Superoxide Dismutase, Glutathione Peroxidase and Vitamin E in Patients with Diabetic Retinopathy

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Abstract: Background: Oxidative stress plays a pivotal role in the development of diabetic complications, both microvascular and cardiovascular. The metabolic abnormalities of diabetes cause several mechanisms that induce diabetic tissue damage. Important mechanism is the production of reactive oxygen species (ROS). Diabetic retinopathy is the leading cause of vision impairment and blinding among working adult. This study **aimed to** assess the level of Superoxide dismutase, glutathione peroxidase and vitamin E in patients with diabetic with and without retinopathy. **Setting:** Departments of Internal Medicine, Ophthalmology, Medical Biochemistry Faculty of Medicine, Zagazig University. **Subjects and Methods:** This study included 128 patients divided into 4 groups. 30 Diabetic patients without retinopathy, 34 diabetic patients with proliferative retinopathy -PDR-, 34 diabetic patients with non proliferative retinopathy-NPDR-, and 30 subjects as a healthy control group. All subjects were subjected to history taking, full clinical and funds examination. Laboratory investigations were done including Superoxide dismutase (SOD) Glutathione Peroxidase (GPx) and vitamin E levels were measured. **Results:** fasting blood glucose level, cholesterol, LDL and triglyceride were higher in diabetic patients and HDL lower in diabetic patients with and without retinopathy while Superoxide dismutase, Glutathione Peroxidase and vitamin E levels were decreased in diabetic patients compared to healthy control with more decrease in diabetic level with retinopathy than those without retinopathy. Correlation of Superoxide dismutase, Glutathione Peroxidase (GPx) with studied parameters in diabetic patients shows negative correlation with fasting blood glucose, LDL, triglyceride and creatinine and significant positive correlation with HDL. While correlation of vitamin E and the studied parameters it shows significant negative correlation with fasting blood glucose, LDL and triglyceride significant positive correlation with HDL and no correlation with creatinine and cholesterol. SOD, GPx and vitamin E are positively correlated with each other. **Conclusion:** Superoxide dismutase, Glutathione Peroxidase and vitamin E are important components in the cell defense against oxidative stress.

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1. Introduction

Hyperglycemia, a relative or absolute lack of insulin action, insulin resistance. The development of diabetes specific pathology in the retina, renal glomerulus, and peripheral nerve are characteristics of diabetes mellitus. It is also associated with accelerated atherosclerotic disease affecting arteries that supply the heart, brain, and lower extremities. Hyperglycemia causes tissue damage through 5 major mechanisms: (1) increased flux of glucose and other sugars through the polyol pathway; (2) increased intracellular formation of AGEs (advanced glycation end products); (3) increased expression of the receptor for AGEs and its activating ligands; (4) activation of protein kinase (PK) C isoforms; and (5) overactivity of the hexosamine pathway. Several lines of evidence indicate that all 5 mechanisms are activated by a single upstream event: mitochondrial overproduction of reactive oxygen species (ROS) [(1).]

Oxidative stress, induced by increased accumulation of ROS and/or decreased antioxidant capacity, plays an important role in the pathogenesis of diabetic retinopathy (DR)[(2,3).] Increased generation of ROS avidly interacts with a large number of molecules including other small inorganic molecules as well as proteins, lipids, carbohydrates, and nucleic acids. Through such interactions, ROS may irreversibly destroy or alter the function of the target molecule[(4).] Oxidative stress is closely related to the vascular changes in diabetic retinopathy. Increased ROS generation in diabetic retina has been confirmed by several studies[(5,6).]

Oxidative stress is associated with damage to lipids, proteins, and nucleic acids. Lipoprotein particles or membranes undergo the process of lipid peroxidation, giving rise to a variety of products including short chain aldehydes such as malondialdehyde[(7).] The formation of lipid hydroperoxides and their metabolites alter membrane structure and function, especially in the retinal portion of eye which is very sensitive to oxidative stress [(8).]

Superoxide dismutase (SOD) is an important antioxidant defense in almost all living cells. Many

studies had demonstrated that SOD plays a pivotal role in protecting injured retinal ganglion cells (RGCs) in variety of animal models of retinal disease [9]. Furthermore, decreased activity and expression of SOD has also been found in the diabetic retina [10]. **Xiao et al., 2012**[11] concluded that *in vitro* application of SOD effectively improves visual functions.

Reduced glutathione caused by the consumption of NADPH which is a cofactor required to regenerate reduced glutathione that is an important scavenger of ROS, this could induce or exacerbate intracellular oxidative stress. Vitamin E is a lipid soluble antioxidant. Several studies in animal models demonstrated that these antioxidants may be a logical choice for reducing diabetes-induced ROS[1]. **Aim:** To study the level of Superoxide dismutase, glutathione peroxidase and vitamin E in patients with diabetic with and without retinopathy

2. Subjects and Methods

The study was carried out in the departments of Internal Medicine, Medical Biochemistry and Ophthalmology, Faculty of Medicine, Zagazig University. It was conducted on 128 subjects.

They were divided into the following groups:

Group 1: 30 Diabetic patients without retinopathy they were (8 males, and 12 females). Their ages ranged from **39-60** years with mean age (**50.8 ± 4.67** years**), they are lean subjects their body mass index (BMI) 30.5 ± 2.9**

Group 2: It included 34 diabetic patients type 2 DM with proliferative retinopathy, 18 males and 16 females, their ages ranged from **42 - 62** years with a mean value ± SD of (**49.7 ± 6.77**), mean value ± SD of **(2.57 ± 1.38), their body mass index (BMI) 33.69±3.76.**

Group 3: It comprised 34 diabetic patients with non proliferative retinopathy (20 males and 14 females), their ages ranged from **42 - 60** years with a mean value ± SD of (**50.9 ± 6.45), their body mass index (BMI) mean** value ± SD **(30.46 ± 2.09)**

Healthy control group: It included 30, (13 males, and 17 females). Their ages ranged from **39 - 60** years with mean age (**49.8 ± 5.67), they are lean subjects their body mass index (BMI) 28.5 ± 1.9.**

Patients were randomly recruited from those attending the outpatient clinic of Zagazig University Hospitals.

After being informed on the purpose and procedures of the study, all subjects signed an informed consent form.

All cases were subjected to the following:

*Thorough history taking.

*Proper clinical examination with stress on:

-Body mass index determination and waist circumference.

- Blood pressure determination.
- Full neurological examination.
- Ophthalmological examination especially fundus examination.

*Laboratory investigations including:

1- **Routine Investigation:**

- Complete blood count.
- Liver functions tests.
- Serum creatinine.
- Lipid profile (HDL, LDL, triglycerides & total cholesterol).

2- **Specific Investigation:**

-Erythrocyte Superoxide Dismutase level (SOD)
-Erythrocyte Glutathione Peroxidase level (GPx)
-Serum Vitamin E
- Flourescene angiography for diabetic patients

Type 2 DM was diagnosed according to American Diabetes Association Guidelines for diagnosis and classification of DM [12].

Statistical analysis:

Data analyzed by SPSS version 16. All data are expressed as means ± SD. To analyze data among groups of three or more, a one way ANOVA was performed using patients' data then least significant difference when there is significant difference between groups. For factor correlation Person Correction was performed.

3. Results

Table (1) shows that mean ± standard deviation of (fasting blood glucose level, cholesterol, LDL and triglyceride) were higher in diabetic patients while patients with retinopathy show higher levels than those without retinopathy. HDL was lower in diabetic patients with and without retinopathy compared to healthy control. As regard, the superoxide dismutase level it shows significant decrease in diabetics without retinopathy than in non diabetics moreover, diabetic patients with retinopathy (especially those with PDR) showed more decrease than diabetics without retinopathy **Table (2).** In **Table (3)** there is a decrease in glutathione peroxidase (GPx) level in diabetic patients with retinopathy(with more decrease in patients with PDR than those with NPDR) than without retinopathy in comparison to healthy control with significant difference between diabetic patients with retinopathy than without retinopathy. **Table (4)** shows significant difference between groups as regard vitamin E levels with significant decrease in diabetic group with and without retinopathy moreover, vitamin E levels shows significant decrease in patients with retinopathy than without In **Table (5)** there is a significant negative correlation between GPx and fasting blood glucose, LDL, triglyceride and creatinine (P = <0.0001) while GPx shows significant positive correlation with vitamin E, SOD

and HDL (P = <0.0001) with no significant correlation with cholesterol (P = >0.05). Correlation between superoxide dismutase level and the studied parameters in diabetic groups shows significant negative correlation with fasting blood glucose. LDL, triglyceride and creatinine (P= <0.0001) and significant positive correlation with vitamin E, GPx and HDL (P=<0.0001) with no significant correlation with cholesterol (P= >0.05) **Table (6)**.

As regard the correlation of vitamin E and the studied parameters it shows significant negative correlation with fasting blood glucose (P=<0.001), LDL (P=<0.0001) and triglyceride (P= <0.001) significant positive correlation with HDL (P = <0.001), SOD (P = <0.0001), GPx (P = <0.001) no correlation with creatinine and cholesterol **Table (7)**.

Table (1): **Mean ± Standard deviation for biochemical parameters among the studied groups**

	Control N=30	**Diabetics without retinopathy N=30**	**Diabetics with** PDR **N=34**	**Diabetics with** NPDR **N=34**		*P*
Fasting blood glucose mg/dl	97.87± 5.53	165.68 ± 5.29	218.77 ± 10.05	206.56± 11.02	1286.49	<0.0001
S.Creatinine mg/dl	0.85± 0.45	0.75± 0.33	1.15± .157	1.06±.128	21.59	<0.001
Cholesterol mg/dl	157.6±5.09	226.8± 5.97	223.15± 4.13	219.23±36.08	102.61	<0.001
Triglycerides mg/dl	92.37± 4.01	189.23± 6.05	222.30± 4.38	218.17±6.31	3916.44	<0.001
HDL mg/dl	40.07± 1.31	28.38± 1.61	24.09±1.99	24.27±2.09	542.68	<0.001
LDL mg/dl	95±5.6	166.68±8.39	243.59± 39.55	230.3±7.53	316.62	<0.0001

Table (2): Mean ± Standard deviation for superoxide dismutase (SOD u/g H) among the studied groups

	Control	**Diabetics without retinopathy**	**Diabetics with** PDR	**Diabetics with** NPDR	*P*	
SOD u/g H	1139.32± 54.16	889.03±17.67	557.82±47.79	735.53±49.48	985.42	<0.0001

Table (3): Mean ± Standard deviation for Gpx (mg/dl) among the studied groups

	Control	**Diabetics without retinopathy**	**Diabetics with** PDR	**Diabetics with** NPDR		*P*
Gpx	66.5±2.2	59.86±2.2	44.93±2	49.78±1.21	795.42	<0.0001

Table (4): Mean ± Standard deviation for Vitamin E (mg/dl) among the studied groups

	Control	**Diabetics without retinopathy**	**Diabetics with** PDR	**Diabetics with** NPDR		*P*
Vit E mg/dl	22.7±0. .43	19.44±0 .81	17.87±2.61	18.96±0.678	62.645	<0.0001

Table (5): Correlation of Glutathione peroxidase with the studied parameters in diabetic groups

	r	*P*
FBG	-0.781	<0.0001
LDL	-0.786	<0.0001
HDL	0.696	<0.0001
S.Cr	-0.793	<0.0001
S.Cr	-0.51	<0.0001
SOD	0.900	<0.0001
Vit E	0.336	<0. 001
Cholesterol	.043	>0.05

Table (6): Correlation of Superoxide Dismutase with the studied parameters in diabetic groups

	r	*P*
FBG	-0.754	<0.0001
LDL	-0.733	<0.0001
HDL	0.664	<0.0001
trig	-0.730	<0.0001
S.Cr	-0.452	<0.0001
GPx	0.900	<0.0001
Vit E	0.444	<0.0001
Cholesterol	-0.004	>0.001

Table (7): Correlation of Vitamin E with the studied parameters in diabetic groups

	r	*P*
FBG	-.317	<0.001
LDL	-.487	<0.0001
HDL	.339	<0.001
trig	-.323	<0.001
S.Cr	-.121	>0.05
GPx	0.336	<0. 001
SOD	0.444	<0.0001
Cholesterol	-.046	>0.05

4. Discussion

Diabetic retinopathy is the most common micro-vascular complication of diabetes and the leading cause of vision impairment and blinding among working adult. The number of people worldwide at risk of vision loss from diabetes is predicted to double in the next 30 years so it is imperative to develop better means to identify prevent and treat retinopathy in its earliest stages rather than wait for the onset of vision threatening lesions[13].

Early detection of retinopathy in individuals with diabetes is critical in preventing vision loss. The control of diabetes associated metabolic abnormalities (hyperglycemia, hyperlipemia) play an important role[14].

Many researchers **Memisogullari *et al.*,**[15] and **Turk *et al.***[16] have studied the comparison of antioxidant enzymes level in diabetic and non diabetic patients. This study aimed to evaluate the level of erythrocytes superoxide dismutase, glutathione peroxidase and vitamin E in diabetic patients with and without retinopathy. In this study fasting blood glucose level, cholesterol, LDL and triglyceride were higher in diabetic patients and HDL lower in diabetic patients with and without retinopathy than healthy control while patients with retinopathy show higher levels than those without retinopathy.

Similar results were reported by others[17]. Chronic hyperglycemia, as well ashyperlipidemia contributes to the pathogenesis of diabetic retinopathy by initiating vascular disruptions through several biochemical mechanisms. High concentrations of glucose leads to an elevation of intracellular sorbitol concentrations with the enzymatic activity of aldose reductase, which cause osmotic damage to vascular cells. Also, high blood glucose leads to the formation of advanced glycation end products (AGEs) which cause vascular disruptions [18].

Oxidative stress is increased in the retina in diabetes; this is mainly due to an imbalance between increased generation of free radicals and impaired antioxidant protective mechanisms. The retina is the neurosensorial tissue of the eye and is extremely rich in polyunsaturated lipids membranes. This feature makes it especially sensitive to oxygen and nitrogen activated species and lipid peroxidation[19]. Antioxidants are substances that neutralize free radicals or their actions. Each cell is armed with adequate protective mechanisms against any harmful effects of free radicals. Superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase are buffering systems in every cell. Also, vitamin E is an essential nutrient which functions as a chain-breaking antioxidant which prevents the propagation of free radical reactions in all cell membranes in the human body. Vitamin E is also part of the protecting mechanism[20].

The present study showed that the erythrocyte SOD level was significantly lower in diabetic patient without retinopathy and diabetic patients with retinopathy either PDR or NPDR when compared to healthy control group. Moreover, the diabetic group with retinopathy shows more significant decrease than diabetic group without retinopathy. Also, PDR group shows significant decrease in comparison to NPDR group.

SOD is an endogenously produced intracellular enzyme that plays a key role in defending the cell against oxygen free radicals toxicity by accelerating the dismutation of the toxic superoxide radical, produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen [21]. The

decrease of SOD activity in diabetic retinopathy might be attributed to; (1) Hyperglycemia activates various biochemical pathways such as glucose autooxidation, nonenzymatic glycation of proteins and activation of protein kinase C, which, in turn, overproduce oxidants like superoxide and hydroxyl radicals as well as hydrogen peroxide; (2) The increase of glycosylated SOD that leads to the inactivation of this enzyme; and (3) Loss of its two factors Zn and Cu which act as cofactors for the antioxidant defence system. They not only constitute the active sites and/or stabilize the conformation of several antioxidant enzymes, but they also compete for iron and copper binding sites and can provide protection against transition metal- mediated and free radical-induced injury [16].

Gupta *et al.*, [20] proved that the level of SOD was significantly decreased in patients with diabetic retinopathy. Moreover, superoxide anion plays an important role in the determination of its level and products of membrane lipid peroxidation. Other oxidants like H_2O_2 may react with SOD resulting in oxidative modification thereby causing loss of its activity.

Regarding erythrocyte **GPx** level, it was significantly lower in diabetic patients without retinopathy and diabetic patients with retinopathy either PDR or NPDR and when compared to healthy control group. Moreover, the diabetic group with retinopathy shows more significant decrease than diabetic group without retinopathy. Also, PDR group shows significant decrease in comparison to NPDR group.

These results are in agreement with **Colak *et al.*,** [22] who attributed the decrease of GPx activity in diabetic patients with complications to the effect of protein glycosylation. However, they found that in diabetic group without complications, the increase of glucose concentration is followed by higher GPx activity as a positive response.

Kumar *et al.*, [23] found that the decreased GPx activity in diabetic patients with microvascular complications could be due to decreased activity of SOD, which is required for scavenging superoxide radicals, leading to their increase and by turn inhibition of GPx. However, **Gupta *et al.*,** [20] found significant increase in erythrocyte activity of GPx in the later part of the disease. GPx is a selenium (Se) dependent enzyme and any alterations in the tissue levels of selenium would alter GPx activity. Insulin deficiency promotes oxidation of fatty acids with resulting increase in **H_2O_2** formation. Thus with increase in the lipid peroxide levels, the paradoxical increase in the levels of GPx could be a compensatory mechanism by the body to prevent tissue damage.

Regarding vitamin **E** level, it was found to be significantly lower in diabetic patients without retinopathy and diabetic patients with retinopathy either PDR or NPDR and when compared to healthy control group. Moreover, the diabetic group with retinopathy shows more significant decrease than diabetic group without retinopathy. However, there was no significant difference between PDR and NPDR groups.

Similar results were found by **Gupta *et al.*,** [20], **Ahmed** [8], and **Van Reyk *et al.*,** [24]. All proved that vitamin E is an important component of cell defense against oxidative stress. Its level is decreased in diabetics and more so in diabetic retinopathy. There is strong evidence that antioxidant therapy with vitamin E might normalizes diabetic retinal hemodynamics, known to be affected in preclinical retinopathy and therefore might be important therapeutically in altering the course of diabetic retinopathy.

The antioxidant function of vitamin **E** is to prevent the peroxidation of membrane phospholipids and avoids cell membrane damage. It is a single oxygen quencher, neutralizing these highly reactive and unstable singlet oxygen molecules..Vitamin E is a lipid phase chain breaking antioxidant, it reacts more rapidly than polyunsaturated fatty acids with peroxyl radicals and hence act to break the chain reaction of lipid peroxidation[25].

The present study showed positive correlation between SOD level and GPx, vit E and HDL and negative correlation between SOD level and fasting blood glucose, creatinine, triglycerides, and LDL and non significant correlation between SOD level and cholesterol There was positive correlation between GPx level and SOD, HDL and vit E and negative correlation between GPx level and fasting blood glucose, creatinine, triglycerides, and LDL and non significant correlation between GPx level and. cholesterol

There was positive correlation between vit E level and SOD, GPx, and HDL and negative correlation between vit E level and fasting blood glucose, LDL and triglycerides and non significant correlation between vit E level and creatinine, cholesterol.

These correlations can confirm the hypothesis that hyperglycemia, hypercholesterolemia and hyperlipidemia lead to generation of oxidative stress and by turn diminished antioxidant protective enzymes such as SOD, GPx, and vit E[26, 20].

Results of this study indicate that ROS in diabetes mellitus are increasing over the time course of the disease and might contribute to the development of diabetic retinopathy. Antioxidant as a protective mechanism might not always be able to compensate for increased oxidation stress. This may be a reason

for divergent results obtained in various studies. Further work is needed to confirm whether there is an association between antioxidant nutrient intake and reduction in the development of diabetic complications particularly retinopathy.

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