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ROLE OF OXIDATIVE STRESS IN DIABETIC RETINOPATHY

Sri Hari Atti¹, Angirekula Saseekala², Varun Siri A.³¹Department of Ophthalmology, Sarojini Devi Eye Hospital, Osmania Medical College, Mehdiapatnam, Hyderabad, India²Department of Biochemistry, Sri Venkateswara Medical College, Tirupati, Chittoor Dt, Andhra Pradesh, India³Sri Venkateswara Medical College, Tirupati, Chittoor Dt, Andhra Pradesh, India

E-mail of Corresponding Author: saseekala.a@gmail.com

ABSTRACT

Objective: Diabetic retinopathy is the most commonest form of complications of diabetes mellitus which leads to vision loss if untreated. It has been hypothesized that oxidative damage may be involved in the pathogenesis of development of diabetic retinopathy. The aim of our present study is to evaluate the role of oxidative stress in diabetic retinopathy patients.

Methods: We tried to assess the role of oxidative stress in patients by estimating the levels of lipid peroxidation assessing plasma Malondialdehyde(MDA) levels and antioxidant status by reduced glutathione(GSH), Vitamin-E, Vitamin-C in blood. For this, we have taken 50 cases of diabetic retinopathy compared with 25 age matched controls.

Results: There were significant increase in the levels of MDA and significant decreases in the levels of antioxidants like GSH, vitamin-E and Vitamin-C in patients when compared with controls.

Conclusion: Our results indicate that oxidative stress is associated with the development of diabetic retinopathy which needs further studies.

Keywords: Diabetes, retinopathy, oxidative stress, antioxidants.

INTRODUCTION

Diabetes mellitus comprises a group of common metabolic disorders that share the phenotype of hyperglycemia. It is the most common endocrine metabolic disorder, affecting about 170 million people worldwide(1). The incidence of diabetes mellitus and its complications are increasing rapidly in India due to an aging population and the westernization of lifestyles. The ophthalmic complications of diabetes include corneal abnormalities, glaucoma, iris neovascularisation, cataracts and neuropathies. However, the most common and the potentially most blinding of these complications is diabetic retinopathy(2).

Diabetic retinopathy is caused primarily by microvascular injury and the duration of diabetes, hyperglycemia, hypertension, and Hyperlipidemia have been shown to be risk factors for diabetic retinopathy(3). It is known that 20-50% of the long duration cases show proliferative diabetic retinopathy. In diabetes, the retina experiences increased oxidative stress(4), and reactive oxygen species are considered as a link between elevated glucose and the metabolic abnormalities important in the development of diabetic complications.(5).Oxidative stress increases in diabetes owing to an increase in the production of oxygen free radicals and a deficiency in the antioxidant defense

mechanisms. The lipid peroxidation of the cellular structures, a consequence of the increased oxygen free radicals is thought to play an important role in the atherosclerosis and the microvascular complications(6). Elevated glucose causes a hypoxia like imbalance by increasing the NADH to NAD ratio. This altered ratio has been hypothesized to be a mechanism for ischemic retinopathy(7) and a cause of increased production of the superoxide ion(8). Greater ischemia manifested by capillary non perfusion of the retina leads to greater risk of extraretinal neovascularization and proliferative diabetic retinopathy, which is partly in response to up regulation and release of VEGF(9). Increased superoxide ion increases the oxidative load with greater reactive oxidative intermediates and advanced glycation end products, which also lead to increased release of VEGF(10) and the risk of neovascularization(11). Both ischemia and increased oxidation can lead to an increased production of lipid peroxides, which are themselves angiogenic(11).

Taking the above facts into consideration, the present study was planned to evaluate the possible role of oxidative stress and antioxidant status in the pathophysiology of diabetic retinopathy patients.

MATERIALS AND METHODS

The present study was conducted at the Department of Ophthalmology and the department of biochemistry at S.V. Medical college, Tirupati. A total of 50 known cases of diabetes mellitus patients of age group 45-75 were selected for this study. Age and sex matched 25 normal healthy adults were selected as controls. Informed consent from all cases and controls were obtained. Ethical clearance was obtained for this study. A detailed medical history was obtained to

identify those with known or suspected systemic hypertension, peripheral or coronary artery disease, venous thrombotic events, cerebrovascular disease, alcohol abuse, smoking and current use of cholesterol lowering drugs or vitamin supplements. All subjects underwent a complete ophthalmoscopic examination including visual acuity, slit lamp examination, intraocular pressure measurement using tonometry, gonioscopy, and dilated fundoscopic examination and systemic examination. Based on ophthalmic and systemic examinations, the study had two groups. Group 1 included 25 age matched healthy adults as controls and Group 2 included 50 patients of type-2 diabetes with retinopathy out of which 23 had non proliferative diabetic retinopathy and 27 had proliferative diabetic retinopathy. 10 ml of fasting blood samples were collected by venipuncture and for the separation of sera, 5ml of blood was centrifuged at 3000rpm for 5min and the remaining 5ml of blood was taken into a plain vial containing EDTA and was centrifuged at 3000rpm for 10min for the separation of plasma. The plasma MDA levels were estimated by using thiobarbituric acid reacting substances(TBARS) by the method of Yagi(12) and Sinnhuber et al(13). Reduced glutathione was determined by the method of Beutler et al(14). Serum Vit-E was measured by the method of Baker on the basis of reduction of ferric ions to ferrous ions by Vit-E & the formation of red colored complex with 2-2' dipyridyl at 520nm(15). The activity of Ascorbic acid was determined by the method of Teitz(16). All the results were expressed as mean \pm SD and statistical comparisons were done using student t-test using the SPSS package and ANOVA correlation study.

RESULTS

Comparison of levels of MDA, enzymatic and non-enzymatic antioxidants in cases and controls

Parameters	Controls (n=25)	Cases (n=50)	p value
MDA (nmol/ml)	2.13±0.11	13.21±0.06	Highly significant (<0.001)
GSH (mg/g Hb)	15.32±0.12	5.11±0.16	Highly significant (<0.001)
Vitamin-C (mg/dl)	2.53±0.14	0.89±0.11	significant (<0.05)
Vitamin-E (mg/dl)	1.34±0.22	0.80±0.22	significant (<0.05)

Evaluation of oxidative stress is done based on the levels of MDA and statistically significant increase in the levels of MDA was observed in diabetics with retinopathy when compared to controls. Statistically significant decreases were observed in the levels of enzymatic antioxidants like GSH and non enzymatic antioxidants like vitamin-E and C in cases when compared to controls.

DISCUSSION

Diabetic retinopathy is one of the most common microvascular complications of diabetes and ranks as a common cause of blindness worldwide(17). Diabetic retinopathy could become a major threat to public health in the future due to the global prevalence of diabetes of diabetes, which is projected to affect 438 million people by 2030(18). Both the duration of diabetes and its metabolic control have been identified as the risk factors most strongly associated with the development of diabetic retinopathy(19). Diabetic retinopathy occurs in 70% of all persons having diabetes for more than 15 years. The prevalence of diabetic retinopathy among urban subjects with diabetes in India was reported to be about 17%(20), whereas in a clinical study it was found to be 34% among patients with type 2 diabetes(21). The prevalence of diabetic retinopathy was 0.5%

in the general rural populations of south India and 10.5% among patients with diabetes(22).

Diabetic retinopathy is characterized by the appearance of vascular lesions of increasing severity, culminating in the growth of new vessels. Several hypotheses have been tested on the cause and pathogenesis of diabetic retinopathy. In non proliferative retinopathy, prolonged hyperglycemia may produce microaneurysms because of the selective decrease of cells in the vicinity of the retinal microvasculature and may also induce thickening of the vascular fundus membrane, endothelial proliferation, microvascular dilatation due to decreased endothelin-1 synthesis, increased vascular permeability and vascular occlusion in microvascular endothelial cells. In addition, prolonged hyperglycemia may increase blood flow and viscosity, resulting in increase of shear stress against the vascular wall, as well as injury of the vascular wall, in severe cases, it is known to induce macular edema(23). However, the main pathogenesis of proliferative retinopathy is known to be neovascularization caused by several factors including insulin like growth factor released from a retinal ischemic area(23). Recently, four biochemical pathways including enhanced glucose flux through the polyol pathway, increased intracellular formation of

advanced glycation end-products, activation of protein kinase C isoforms, and stimulation of the hexosamine pathway have been suggested for eye disorders caused by hyperglycemia induced vascular injury in diabetes. These pathogenic mechanisms also appear to be associated with superoxide overproduction by the mitochondrial electron transport chain(24).

In our study it was found that MDA levels of subjects with diabetic retinopathy were significantly higher than the levels of healthy controls. MDA level acted as markers of lipid peroxidation which represents oxidative stress and the reasons for increased lipid peroxidation products may be due to increased oxygen products as a result of auto-oxidation of glucose and glycosylated proteins, polyol pathways and decreased non enzymatic antioxidants. In addition, hyperglycemia increases the formation of triose phosphate, whose oxidation causes the formation of two free radicals alpha aldehyde and hydrogen peroxide(25). The increased levels of lipid peroxides can cause oxidative injury to blood cells, cross linking of membrane lipids and proteins, increasing of cell ageing, imbalance of prostacyclin/prostaglandin and vasoconstriction(26).GSH has a key role in enzymatic defense systems and helps in removing peroxides. In our study, there was statistically significant decrease in the levels of reduced glutathione when compared to healthy subjects. In the literature, different results have been reported in different organs and tissues regarding the levels of these two enzymes in diabetes mellitus. The low levels of antioxidant enzymes in diabetes mellitus may be because of the inactivation of the antioxidant enzymes by non enzymatic glycation due to persistent hyperglycemia or GSH could be overused implying a reduction of protection against reactive oxygen species,

possibly due to a defective redox cycle or it may be due to defective intracellular synthesis. Statistically significant low levels were observed in the levels of vitamin- C and E in cases when compared to controls. Vitamin-C is water soluble vitamin and vitamin-E is fat soluble and they are the most important antioxidant vitamins, inactivates free radicals found in the cytosol, plasma and extracellular environment. The low levels of these vitamins suggest the overusage which again increases the oxidative stress. Thus, this study concluded that the increased oxidative stress and the decreased antioxidant status can predict the micro-vascular complications in diabetes mellitus. The raised MDA levels indicate the oxidative stress and the decreased GSH, Vitamin-C and E levels indicate the reduced anti oxidative status in diabetic retinopathy.

CONCLUSION

Hence, for the early detection and prevention of diabetic retinopathy, it is advisable to estimate the oxidative stress markers. It is also advised that vitamin supplements should also be taken to allow the non enzymatic and enzymatic antioxidant systems to respond to oxidative stress.

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