Original Article

Oxidative Stress in Diabetic Retinopathy

Biochemistry Section

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ABSTRACT

Diabetic retinopathy (DR) is one of the micro-vascular complications of diabetes which leads to blindness among the working age individuals. Chronic hyperglycaemia and dyslipidaemia cause oxidative stress and increase the free radicals. Oxidative stress is one of the important causes in the genesis of microangiopathy. Twenty five patients of Type II Diabetes mellitus (DM) without retinopathy, 50 patients of Type II Diabetes mellitus with non-proliferative diabetic retinopathy and proliferative diabetic retinopathy and 25 normal subjects were included in the study. Fasting plasma glucose (FPG), post prandial plasma glucose (PPPG), glycosylated haemoglobin, lipid profile, plasma malondialdehyde (MDA) and Vitamin C were estimated. The plasma MDA levels were significantly elevated ($p< 0.001$) and the Vitamin C levels were significantly decreased ($p<0.01$) as compared to the controls. The study revealed a significant positive association between plasma MDA and both FPG and PPPG ($r=0.438$, $p<0.01$, $r=0.455$, $p<0.01$) , a positive correlation between plasma MDA and HbA1c and also, a positive correlation between plasma MDA and serum triglycerides ($r=0.028$, $p<0.01$, $r=0.454$, $p<0.01$) and a negative correlation between MDA and Vitamin C ($r=0.241$, $p<0.01$). The results suggest that increased lipid peroxidation and a decline in the antioxidant defense mechanisms plays a very important role in the initiation and progression of micro-vascular complications like diabetic retinopathy.

Key Words: HbA1c, MDA, Vitamin C, Oxidative stress

INTRODUCTION

Diabetes mellitus (DM) is a major medical problem throughout the world. Diabetes causes an array of long-term systemic complications which have a considerable impact on both the patient and the society, because it typically affects individuals in their most productive years. The ophthalmic complications of diabetes include corneal abnormalities, glaucoma, iris neo-vascularization, cataracts and neuropathies. However, the most common and the potentially most blinding of these complications is diabetic retinopathy [1]. Diabetes mellitus is characterized by hyperglycaemia, together with the biochemical alteration of glucose and lipid peroxidation. DM is considered to be one of a rank of free radical diseases which propagate their complications with increased free radical formation. Oxidative stress is increased in DM, owing to an increase in the production of oxygen free radicals and a deficiency in the antioxidant defense mechanisms. The lipid per-oxidation of the cellular structures, a consequence of the increased oxygen free radicals, is thought to play an important role in the atherosclerosis and the micro-vascular complications [2].

Patients with long standing Type II DM have a higher prevalence of severe visual impairment which is usually associated with diabetic retinopathy. The incidence of retinopathy which increases with an increasing duration of diabetes and sustained hyperglycaemia has been identified as the major risk factor in the development of this micro-vascular complication. It is known that 20–50% of the long duration diabetes cases show proliferative diabetic retinopathy. This type of retinopathy progresses from mild non-proliferative abnormalities to proliferative retinopathy which is characterized by the growth of new vessels on the retina and on the posterior surface of the vitreous. The exact biochemical mechanism which causes the initiation and progression of diabetic retinopathy has been poorly understood. It has now been proved that the blood supply to the essential organs including the retina is reduced in long standing diabetes mellitus, owing to a failure in the auto-regulatory mechanisms. We assume that ischaemia in the inner retinal tissues produces some biochemical changes which are responsible for the morphological changes in the retina [3].

Oxidative stress is increased in diabetes mellitus, owing to an increased production of free radicals such as the super oxide radical, hydrogen peroxide and the hydroxide radical and free radical induced lipid per oxidation.

Taking into consideration the above facts, that chronic hyperglycaemia leads to the formation of free radicals and free radical induced lipid peroxidation, which cause microangiopathic changes like retinopathy in DM, we conducted this study with an objective to evaluate the role of oxidative stress and its correlation with hyperglycaemia in patients of Type II DM with and without retinopathy [3, 4].

MATERIALS AND METHODS

The present study was conducted at the Department of Biochemistry, NRI Medical College and General Hospital, Chinakakani, Guntur Dist, Andhra Pradesh. The diabetic patients who attended the Ophthalmology Department, who were diagnosed on the basis of their history and ophthalmoscopic examination, were included in the present study. The criteria for the diagnosis of NIDDM were those which were proposed by the National Diabetes Data Group (NDDG) of the National Institutes of Health in the 1980/ WHO criteria. Patients with acute and chronic infections, cerebro-vascular accidents, renal diseases, smoking, alcohol abuse and hypertension were excluded from the study. The present study had three groups. Group I included 25 age and sex matched healthy

controls with a mean age of (50.4 ± 12.4), Group II included 25 patients of Type II DM without retinopathy with a mean age of (48.6 ± 10.5) and Group III included 50 patients of Type II DM with non-proliferative diabetic retinopathy and proliferative diabetic retinopathy with a mean age of (53.6 ± 9.3). The present study was conducted with prior ethical clearance from the institutional ethical committee (IEC) , NRIAS and an informed consent was taken from the study participants.

All the three diabetic groups were on hypoglycaemic drugs .None of the subjects were on antioxidant or lipid lowering drugs. Both the cases and controls were subjected to the estimation of biochemical parameters like fasting plasma glucose, 2 hrs post prandial plasma glucose, HbA1c, total cholesterol and serum triglycerides [(TGL), HDL, LDL and VLDL cholesterol]. Specific tests for Plasma MDA (Malondialdehyde) and Vitamin C were performed in all the subjects. The lipid peroxidation product, MDA, was measured by using thiobarbituric acid reactive substances (TBARS). MDA reacts with thiobarbituric acid at 100°C in an acidic medium to give a pink coloured complex. The colour intensity of the MDA - TBA complex was measured at 535 nm by using a spectrophotometer. The concentration of MDA was calculated by using the molar extinction co-efficient of the MDA-TBA complex (1.56×105 $Lmol^{-1}cm^{-1}$) [5]. The plasma Vitamin C levels were measured by the conversion of Vitamin C to dehydroascorbic acid by acidic 2,4 Dinitrophenylhydrazine in the presence of thiourea as a mild reducing agent to form a red coloured compound, bis–hydra zone, which was measured at 520 nm by using a spectrophotometer. HbA1c was measured, based on the latex agglutination method by using a RANDOX fully automated instrument. All other serum parameters were analyzed by using a DADE DIMENSION instrument. The results were analyzed by using the t test and an ANOVA correlation study.

RESULT

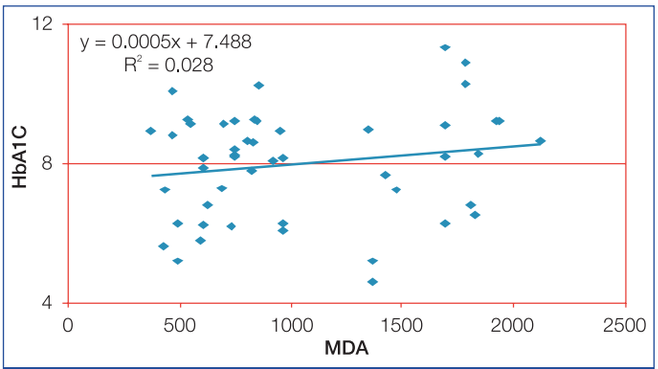
The results which were obtained from the controls, diabetics without retinopathy and diabetics with proliferative retinopathy and non- proliferative retinopathy are shown in [Table/Fig-1]. The levels of FPG, PPPG, HbA1c, cholesterol, triglycerides and MDA were significantly higher in the diabetics without retinopathy, while the HDL and Vitamin C levels were lower as compared to those of the control group ($p< 0.001$). The levels of FPG , PPPG, HbA1c, cholesterol, triglycerides and MDA were significantly higher in the diabetics with retinopathy, while the HDL and Vitamin C levels were lower as compared to those in the diabetes without retinopathy ($p< 0.001$).

The plasma MDA levels were significantly elevated ($p<0.001$) and the Vitamin C ($p<0.01$) levels were decreased in all the diabetic groups as compared to those in the controls. Our correlation study revealed a significant positive association of plasma MDA with fasting plasma glucose, 2 hr post prandial plasma glucose ($r=0.438$, $p<0.01$, $r=0.455$, $p<0.01$) and HbA1c ($r=0.277$, $p<0.01$), thus suggesting the role of hyperglycaemia in free radical production. The plasma MDA levels also indicated a significant positive relation ($p<0.001$) with all the lipid parameters except serum HDL, thus explaining the role of dyslipidaemia in the liberation of the free radicals. A stastically significant positive correlation was found between HbA1c and MDA ($r = 0.028$), as shown in [Table/Fig-2] and between the triglycerides and MDA ($r = 0.454$), as shown in [Table/Fig-3]. There was a negative correlation between MDA and Vitamin C ($r = 0.068$) in the cases of diabetic retinopathy, as shown in [Table/Fig-4].

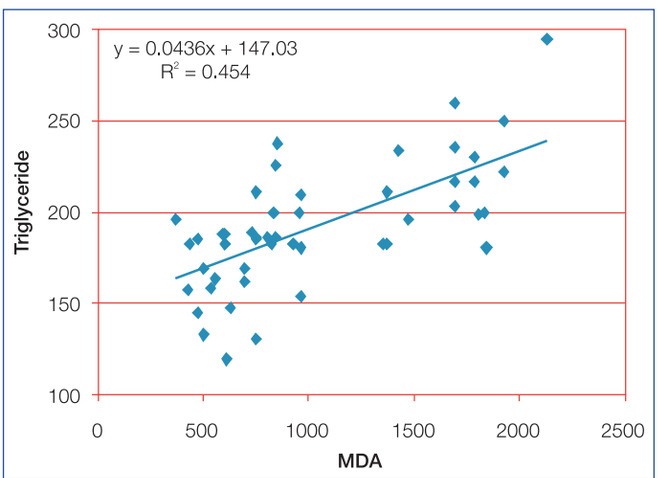
The results of the ANOVA and the post ANOVA t tests showed a significant difference in all the three groups for MDA, as has been represented in [Table/Fig-5]. The results of the ANOVA and the

Parameters	Group I	Group II	Group III
FPG (mg/dl)	100.24 ± 9.3	212.88 ± 13.9	234.1 ± 86.45
PPPG (mg/dl)	131.3±16.1	299.4 ± 11.8	302.30 ± 87.9
TGL (mg/dl)	104.44 ± 29.9	197.6 ± 36.6	245.52 ± 41.8
HDL (mg/dl)	43.64 ± 9.5	40.48 ± 11.9	40.10 ± 8.75
HbA1C (%)	5.45 ± 0.74	7.97 ± 1.39	8.34 ± 1.05
MDA (nmol/dl)	204.87± 27.4	437.0 ±111.65	1041.9± 664.2
VITAMIN C (mg/dl)	0.99 ± 0.22	0.87 ± 0.22	0.80 ± 0.25

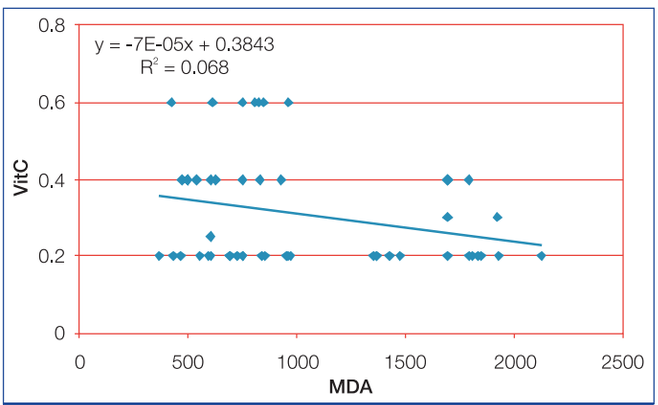
[Table/Fig-1]: Biochemical Parameters in the Study Population (Mean ± SD)



[Table/Fig-2]: Correlation between HbA1C and MDA



[Table/Fig-3]: Correlation between Triglyceride and MDA



[Table/Fig-4]: Correlation between Vitamin C and MDA

Group	Mean ± SD	Group I	Group II
I Controls (n = 25)	204.87 ± 7.41	–	–
II Diabetics without Retinopathy (n = 25)	473.01 ± 111.65	Significant (p<0.001)	–
III Diabetics with Retinopathy (n = 50)	1041.9± 664.2	Significant (p<0.001)	Significant (p<0.001)

[Table/Fig-5]: ANOVA data for MDA in Different Groups

Group	Mean ± SD	Group I	Group II
I Controls (n = 25)	0.99 ± 0.22	–	–
II Diabetics without Retinopathy (n = 25)	0.87 ± 0.22	Significant (p<0.001)	–
III Diabetics with Retinopathy (n = 50)	0.80 ± 0.25	Significant (p<0.001)	–

[Table/Fig-6]:ANOVA data for VITAMIN C in Different Groups

post ANOVA t tests for Vitamin C showed a significant difference in all three groups, as has been represented in [Table/Fig-6].

DISCUSSION

Diabetic retinopathy is the most specific of all the diabetic micro-vascular complications. The prevalence of retinopathy is related to the duration of diabetes. Diabetic retinopathy is the leading cause of new blindness in persons who are aged 25-74 years. The incidence of retinopathy is rarely detected in the first few years of diabetes, but the incidence increases to 50% by 10 years and to 90% by 25 years of having diabetes. The prevalence of diabetic retinopathy is increasing due to the prolonged survival of the diabetic patients. In south India, diabetic retinopathy was detected in 1.78% of the patients who were screened and it was projected to become a significant cause of blindness in the coming decades. Subjects with a duration of diabetes which was <5 years had 22.9% of diabetic retinopathy, while those with diabetes for >5 years had 33.5% of diabetic retinopathy. Younger subjects (<40 years) had 17.9% of diabetic retinopathy, while those who were >40 years of age had 36% of diabetic retinopathy. Subjects with HbA1c which was < 7% had a 12.5% prevalence as compared to those with HbA1c which was > 7%, who had a prevalence of 40.5% [3].

The pathogenesis of DR has not been completely understood, but the established risk factors include poor glycaemic control, hypertension, increasing age and the duration of diabetes [7]. A study on the epidemiology of diabetes complications demonstrated that high triglyceride and high LDL levels at the baseline were associated with the subsequent progression of retinopathy over 2 years [6]. It is found that a poor metabolic control which was demonstrated by high HbA1c levels was directly proportional to the prevalence of DR, which has been documented by Klein et al. in 1988. A longer duration of diabetes has been seen to have a stastically highly significant correlation with the prevalence of DR [4]. Oxidative stress plays a major role in the onset of diabetes mellitus, as well as in the development of vascular and neurological complications of the disease [8]. The source of the oxidative stress is a cascade of Reactive Oxygen species (ROS) which leak from the mitochondria [9]. Hyperglycaemia contributes to micro-vascular complications. The prominent biochemical pathways which explain how diabetes causes damage to the micro-vasculature system include: (1) an increased polyol pathway flux (2) the production of advanced glycation end products (AGE) (3) the generation of reactive oxygen species (ROS) and (4) the activation of the diacyl glycerol and the protein kinase C isoforms [10,11]. AGEs are the products of glycation and oxidation and they are responsible for the liberation of superoxide radicals. Excess glucose enters the polyol pathway, resulting in excess sorbitol production, with a concomitant decrease in the NADPH levels. Low levels of NADPH can decrease nitric oxide production in the endothelial cells and can adversely affect the cellular redox balance, thereby resulting in deleterious metabolic consequences. NADPH is required for regenerating the reduced glutathione and the consumption of NADPH could contribute to an intracellular increase in the formation of the reactive oxygen species, thus leading to oxidative stress and resultant diabetes related vascular damage. Therefore, an increased flux through the polyol pathway can lead to micro-vascular damage by contributing to AGE formation, specific protein kinase C activation and the generation of reactive oxygen species (ROS).

Oxidative stress in hyperglycaemia is amplified by the metabolic stress [11]. In the present study, the production of the oxygen free radicals was directly related to hyperglycaemia and the duration of diabetes. The MDA levels acted as a marker for lipid peroxidation i.e. oxidative stress and it was significantly increased in the cases as compared to the controls (P<0.001). In a state of poor metabolic control, increased serum MDA levels are expected. A positive correlation was found between the mean serum MDA levels and the mean HbA1c levels in the diabetic patients [12]. The longer the duration of the disease, the higher were the lipid peroxide levels. This was in agreement with the findings of Gruler B, Kesavulu M and Turk et.al. Thus, the increase in the lipid peroxides in blood, coupled with the weakness of the defense antioxidant system in diabetics with complications, probably served as a background for the pathogenesis of the endothelial dysfunction which was associated with diabetes.

The retina is highly susceptible to oxidative stress because of its high consumption of oxygen, its proportion of polyunsaturated fatty acids (PUFA) and its exposure to visible light. Studies have consistently shown that photochemical injury is attributable to oxidative stress and that the antioxidants, Vitamins A, E and C protect against this type of injury [13]. The levels of Vitamin C, which is a measure of the antioxidant status, is significantly decreased in diabetic patients (P<0.05).

In the present study, HbA1c was significantly elevated in diabetic patients, which indicated their glucose status for the past 3 months. There was a significant elevation of triglycerides in patients with complications, indicating dyslipidaemia. Thus, this study concluded that the poor glycaemic control, the long duration of diabetes and dyslipidaemia contributed to the increased oxidative stress. The increased oxidative stress and a decreased antioxidant status can predict the micro-vascular complications in diabetes mellitus. The raised MDA levels indicate the oxidative stress and the decreased Vitamin C levels indicate the reduced antioxidant status in diabetic retinopathy. Hence, for the early detection and prevention of diabetic retinopathy, it is advisable to estimate the oxidative stress markers. The diet of diabetic patients should contain a recommended dietary allowance of vitamins to allow the non-enzymatic as well as the enzymatic antioxidant systems to respond to oxidative stress, which is observed among the diabetic changes.

ABBREVIATIONS

NIDDM: Non Insulin Dependent Diabetes Mellitus
MDA: Malondialdehyde
HDL: High Density Lipoprotein
LDL: Low Density Lipoprotein
VLDL: Very Low Density Lipoprotein
HbA1c: Glycosylated hemoglobin
ANOVA: Analysis Of Variance

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