measured using a standard mercury sphygmomanometer on the right arm with the subject in supine position after 10 minutes rest. Systolic blood pressure was determined by the onset of the tapping Korotkoff sounds. The disappearance of Korotkoff sounds was characterized as diastolic blood pressure. Mean values were determined from two independent measurements taken at 5 minutes intervals. Hypertension generally is defined as values ≥140 mmHg systolic blood pressure (SBP) and/or ≥ 90 mmHg diastolic blood pressure (DBP).

**3- Laboratory investigations:** Fasting blood sugar, postprandial blood sugar, HbA1c measurement by using high performance liquid chromatography (HPLC) done according to method of Glycated hemoglobin analysis was performed using Bio-Rad D-10 HbA1c Testing System; Serum lipid profile including serum triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and total cholesterol level which were estimated by colorimetric technique; Liver enzymes (serum SGOT and SGPT); 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 malondialdehyde levels as a lipid peroxidation residue in diabetic retinopathy measurement; serum paroxonase-1 level as an antioxidant in diabetic retinopathy measurement.

Blood samples were obtained at the morning after 12 hours fasting for estimation of all parameters. A blood sample was aspirated, containing k-EDTA for HbA1c measurement and the second contained no additive for serum separation. Lipid profile, SGOT, SGPT, urea and creatinine were estimated immediately within a suitable time.

**Oxidative stress parameters in the blood:**

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

**Statistical analysis**

Data were checked, entered and analyzed using SPSS version 23 for data processing. The following statistical methods were used for analysis of results of
the present study. Data were expressed as number and percentage for qualitative variables and mean ± standard deviation (SD) for quantitative one. For all above-mentioned statistical tests done, the threshold of significance was fixed at 5% level (p-value): p value of > 0.05 indicates non-significant results, p value of < 0.05 indicates significant results. The smaller the p value obtained the more significant are the results.

**Results**

This case control study was conducted to assess the effect of vitamin E on oxidative stress indicated by malondialdehyde and paroxanase 1 level 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

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control No=30 mean ± SD</th>
<th>Case No=30 mean ± SD</th>
<th>t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>54.6±10.4</td>
<td>56.8±11.1</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>27.8±3.5</td>
<td>29.5±6.2</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>14 (46.7%)</td>
<td>12 (40.0%)</td>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Female</td>
<td>16 (53.3%)</td>
<td>18 (60.0%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control No=30 mean ± SD</th>
<th>Case No=30 mean ± SD</th>
<th>t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HbA1c (%mmol/ml)</strong></td>
<td>6.4±1.9</td>
<td>6.8±2.1</td>
<td>1.2</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Total cholesterol (mg/dl)</strong></td>
<td>213.5±34.1</td>
<td>225.4±42.3</td>
<td>1.5</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>HDL (mg/dl)</strong></td>
<td>56.1±10.6</td>
<td>50.2±5.6</td>
<td>3.6</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td><strong>LDL (mg/dl)</strong></td>
<td>124.5±37.1</td>
<td>131.2±30.5</td>
<td>1.4</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Triglycerides (mg/dl)</strong></td>
<td>131.5±46.2</td>
<td>156.1±50.4</td>
<td>1.19</td>
<td>0.6</td>
</tr>
</tbody>
</table>

**Highly statistically significant different**
This table shows that there was highly statistically significant increase on HDL level among T2DM with than without retinopathy. Otherwise, there was no statistically significant difference regarding HbA1c, total cholesterol, LDL and triglycerides.

### Table 3
Malondialdehyde, paroxanase 1 and hydroperoxide levels among the two studied groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control No=30 mean ± SD</th>
<th>Case No=30 mean ± SD</th>
<th>t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malondialdehyde (mmol/ml)</td>
<td>3.71±0.45</td>
<td>4.28±0.13</td>
<td>6.6</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Paroxanase (mmol/min/ml)</td>
<td>158.4 (101-443.5)</td>
<td>114.3 (40.5-419.6)</td>
<td>4.7</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydperoxide (mmol/mg)</td>
<td>31.4±6.1</td>
<td>38.1±8.5</td>
<td>3.5</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

**Highly statistically significant different

This table shows that there was highly statistically significant increase on malondialdehyde and hydroperoxide levels among T2DM with than without retinopathy. Oppositely, paroxanase was statistically significant decreased among T2DM with than without retinopathy.

### Table 4
Correlation between paroxanase 1 level with hydroperoxidase and HDL among the T2DM with retinopathy group

<table>
<thead>
<tr>
<th>Variables</th>
<th>Serum paroxanase 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
</tr>
<tr>
<td>HDL</td>
<td>0.6</td>
</tr>
<tr>
<td>Hydperoxidase</td>
<td>-0.8</td>
</tr>
</tbody>
</table>

** 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. Oppositely, paroxanase 1 level was statistically significantly negatively correlated with hydroperoxidase.
Table 5
Malondialdehyde level before and after 3 months administration of vitamin E as antioxidants among the T2DM with retinopathy group with different diabetic stages

<table>
<thead>
<tr>
<th>Malondialdehyde (mmol/ml)</th>
<th>Before VIT E mean ± SD</th>
<th>After VIT E mean ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I No=8</td>
<td>3.7±0.19</td>
<td>3.46±0.15</td>
<td>0.001**</td>
</tr>
<tr>
<td>Stage II No=6</td>
<td>4.02±0.09</td>
<td>3.75±0.11</td>
<td>0.001**</td>
</tr>
<tr>
<td>Stage III No=6</td>
<td>4.17±0.12</td>
<td>4.04±0.86</td>
<td>0.001**</td>
</tr>
<tr>
<td>Stage IV No=10</td>
<td>4.65±0.21</td>
<td>4.31±0.1</td>
<td>0.001**</td>
</tr>
</tbody>
</table>

**Highly statistically significant different

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

Table 6
Paroxanase 1 level before and after 3 months administration of vitamin E as antioxidants among the T2DM with retinopathy group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before VIT E Mean ± SD</th>
<th>After VIT E Mean ± SD</th>
<th>p-value ^</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paroxanase 1 level No=30 (mmol/min/ml) Median (IQR)</td>
<td>114.3(40.5-419.6)</td>
<td>157.1(101-487.5)</td>
<td>0.001**</td>
</tr>
</tbody>
</table>

^ Mann-Witenny U test

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

Discussion

Diabetes mellitus (DM) is associated with macro-vascular diseases and micro-vascular complications, such as neuropathy, nephropathy and retinopathy, induced by chronic hyperglycemia and subsequent hypoxia. Diabetic retinopathy represents a major cause of loss of vision in the working age in the developed countries. Thus, there is a need to implement effective strategies being able to prevent DR and to identify specific and early predictors. The etiology of DR is multifactorial, but reported risk factors include increased duration of diabetes mellitus, as well as severity of hypertension and hyperglycemia (Malaguarnera et al., 2013).

Diabetic retinopathy is present in almost all type 1 DM patients, as well as in 60% of type 2 DM patients 20 years after the onset of the disease. High levels of glucose, causing apoptosis in vascular endothelial cells, and a great variety of hemodynamic changes i.e., increased blood viscosity, increased erythrocyte aggregation, alteration in erythrocyte permeability and increased adhesion of
erythrocytes to endothelial cells, are considered to play an important role in the pathogenesis of DR (Tarr et al., 2013).

Furthermore, several candidate genes and gene polymorphisms have been implicated in the pathogenesis of DR, while epigenetic mechanisms are thought to be also responsible for DR (Petrovic et al., 2015). Specifically, in a study by Agardh et al. (2015), DNA methylation was found to be a prospective marker of proliferative DR, predicting the development of DR.

Hyperlipidemia is a powerful risk factor for atherosclerosis and related disorders such as ischemic heart disease, cerebrovascular diseases and retinal atherosclerosis (Gnaneswaran et al., 2013).

In many developing countries the incidence and prevalence of type-2 diabetes mellitus (type-2 DM) far exceed rates in the developed world but facilities for the detection and treatment of retinopathy are limited. Therefore, diabetic retinopathy has the potential to become many serious public health problems in populations. DR which is the leading cause of vision loss in adults in industrialized country, cataract, neovascular glaucoma, oculomotor nerve palsy, lid infection etc are some of the typical complications seen in diabetes mellitus. Apart from these, symptoms like dry eyes and burning sensation were also observed in patients (Zimmet et al., 1990).

Al-Shabrawey and Smith (2010) have focused on oxidative stress on DR, according to the “free radicals” hypothesis. Oxidative stress is described as the imbalance between excess production and/or impaired removal of reactive oxygen species (ROS).

A variety of stimuli, such as hyperglycemia and hypoxia-hyperoxia, might increase the production of ROS at the retinal level, generating oxidative stress. Under these conditions a number of hyperoxides and aldehydes are produced, leading to microangiopathy in diabetes. Accumulation of ROS contributes not only to the pathogenesis of DR but also to the resistance of DR to reverse even after good glycemic control is achieved (metabolic memory phenomenon). Chronic oxidative stress causes damage in cell proteins, membrane lipids and nucleic acids, disrupting cellular homeostasis. In order to compensate the effects of “free radicals”, there are defence mechanisms in the organism called anti-oxidants. The deficiency of anti-oxidant protection in DM patients increases the vulnerability to oxidative alterations of the retinal tissue and development of complications, as it may be the biochemical background for DM-associated endothelial dysfunction. On the other hand, the potential effect of vitamin E, the major antioxidant in lipid phase, has been shown in DR by its free radicals scavenger activity outside the cell through nonenzymatic mechanisms (Kumari et al., 2008).

Reactive oxygen species (ROS) are atoms or molecules with one or more uncoupled electrons, that may damage cell compounds such as lipids, proteins, carbohydrates and DNA. ROS induced oxidation of polyunsaturated fatty acids (PUFA) in biological systems results in the formation of lipid peroxidation products such as Malondialdehyde (MDA). Malondialdehyde is a product of lipid peroxidation and has been used as a biomarker of oxidative stress. It has been
widely used clinically and in environmental epidemiology, especially in the
evaluation of oxidative stress as a response to air pollution exposure (Ho et al.,
2013).

The detection and measurement of lipid peroxidation products such as MDA
provides the evidence most frequently cited to support the involvement of free
radical reaction in diseases including type 2 diabetes mellitus with retinopathy
(Pastore and Korkina, 2010).

Pan et al. (2008) reported higher levels of MDA in patients with DM compared
with controls and in patients with DR versus those without DR, also suggesting
that oxidative stress may play a significant role in the development of DR. As a
result, levels of MDA are used as an index of LPO and in consequence of oxidative
stress.

Human serum paraoxonases (PON1, PON2 and PON3) are a family of calcium-
dependent hydrolases that are involved in antioxidant defense and the
metabolism of various organophosphorus compounds, including paraoxon,
neurotoxins and insecticides. PON1 is a member of the paraoxonase family, and it
encodes a protein of an enzyme with lactonase and ester hydrolase activity. It is
an antioxidant defensive factor that is relevant in the pathogenesis of several
inflammatory diseases (Moya and Manez, 2018).

PON1 has been associated with diseases characterized by high oxidative stress
such as cardiovascular disease and diabetes. Oxidative stress downregulates
serum PON1 expression due to the changes in the redox status (Aviram et al.,
2004). There is no existing report on PON1 activity in diabetic nephropathy.

This case-control study was conducted to assess the effect of vitamin E on
oxidative stress indicated by malondialdehyde and paronxanase 1 level 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).

Our study showed that there was no statistically significant difference between the two
studied groups regarding age. According to Hyun et al. (2014), there was no
significant age difference between groups.

In our study, there was no statistically significant difference between the two
studied groups regarding BMI. In agreement with our study, no BMI difference
was found in Hyun et al. (2014) study. But, our result disagreed with Price et al.
(2014) study as obesity with a BMI of > 30 kg/m² is major risk factor for DR.
Our study showed that there was no sex effect on the development of diabetic
retinopathy in diabetic patients. This was in agreement with Hyun et al. (2014)
and Zhou and Hu (2016) studies.

We found that there was no statistically significant difference regarding HbA1c. In
agreement with our study, Agroiya et al. (2013) found that there was no
association between HbA1c and DR.
Glycated hemoglobin (HbA1c) is a recognized indicator of time-integrated glycemia (Fong et al., 2004). Wat et al. (2016) found that a higher HbA1c is associated with both increased incidence as well as progression of diabetic retinopathy. High blood glucose affects morphology and physiology of retinal vascular cells including endothelial cells, pericytes, and astrocytes leading to dysfunction of retinal vasculature. Sharma et al. (2016) found that increased HbA1c levels (mean 11.2±3.26) in DR group as compared to control healthy group (4.7±2.24) and the results were statistically significant (P value < 0.001).

HbA1c is a useful indicator of how the blood glucose level has been kept in check in the recent past. The indication of glycemic control is better provided by HbA1c than blood or urinary glucose determinations. Poor long term glycemic control can contribute to complications like diabetic retinopathy in subjects (Jin et al., 2003).

We found highly statistically significant increase on HDL level among T2DM with than without retinopathy. Otherwise, we found no statistically significant difference regarding total cholesterol, LDL and triglycerides between cases and control group. Gnaneswaran et al. (2013) showed that there is no association of serum lipids with diabetic retinopathy. Also, Tomić et al. (2013) reported no significant association between DR, triglycerides, HDL and total cholesterol in diabetic population in Denmark. Sharma et al. (2016) found that lipid profile (serum total cholesterol, triglyceride and LDL) was higher in diabetic retinopathy patients and whereas HDL lower in these diabetic patients than healthy control and the results were statistically relevant (P value <0.001).

But, Yau et al. (2012) reported that a higher prevalence of diabetic macular edema and diabetic retinopathy associated with elevated total serum cholesterol. Also, Gnaneswaran et al. (2013) observed significant differences between the levels of total cholesterol and LDL-C in diabetic patients with DR. More severe retinopathy was found to be associated with higher total triglyceride levels. Lyons et al. (2004) pointed out a significant association between elevated serum lipid levels and increased risk of retinal hard exudates. The severity of retinopathy was positively associated with triglycerides as well as with VLDL-cholesterol and negatively associated with HDL-cholesterol.

Sharma et al. (2016) evaluated the status of antioxidant markers and oxidative stress in diabetic retinopathy of type-2 diabetic mellitus subjects, compared to non diabetic healthy subjects. The values of all these biochemical parameters except HDL, antioxidant-SOD, GPx and vitamin E were elevated in diabetic retinopathy patients as compared to healthy subjects and the differences were found to be statistically significant.

Our study showed that there was highly statistically significant increase on malondialdehyde and hydroperoxide levels among T2DM with than without retinopathy. Oppositely, paroxanase was statistically significant decreased among T2DM with than without retinopathy.

Our findings are in line with Ramakrishna and Jailkhani (2007), who have also found increased levels of oxidative stress markers in insulin-dependent DM patients and correlation of them with vascular complications of DM.
Increased LPO in DM has been associated with a variety of metabolic alterations; the most significant among them is hyperglycaemia, inducing the formation of thiose phosphate, whose oxidation causes overproduction of free radicals, leading to oxidative injury to blood cells, cross-linking of membrane lipids and proteins, increasing of cell aging, and vasoconstriction. Therefore, the oxidative stress-mediated retinal neurodegeneration and the free radicals production, leading to progression of DR, may act in a common pathway to DM per se and to its complications (Mandal et al., 2013).

Our study showed 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. Oppositely, paroxanase 1 level was statistically significantly negatively correlated with hydroperoxidase.

Our study showed 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 group. As vitamin E is also part of the protecting mechanism as it is an essential antioxidant which prevents the propagation of free radical reactions in all cell membranes in the human body by acting as a chain breaker, we found that there was 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.

There are very few studies showing the association between serum malondialdehyde levels and diabetic retinopathy. Mancino et al. (2011) reported that increased MDA is associated with oxidative stress and poor antioxidant defense, which promotes the progression of DR to its proliferative form. This finding speculated that retinal microvascular complications is closely related to the severity of oxidative stress, as expressed as increased level of MDA among PDR patients. Indeed, the exact mechanism by which the oxidative stress contributes to diabetic complications remains unclear, but all biochemical alterations due to DM lead to anatomical and functional impairment in the retinal microvascular network, such as changes in blood flow in the retina, disruption of the blood-retina-barrier and consequently capillary occlusion and ischemia.

Chatziralli et al. (2017) investigated the impact of oxidative stress on progression of diabetic retinopathy (DR) in insulin-dependent type 2 DM patients, measuring serum malondialdehyde (MDA), as well as to examine the effect of vitamin E on DR progression in the above-mentioned patients. All participants presented DR, staged by an ophthalmologist, according to the Early Treatment Diabetic Retinopathy Study (ETDRS) criteria, as nonproliferative mild DR (Group I), moderate DR (Group II), severe DR (Group III) and proliferative DR (Group IV). Serum MDA was positively associated with DR stage. Therefore, patients with proliferative DR (Group IV) had more increased MDA levels than those with non proliferative DR (Groups I, II, III). There was a statistically significant difference pre- and post- intake of vitamin E in all DR stages.

Progressive reduction in capillary perfusion results in cotton-wool spots formation, venous beading, intraretinal microvascular abnormalities and
neovascularization, as signs of DR and its progression to proliferative stage. As a result, the early detection of hypoxia is very important of preventing potential severe retinal complications \(\text{(Kowluru and Chan, 2007).}\)

Decreased activity of the antioxidant enzymes may increase diabetic patients’ susceptibility to oxidative damage. The potential benefit of vitamin E, the major antioxidant in lipid phase, has been shown in diabetic patients by its free radical scavenger activity outside the cell through non-enzymatic mechanisms \(\text{(Baburao and Anand, 2012).}\)

It is worthy to note that vitamin E supplementation remains controversial, as \text{Shinde et al. (2011)} have found detrimental instead of beneficial effect due to prooxidant effect of vitamin E. However, prooxidant effect depends on doses of vitamin E, duration of intake and duration of DM. Therefore, appropriate support for enhancing antioxidant supplies may help preventing complications of DM. Furthermore, as MDA is a biomarker, it could be affected not only by oxidative stress but also by lifestyle factors, such as smoking and alcohol use, exercise and diet.

\text{Sharma et al. (2016)} concluded relatively high levels of diabetic retinopathy in subjects with diabetes diagnosed during screening. Independent risk factors identified were duration of diabetes, fasting plasma glucose, HbA1c (Glycosylated hemoglobin), lipid profile, oxidative stress marker (MDA), and vitamin E level. As in other populations of the developing world with high rates of type-2 diabetes mellitus, effective primary prevention, early detection of diabetes, and quality clinical care and education, focusing particularly on glycemic control and oxidative stress control, provide the key to averting an epidemic of blindness and other diabetic complications.

\text{Chatziralli et al. (2017)} demonstrated that oxidative stress has been found to play significant role in the pathogenesis and progression of DR, while vitamin E seems to reduce MDA levels and subsequent oxidative stress, suggesting that it might have protective role in DR progression.

Our study showed that there was statistically significant increase on paroxanase 1 level after 3-month vitamin E application as an antioxidant among the T2DM with retinopathy group.

Results of this study indicate that oxidative stress are increasing over the time course of the disease and might lead to the development of diabetic retinopathy. The increased oxidation stress may or may not be effectively compensated by the present antioxidants. This may be a reason for divergent results obtained in various studies.

\text{Conclusion}

From our study, we concluded that serum MDA levels were associated with DR stage, suggesting that oxidative stress play a significant role in DR progression. On the other hand, MDA levels were reduced and proliferative DR patients after vitamin E intake for 3 months. As a result, antioxidant supplementation, such as vitamin E, may be used as adjunctive treatment in patients with DR to reduce
oxidative stress and to potentially protect from subsequent complications of DM; poor glycemic control significantly associated with high incidence of diabetic retinopathy and its severity; longer diabetes duration is associated with progression of DR.

References


