

A Study of Oxidative Stress in Diabetic Retinopathy

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Abstract:

Introduction : Diabetes mellitus comprises a group of common metabolic disorders that share the phenotype of hyperglycaemia due to impaired insulin secretion, variable degree of insulin resistance, and increased glucose production. Due to insulin resistance there is alteration in lipid metabolism and hyperglycaemia leads to oxidative stress in pancreatic β cells, overload of ROS leads to various complications like diabetic retinopathy.

AIM: To assess the oxidative stress in diabetic retinopathy patients.

Materials and methods: The study was conducted in SVS medical college between January and March 2014. A total 50 persons of diabetes for more than 5 yrs were taken as controls and 50 persons with uncontrolled diabetes for more than 3 yrs on oral hypoglycemic drugs and insulin therapy having diabetic retinopathy are taken as cases.

Results: serum MDA level was significantly higher than in cases compared to control subjects ($P < 0.001$).

Serum vit C level was lower in cases compared to controls ($P < 0.001$).

Serum vit E was lower in cases compared to controls ($P < 0.001$).

CONCLUSION: Monitoring MDA, vit C and vit E levels help in preventing diabetic complications like diabetic retinopathy which leads to blindness.

Key words: malondialdehyde(MDA), Diabetic retinopathy.

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I. Introduction:

Diabetes mellitus comprises a group of common metabolic disorders that share the phenotype of hyperglycaemia due to impaired insulin secretion, variable degree of insulin resistance, and increased glucose production and decreased glucose utilisation. DM is classified into type 1 (IDDM) and type 2 (NIDDM). At present 90% of the patients are type 2 DM. The global diabetes prevalence in 2019 is estimated to be 9.3% (463 million people), rising to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045. The prevalence is higher in urban (10.8%) than rural (7.2%) areas, and in high-income (10.4%) than low-income countries (4.0%)¹. Approximately 25% of patients with diabetes mellitus have been shown to be affected with retinopathy with incidence increasing to 60% after 5 years and 80% after 10 to 15 years of affliction⁽²⁾. Environmental factors such as lack of exercise, high calorie intake, sedentary life, stress, smoking and alcohol consumption leads to hyperinsulinemia and insulin resistance. Hyperglycaemia and dyslipidaemia in DM induces increased lipid peroxidation and reactive oxygen species formation, an important mechanism in the pathogenesis of micro angiopathy⁽²⁾. The levels of intermediate reduction products of oxygen metabolism (superoxide, hydroxyl radical, and H_2O_2) are controlled by cellular defence mechanism consisting of enzymatic (SOD, Glutathione peroxidase) and non enzymatic (vit E, Glutathione) scavenger components. The oxidative stress is due to imbalance between excess reactive oxygen species formation and impaired removal of reactive oxygen species by antioxidant defense system of the cells. The resulting endogenous oxidative stress causes damage to proteins, lipids and DNA, which is thought to be an important etiological factor in the pathophysiology of complications of diabetes mellitus⁽³⁾.

Oxidative stress is associated with number of pathological conditions, such as inflammation, carcinogenesis, aging, atherosclerosis, and reperfusion injury⁽⁴⁾. Expression of antioxidant enzymes in the pancreatic islets is reportedly low⁵. The pancreatic islets therefore might be especially vulnerable to the attacks by reactive oxygen species. ROS are produced by glucose auto-oxidation⁶ and by non enzymatic protein glycation in various tissues⁷. Diabetic retinopathy is a progressive disorder and is the most common cause of blindness in people aged 30-60 years. The retina has high content of polyunsaturated fatty acids (PUFA) and has the highest oxygen uptake and glucose oxidation relative to any other tissue. This phenomenon renders retina more susceptible to oxidative stress. Several studies have consistently shown that photochemical retinal injury is attributable to oxidative stress and that the antioxidant vitamins A, E and C protect against this type of injury⁽³⁾. Vitamin C is an important reducing agent and acts as a powerful antioxidant and can scavenge physiologically important reactive oxygen species and reactive nitrogen species. Ascorbate can regenerate other small molecule antioxidants, including α -tocopherol reduced glutathione, urate and β -carotene from their radical species and prevent oxidative damage to biological macromolecules including DNA, lipid and protein.

Vitamin E acts as chain breaking antioxidant which protects LDL and polyunsaturated fats in membranes from oxidation.

Diabetic retinopathy is a major complication of DM and a leading cause of visual disability and blindness. Hence assessing oxidative stress helps in preventing diabetic retinopathy and blindness. It occurs as a result of oxidative damage to micro vasculature. Hyperglycemia hyperinsulinemia and oxidative stress contribute to diabetic complications and diabetic retinopathy.

AIM OF THE STUDY: The aim of the study is to assess oxidative stress in diabetic retinopathy patients.

II. Materials And Methods:

The present study was carried out in department of biochemistry and department of ophthalmology,SVS medical college and hospital, mahabubnagar, over a period of 3 months.

A total 50 persons of diabetes for more than 5 yrs were taken as controls and 50 persons with uncontrolled diabetes for more than 3 yrs on oral hypoglycemic drugs and insulin therapy having diabetic retinopathy are taken as cases.

Fasting venous blood samples were collected(5ml)under aseptic conditions after 12 hr overnight fast. About 1 ml is dispensed into dry tubes with NaF and potassium oxalate for estimation of plasma glucose. The rest of blood is used for obtaining serum which is used for estimation of MDA, Vit C, and Vit E.

Estimation of blood glucose was done by GOD-POD method. Estimation of serum Vit E was done by Backer and Frank's method, Vit C was estimated by 2,4 Dinitrophenyl hydrazine method and estimation of serum MDA was done by Thiobarbituric acid .

Normal values:

FBS:70-100 mg/dl.

Serum Vit E : 0.5-1.8 mg/dl.

Serum Vit C : 0.4-1.5 mg/dl.

Serum MDA: 247±35 mg/dl.

III. Results:

parameter	cases	controls	P value
FBS	193.7±24.5	96.9±12.49	<0.001
MDA	679.47±106.82	233.5±13.5	<0.001
Vit C	0.3018±0.07	0.956±0.52	<0.001
Vit E	0.54±0.30	1.424±0.26	<0.001

In this study fasting blood sugar and MDA levels were elevated in cases and Vit C ,Vit E levels were reduced.

IV. Discussion:

Diabetes mellitus is characterised by state of chronic hyperglycaemia resulting from diverse etiologies, environmental and genetic ,acting together as causative factors. The underlying cause of the diabetes is the defective production or action of insulin . Chronic vascular complications represent the main cause of morbidity and mortality in diabetes mellitus. Free radicals and oxidative stress are found to be responsible for the development of diabetic macroangiopathy and microangiopathy. The impact of microangiopathy in diabetes mellitus includes nephropathy, retinopathy and neuropathy⁽³⁾. Diabetic retinopathy is the major cause of blindness in adults. It is a duration dependent disease which develops in stages. In the early stages the diabetic retinopathy is nonproliferative which if left untreated progresses to proliferative diabetic retinopathy

Type 2 DM is the predominant form accounting for 90% of cases globally among diabetics. Although there is increase in incidence and prevalence of DM globally, dramatic rise is seen in countries with economic transition and in developing countries.

The present study was carried out in 50 controls and 50 cases between age group of 45-65 yrs. FBS, MDA, Vit C ,Vit E levels were measured.

In all controls, Mean±SD of FBS is 96.9±12.49 and in cases it is 193.7±24.5 .p value is <0.001 which is significant. It shows hyperglycemia for prolonged period is the causative factor for diabetic retinopathy. Findings of the present study correlated with study done by Rema et al⁽⁸⁾.

The levels of MDA are significantly elevated in patients with diabetic retinopathy compared to those in controls. Mean ±SD of MDA levels in controls is 233.5±13.5 and in cases is 679.47±106.82 and p value is <0.001which is highly significant. Malondialdehyde is one of the final product of polyunsaturated fatty acids peroxidation in the cells. Increase in free radicals causes an overprduction of MDA. MDA ia a reactive aldehyde and is one of the many reactive electrophile species that causes toxic stress in the cells and forms covalent

protein adducts which are referred to as advanced lipoxidation end products. The production of aldehyde is used as a biomarker to measure the level of oxidative stress in organisms.

Uncontrolled lipid peroxidation may contribute to various disease process via disruption of membrane lipids and cell components⁽⁹⁾. A number of reports indicate that blood levels of lipid peroxidation products are elevated in diabetic retinopathy.⁽¹⁰⁾ Vivian Samuel et al, also demonstrated higher levels of MDA in diabetic retinopathy cases as compared to those in diabetics without retinopathy and healthy controls⁽¹¹⁾. Gurler B et al and Kesavulu MM have shown increase in lipid peroxide levels with increase duration of disease.^(12,13)

Vitamin E levels are decreased in cases compared to controls. Mean \pm SD of vit E in controls is 1.424 ± 0.26 and in cases is 0.54 ± 0.30 and the p value is < 0.001 which is highly significant. Vitamin E acts as chain breaking antioxidant which protects LDL and polyunsaturated fats in membranes from oxidation.

Vitamin C levels are decreased in cases compared to controls. Mean \pm SD of vit C in controls is 0.956 ± 0.52 and in cases it is 0.3018 ± 0.07 and the p value is < 0.001 which is highly significant. Vitamin C is an important reducing agent and acts as a powerful antioxidant and can scavenge physiologically important reactive oxygen species and reactive nitrogen species. Ascorbate can regenerate other small molecule antioxidants, including α -tocopherol reduced glutathione, urate and β -carotene from their radical species and prevent oxidative damage to biological macromolecules including DNA, lipid and protein.

The above study explains that due to oxidative stress as measured by MDA is more in diabetic retinopathy, to compensate the elevated MDA levels the antioxidants Vit C and Vit E are utilised and decreased.

The combination of antioxidants will improve the antioxidant status which will help in prevention of Diabetic retinopathy.

In our study, the antioxidants vit C and vit E were decreased, this correlates with the previous study done by Bulent Gurler et al (2000).

V. Conclusion:

Our studies show there was increased level of FBS and MDA and decreased levels of Vit C and Vit D in patients of diabetic retinopathy compared to controls. Hyperglycemia and oxidative stress in Diabetes contribute to complications like diabetic retinopathy. Hence assessing FBS, MDA, Vit C and Vit E help in preventing diabetic complications like diabetic retinopathy which leads to blindness.

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