STUDY OF ANTIOXIDANT STATUS IN TYPE-II DIABETIC RETINOPATHY CASES
Bhaskar Balasundaram¹, H. R. Surendra²

HOW TO CITE THIS ARTICLE:
Bhaskar Balasundaram, H. R. Surendra. “Study of Antioxidant Status in Type-II Diabetic Retinopathy Cases”. Journal of Evidence based Medicine and Healthcare; Volume 2, Issue 27, July 06, 2015; Page: 4013-4021.

ABSTRACT: Diabetic Retinopathy is a progressive disorder. It is the most common cause of blindness in people aged 30-60 years. The retina has high content of polyunsaturated fatty acid and glucose oxidation relative to any other tissue. Hyperglycemia and dyslipidemia in diabetes mellitus induce increased lipid peroxidation and reactive oxygen species formation, an important mechanism in the pathogenesis of micro-angiopathy. The oxidative stress is an imbalance between excess oxidative species formation and impaired removal of the reactive oxygen species by antioxidant defence system like vitamin A, vitamin C, vitamin E, superoxide dismutase, glutathione peroxidise and catalase. Hence the study over a period of one and a half month from 1st May to June 15th 2015 with 20 diabetic retinopathy cases and 20 control cases was undertaken to evaluate the oxidative status and serum vitamin antioxidants levels in diabetic retinopathy cases. Our study using descriptive statistical analysis has shown positive correlation between hyperglycemia and MDA levels and oxidative stress with simultaneously decrease in antioxidant levels and serum vitamin like A, C and E.

KEYWORDS: Antioxidant status, Type II Diabetic Retinopathy, FBS level, PPBS level, MDA in serum by thiobarbiturate method, Serum Vitamin A, E and C levels, HbA1C level in serum.

INTRODUCTION: Diabetes Mellitus is one of the major problems in medicine throughout the world. The incidence appears to be increasing not only among the adults but also among the children. Diabetes causes an array of systemic complications, which have considerable impact on both the patient and the society because it typically affects individuals in their most productive years. Diabetes is a disorder of carbohydrate metabolism characterized primarily by hyperglycemia and glycosuria due to lack of or diminished efficiency of endogenous insulin. Sushruta called the disease “honey urine” explaining it as melting down of the flesh and limbs to urine. In 1776, Dobean demonstrated sugar in urine. In 1876, Michael Eugine described the glycosuria. Micknosky produced a similar state as diabetes after removing the pancreas in dogs. In 1921 – Best, Banting and Charles isolated insulin from the beta cells of Langerhans. Diabetes attacks both the micro and macro vessels throughout the body. It has reached the epidemic proportion and has become one of the most challenging health problems of the 21st century. Prevalence of Diabetes in adults in worldwide is estimated to be 4.0% in 1995 and expected to rise to 5.4% by 2025. The worldwide prevalence of diabetes mellitus has risen dramatically over the past 2 decades from the estimated 30 million cases in 1985 to 177 million cases in 2000 and estimated that >350 million people will have diabetes by the year 2030. In India alone, diabetes is expected to increase from 40.6 million in 2006 to 79.4 million by 2030. Long term vascular complications represent the main cause of morbidity and mortality in diabetic people.
Hyperglycemia and dyslipidemia in diabetes mellitus induce increased lipid peroxidation and reactive oxygen species formation, an important mechanism in the pathogenesis of microangiopathy.\textsuperscript{1,2} The oxidative stress is an imbalance between excess oxidative species formation and impaired removal of the reactive oxygen species by antioxidant defence system like vitamin A, vitamin C, vitamin E, superoxide dismutase, glutathione peroxidise and catalase.

Diabetic Retinopathy is a progressive disorder.\textsuperscript{3} It is the most common cause of blindness in people aged 30-60 years. The retina has high content of polyunsaturated fatty acid and glucose oxidation relative to any other tissue. This phenomenon renders the retina more susceptible to oxidative stress.

Hence the study was undertaken to evaluate the oxidative status and serum vitamin antioxidants levels in diabetic retinopathy cases.

**MATERIALS AND METHODS:** The study was spanned over a period of one and a half month from 1\textsuperscript{st} May to June 15\textsuperscript{th} 2015. It includes the following inclusion and exclusion criteria’s for the selection of the patients for the study.

**INCLUSION CRITERIA:** 20 clinically diagnosed case of diabetic retinopathy type II were taken for case study after referring from the medical department to the ophthalmology department of the Saptagiri Medical College and another 20 age and sex matched individuals for controls from the medical department of the Saptagiri Medical College.

**EXCLUSION CRITERIA:** Patients suffering from acute and chronic inflammatory disorders, other metabolic conditions like ketoacidosis, cerebro-vascular accidents or renal diseases as well as smokers, alcoholics, patients with psychiatric disorders and primary hypertensive disorders are excluded from the study.

So a total of 40 patients participated in the study. Detailed medical history and relevant clinical examination was done for the 20 cases of diabetic retinopathy cases and for rest 20 age and sex matched control subjects, who were healthy non-smokers at the time of the study.

**COLLECTION OF THE BLOOD SAMPLE:** Blood samples were collected in the fasting state and were analysed for fasting blood glucose, serum malondialdehyde, serum vitamin A, vitamin E, serum glycated haemoglobin. 8 ml of the sample was drawn under aseptic precautions from clinically diagnosed cases of diabetic retinopathy type II and controls and divide into 3 test tubes and marked as 1, 2 and 3.

1. Test tube 1 consisting of 2 ml of blood with anticoagulant, which is used for estimation of blood glucose. (Glucose Oxidase Method).
2. Test tube 2 consisting of whole blood that is used for estimation of Glycated haemoglobin (Affinity chromatography).
   Normal range = 4.2% - 6.2%.
3. Test tube 3 consisting of 6 ml of blood with no anticoagulant that is allowed to clot and serum is separated. Serum is used for measurement of:
   • Serum malondialdehyde (Thiobarbituric method).
     Normal range = 2.73+0.739 nmol/ml.
• Serum Vitamin A (Spectrophotometric method).
  Normal range = 30 – 90 ug/dl.
• Serum Vitamin E (Baker and Frank method)
  Normal range = 6 – 19 mg/dl.
• Serum Vitamin C (2,4 dinitrophenyl hydrazine method)
  Normal range = 4 – 20 mg/dl.

4. Test tube 4 consisting of 2 ml blood, which was collected with anticoagulant after 2 hours of meal, which was used for estimation of post prandial blood sugar.

STATISTICAL ANALYSIS: Descriptive statistical analysis is carried out in our present study. Results on continuous measurements are presented on Mean+ SD and results on categorical measurements are presented as number (%). Chi-square (x²) test for categorical data and student t-test was used for independent data. The Fisher exact test looks at the contingency table which displays how different treatments have produced outcomes. The statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, Medcalc 9.0.1, Syatat 12.0 and R environment were used to generate tables.

RESULTS: A comparative study was done with 20 diabetic retinopathy cases and 20 controls. The purpose of study was;
• To know the oxidative stress in diabetic retinopathy type II patients by measuring serum MDA levels.
• To know the antioxidant status in diabetic retinopathy type II patients by measuring vitamin A, vitamin E, vitamin C.
• To study the correlation between MDA and other parameters in MDR and Diabetic retinopathy type II.

As diabetes is a most common chronic metabolic disorder associated with a variety of metabolic abnormalities, principle among them is hyperglycemia.

There is an increased oxidative stress in diabetes which plays an important role in pathogenesis of diabetic complications and promotes development of diabetic retinopathy in type II patients. Simultaneously stress is accompanied by decreased antioxidant levels of antioxidant vitamins in diabetic retinopathy type II patients. A statistically significant difference was observed in the values regarding diabetes prevalent in various age groups (Table 1) and their gender predisposition. (Table 2)

| Age in years | Controls | Cases |
|--------------|----------|-------|
| 40–50        | 1        | 2     |
| 51–60        | 6        | 7     |
| 61–70        | 11       | 9     |
| 71 and above | 2        | 2     |
| Total        | 20       | 20    |

| Mean +/- SD  | 62±9.11  | 62.12 ±7.72 |

Table 1: Age distribution
It shows the age distribution patterns of controls and diabetic retinopathy type II cases under study which ranges from 40 years to 70 years with mean age of 62.0±9.11 in controls and 62.12±7.72 in cases.

| Gender | Control | Cases |
|--------|---------|-------|
| Male   | 8       | 9     |
| Female | 12      | 11    |
| Total  | 20      | 20    |

Table 2: Gender distribution

Samples are gender matched as diabetic retinopathy type II changes are more in the females than males both in controls and cases.

In our study a positive correlation of MDA with FBS and PPBS pointed out the contributory role of hyperglycemia towards the oxidative stress in diabetic retinopathy type II patients. (Table 3, 4, 5)

| Variables | Control | Cases | P value |
|-----------|---------|-------|---------|
| FBS       |         |       |         |
| <110      | 20 (100%) | 3(%)  | <0.001  |
| >110      | 17 (84%)   |       |         |

Table 3: Frequency and percentage distribution of FBS at different levels in two groups.

The percentage distribution of FBS values at different levels in controls and cases show that 100% controls had FBS value <110 mg/dl whereas in cases of diabetic retinopathy type II 16 % have FBS value less than 110 and rest 84 % have value >110 mg/dl.

| Variable | Control | Cases | P value |
|----------|---------|-------|---------|
| PPBS     |         |       |         |
| <140     | 20(100%) | 1(2%) | <0.001  |
| >140     | 19(98%)  |       |         |

Table 4: Frequency and percentage distribution of PPBS at different levels in 2 groups.

The percentage distribution of PPBS values at different levels in controls and cases show that 100% controls had PPBS value <140 mg/dl whereas in cases of diabetic retinopathy type II 2% have PPBS value less than 140 and rest 98% have value >140 mg/dl.

A small positive correlation was found between HbA1c and MDA in both cases and controls in our study which indicate that poor glycemic control increases oxidative stress (Table 5).
The percentage distribution of HbA1C values at different levels in controls and cases show that 100% controls had HbA1C value in between 4.2-6.2% whereas in cases of diabetic retinopathy 100% HbA1C value is more than 6.2%.

The percentage distribution of MDA values at different levels in controls and cases show that 90% controls had MDA value less than 2.73% and 10% has value >2.73 whereas in cases of diabetic retinopathy type II has 100% MDA value is more than 2.73%.

The moderate negative correlation between MDA and vitamin A levels was observed in our study that indicates that as oxidative stress increases it leads to deceased levels of vitamin A. (Table 7)

The percentage distribution of vitamin A values at different levels in controls and cases show that 100% controls had vitamin A value between 30 to 90 whereas in cases of diabetic retinopathy type II 52% has vitamin A value less than 30 and 48% that is only 9 of cases have vitamin A value between 30 -90.

Also there was a small negative correlation between plasma MDA and vitamin C in cases which shows that increase in oxidative stress also leads to decreased levels of antioxidant vitamin C. (Table 8)
The percentage distribution of vitamin C values at different levels in controls and cases show that 100% controls had vitamin C value more than 4 mg/l whereas in cases of diabetic retinopathy 62% has vitamin C value less than 4 and 38% that is 7 of cases have vitamin C value between 4-20.

Values of MDA and vitamin E also correlates that vitamin E as an antioxidant reduces in oxidative stress in diabetic retinopathy patients. (Table 9)

DISCUSSION: Diabetes mellitus as a disease is familiar to mankind from times immemorial. Diabetes mellitus refers to a group of common metabolic disorders that share the phenotype of hyperglycemia leading to insulin deficiency or resistance to the action of insulin. There are of two types: Type 1 and Type 2.

Type 1: It result from mostly the interaction of genetic factors most commonly HLA region in chromosome 6, environmental factors including various viruses bovine milk products and nitrosoureas and immunological factors like ICAs (Islet cell antibodies) that ultimately lead to destruction of the pancreatic b cells, most of them include the immune mediated destruction of b cells but not all.

Type 2: The two metabolic defects that characterize type 2 diabetes are (1) insulin resistance (2) b cell dysfunction that is manifested as inadequate insulin secretion in the face of insulin resistance and hyperglycemia. In most of the cases insulin resistance is primarily followed by increasing degrees of b cell destruction.
Environment influences such as sedentary lifestyles, dietary habits clearly have a role which is evident when obesity is considered as diabetes increases body mass index which further increases insulin resistance. Genetic polymorphisms associated with type 2 diabetes also have been found in the genes encoding for peroxisome proliferators-activated receptor, inward rectifying potassium channel expressed in beta cells, zinc transporter expressed in beta cells IRS (Insulin receptor substrates) and calpain.  

In our study we are dealing with all cases of diabetic Type II only. Metabolic effects of insulin causes: uptake of glucose by tissues mainly muscles and adipose tissues, it favours the synthesis of fatty acid from the glucose and increases glucose utilization, it lowers blood glucose levels by promoting its utilization and storage and stopping gluconeogenesis, it increases fat in body by favouring the lipogenesis and stopping lipolysis by inhibiting hormone sensitive lipase. Insulin depresses HMG COA synthase and decreases the ketogenesis. It promotes protein synthesis and retards its degradation by acting as an anabolic hormone.  

The present criteria for diagnosis of diabetes mellitus emphasize that FPG is the most reliable and convenient test of identifying DM in asymptomatic individuals. It includes following criteria’s:

1. Symptoms of diabetes plus plasma glucose concentration >/ 200 mg/dl (11.1 mmol/l). Casual is defined as any day without regard to time since last meal. The classical symptoms of diabetes include polyuria, polydipsia and unexplained weight gain.
2. FPG >/ 126 mg/dl (7.0 mmol/l). Fasting is defined as no caloric intake for atleast 8 hour.
3. 2-hour PPBS >/200 mg/dl (11.1mmol/l) during OGTT. The test should be performed as described by WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

DIABETIC RETINOPATHY: Diabetic retinopathy is a well characterized sight threatening chronic micro vascular complication that eventually affects all patients with diabetes mellitus. It is the leading cause of acquired blindness. It is defined as the progressive dysfunction of retinal vasculature caused by chronic hyperglycemia.

CLASSIFICATION OF DIABETIC RETINOPATHY: The American Academy of Ophthalmology established international classification of diabetic retinopathy. It has five clinical levels:

1. No apparent retinopathy (No abnormalities).
2. Mild non-proliferative diabetic retinopathy (Micro-aneurysms only).
3. Moderate non-proliferative diabetic retinopathy (More than micro aneurysms only but less than severe non-proliferative diabetic retinopathy).
4. Severe non-proliferative diabetic retinopathy (any of the following >20 inter retinal haemorrhages in each of 4 quadrants, prominent inter retinal micro- vascular abnormalities in one or more quadrants).
5. Proliferative diabetic retinopathy (one or more of retinal neovascularization, vitreous haemorrhage or preretinal haemorrhage).
The earliest histopathological effect in diabetes mellitus in eye includes loss of pericytes, thickening of vascular endothelium basement membrane and alteration of retinal blood flow. Due to loss of retinal pericytes, there develops lot of retinal out pouching’s namely micro-aneurysms and become fragile which results in retinal haemorrhages, retinal edema, hard exudates. With increasing time, sclerosis and endothelial cell loss causes narrowing of retinal vessels which in turn decreases the retinal blood flow and causes areas of ischemia which is a potent inducer for angiogenic growth factor like insulin like growth factor, fibroblastic growth factor and vascular endothelial growth factor. These factors promote neovascularization in iris and retina. In iris it extends to anterior chamber leading to glaucoma. In retina proliferative neovascularization has tendency to bleed causing vitreous or retinal haemorrhages and further retinal detachment due to proliferation into vitreous. Free radicals and oxidative stress are found to be responsible for the development of diabetic macroangiopathy and microangiopathy.  

The parameters which were recorded to define hyperglycemia were FBS and PPBS values which measure the blood glucose levels. Glycated haemoglobin HbA1C is the marker of both severity and long term control of the disease. It reflects the average level of blood glucose concentration over the preceding 6-8 weeks and is unaffected by diet, insulin therapy and other drugs. The values in our study are in accordance with several studies which have shown increase in HbA1c levels in diabetes.

MALONDIALDEHYDE: Free radicals or reactive oxygen species (ROS) causes the oxidative stress which leads to development of complications of diabetes mellitus, so an imbalance due to increased production of reactive oxygen and as well as reduction in antioxidant defenses which alter cellular redox status.

MDH is highly toxic compound formed by lipid peroxidation due to free radical damage. Many studies have shown increase in MDA levels in diabetes mellitus type II correlating with poor glycemic control.

Madhur M Gupta and Suresh Ghai, in their study showed a significant increase in MDA levels in diabetic retinopathy cases when compared to diabetics without complications and healthy controls.

VITAMINS: Vitamin A being a component of photoreceptor pigment is needed to maintain the visual process, b carotene protects the different tissues including retina from degeneration caused by active free radical by their antioxidant property. So any decrease in its levels leads to progression of retinopathy changes.

Vitamin C is a water soluble vitamin and acts as a aqueous phase antioxidant against ROS causing lipid peroxidation. S Kumari et al showed significantly fall in vitamin C levels in both diabetic with retinopathy or without retinopathy. Similarly Vitamin E is another lipophilic membrane antioxidant preventing lipid peroxidation particularly PUFAS.

Hence our study has shown positive correlation between hyperglycemia and MDA levels and oxidative stress with simultaneously decrease in antioxidant levels and serum vitamin like A, C, E.
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AUTHORS:
1. Bhaskar Balasundaram
2. H. R. Surendra

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Medicine, Saptagiri Institute of Medical Sciences & Research Centre, Bangalore.
2. Professor, Department of Ophthalmology, Saptagiri Institute of Medical Sciences & Research Centre, Bangalore.

NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Bhaskar Balasundaram,
# 1402, Brigade Gate Way,
Malleshwaram West,
Bangalore-560055.
E-mail: bhassush@yahoo.com

Date of Submission: 29/06/2015.
Date of Peer Review: 30/06/2015.
Date of Acceptance: 02/07/2015.
Date of Publishing: 06/07/2015.