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PLASMA MDA AND ANTIOXIDANT VITAMINS IN DIABETIC RETINOPATHY

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ABSTRACT

Hyperglycemia and dyslipidemia in diabetes mellitus induce increased lipid peroxidation and peroxyl radical formation, an important mechanism in genesis of microangiopathy. We took up a study on oxidative stress, measured by plasma MDA and antioxidant vitamin status in type - 2 DM patients with and without retinopathy and compared them with a control non-diabetic group. Lipid peroxidation marker MDA was significantly elevated ($p < 0.001$) in both the diabetic groups whereas, serum vitamin E and vitamin C registered a significant fall ($p<0.001$) as compared to controls. Our correlation study revealed a significant positive association between plasma MDA with both fasting and 2hr post prandial plasma glucose ($r=0.81$, $p < 0.001$, $r=0.92$, $p <0.001$) suggesting the role of hyperglycemia in free radical production. Plasma MDA also depicted significant positive relation ($p< 0.001$) with all lipid parameters except serum HDLc pointing the role of dyslipidemia towards lipid peroxidation. Plasma MDA level was also found to be negatively correlated with both the vitamins ($p<0.001$, $p<0.001$) in the study group explaining their protective consumption in the oxidative process prevailing in diabetic retinopathy.

KEY WORDS

Oxidative stress, Microangiopathy, Peroxy radical.

INTRODUCTION

Long term vascular complications represent the main cause of morbidity and mortality in diabetic patients. Approximately 25% of patients with type-1 DM have been shown to be affected with retinopathy, with the incidence increasing to 60% after 5 years and 80% after 10 to 15 years of affliction. Moreover there are more adult onset cases than juvenile ones, type-2 DM accounts for a higher proportion of patients with visual impairment(1).

Vascular occlusion caused by thickened basement membrane, platelet aggregation and leucocyte activation results in non-perfusion and hypoxia. Autoregulation of blood flow that leads to dilatation of arterioles and increased pressure, damages blood retinal barrier, thus increasing vascular permeability, leading to retinal hemorrhage and extracellular accumulation of fluid, lipids and lipoproteins (hard exudates). Neovascularization resulting from occlusion of capillaries are fragile and frequently results in preretinal and vitreous hemorrhage in case of vitreous detachment. Increased traction on retina results in localized area of retinal detachment that leads to irreversible visual loss(2).

In recent years, free radicals have assumed an overwhelming importance for the aetiopathogenesis of diabetic retinopathy, the most common damaging effect being lipid peroxidation. This self-perpetuiting process as a result of hyperglycemia and dyslipidemia produces a number of reactive hydroperoxides and aldehydes leading to genesis of microangiopathy in diabetes(3).

The potential benefit of vitamin E, the major antioxidant in lipid phase has been shown in diabetic retinopathy by its free radical scavenger activity outside the cell through non-enzymatic mechanisms(4). Evidences suggest the role played by aquous phase antioxidant ascorbate and its interaction with vitamin-E in reducing protein glycosylation, both in vivo as well as in-vitro. They both act as scavengers of free radicals generated by glycosylated proteins(5).

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The best predictor of diabetic retinopathy is the duration of disease. The oxidative stress, amplified by metabolic stress is related to the severity and duration of diabetes. Studies on patients with long term and poorly controlled diabetes suggest that free radicals in diabetes mellitus and increasing over time may play a role in the development of diabetic retinopathy(6).

Taking into consideration the above facts, the present study was conducted with an objective to evaluate the oxidative status and serum vitamin antioxidant levels in diabetic retinopathy cases & to correlate them with the severity of the disease process.

MATERIALS AND METHODS

The present study was conducted in the Department of Biochemistry, SCB Medical College, Cuttack from 2004 March to 2005 November. Eighty six type-2 DM patients within age group of 40-65 years attending the OPD and Indoor of Department of Ophthalmology, SCB Medical College, Cuttack were included in this study. Type-2 DM patients were diagnosed on the basis of history, physical examination, biochemical investigations and according to the biochemical criteria laid down by the National Diabetes Data Group (NDDG) of the National Institute of Health in 1980 / WHO criteria(7). Thirty six cases were of type-2 DM without retinopathy and rest 50 cases were of diagnosed diabetic retinopathy with an clinical exclusion of renal and cardiovascular involvement.

Thirty two age, sex matched apparently healthy individuals with normal plasma glucose, normal vision & with no symptoms suggestive of DM were taken as controls. Patients with acute & chronic inflammatory conditions, other metabolic conditions like ketoacidosis, cerebrovascular accident or renal diseases as well as smokers, alcoholics, and primary hypertensives were excluded from the study. All the diabetics were on hypoglycemic drugs. None of the subjects were on antioxidant supplementation or lipid lowering drugs. The study was approved by the institutional ethical committee.

Both cases and controls were subjected to estimation of biochemical parameters like fasting plasma glucose(8), 2 hrs post prandial plasma glucose(8) and Total Cholesterol (9), HDL, LDL and VLDL Cholesterol (10,11) and triglyceride (12). Specific tests for plasma MDA, serum vitamin E and serum vitamin C were also performed in all subjects. The lipid peroxidation product MDA formed a characteristic chromogenic adduct with TBA which was measured spectrophotometrically after butanol extraction(13). Plasma vit-C was measured by conversion to dehydroascorbic acid which reacted with acidic 2,4 dinitrophenyl hydrazine in presence of thiourea as a mild reducing reagent to form a red coloured compound bis-hydrazone, which was measured at 520nm in spectrophotometer(14). Serum vitamin-E was estimated by method of Baker & Frank after xylene extraction & reduction of ferric to ferrous ions, which then forms a red coloured complex with α-α' Dipyridyl(15). Absorbance was read at 460nm by spectrophotometer and a correction for the carotenes was made after adding ferric chloride and measured at 520nm. The results were analyzed using student's 't' test and pearson correlation study.

RESULTS

The clinical data in the present study revealed (Table1) that diabetic retinopathy is mostly seen in middle life or in elderly people. The mean of weight and body mass index were more or less same in both the diabetic groups in relation to control. The duration of diabetes has shown a relation with the incidence and occurrence of diabetic retinopathy.

Table 1 : Clinical Data of Control and Diabetics withand without Retinopathy (Mean±SD)

Parameters	Control (n=32)	Diabetes without retinopathy (n=36)	Diabetes retinopathy (n=50)
Age (yrs)	56.84±7.45	55.30±6.49	63.04±4.83
Weight (kg)	60.21±4.42	61.80±6.99	60.24±4.71
Body mass Index (kg/m^2)	23.31±2.82	24.47±2.81	24.40±2.57
Duration of DM (yrs)	Nil	6.37±1.78	11.44±1.77

Table 2 : Biochemical Parameters in the Study Population (Mean±SD)

Parameters	Control (n=32)	Diabetes without retinopathy (n=36)	Diabetes retinopathy (n=50)
FPG (mg/dl)	90.15±6.50	181.27±32.13**	216.70±47.84**
2 hr PPPG (mg/dl)	102.62±5.74	229.52±32.06**	302.28±14.65**
Total cholesterol (mg/dl)	178.28±7.48	200.13±12.90**	207.18±17.13**
HDLc (mg/dl)	45.62±2.87	38.61±2.20**	29.74±1.48**
LDLc (mg/dl)	115.31±8.88	136.69±11.87**	142.74±17.01**
VLDLc (mg/dl)	20.81±1.51	28.02±1.01**	30.25±1.22**
TAG (mg/dl)	105.25±5.09	139.25±5**	148.76±7.39**

** $p < 0.001$

Routine Biochemical investigations registered a significant rise ($p < 0.001$) of both fasting plasma glucose and 2 hr post prandial plasma glucose (PPPG) in both diabetic groups, when compared to control, indicating a poor control of diabetes (Table2). Significant alterations in lipid parameters explained dyslipidemia associated with DM. Plasma MDA was raised markedly ($p < 0.001$) in association with significant fall in both vitamin-E and vitamin-C respectively in both diabetic groups, which was more distinct in retinopathy cases (Table3).

Table 3 : Plasma MDA, Vitamin-E and Vitamin-C in Study Groups

Parameters mean±SD	Control (n=32)	Diabetes without retinopathy (n=36)	Diabetes retinopathy (n=50)
Plasma MDA (nmol/ml)	0.93±0.13	1.67±0.14**	2.02±0.14**
Serum vitamin-E (mg/dl)	1.04±0.16	0.70±0.10**	0.49±0.12**
Serum vitamin-C (mg/dl)	1.10±0.29	0.68±0.22*	0.53±0.19**

** $p < 0.001$; * $p < 0.01$

Correlation study revealed a significant positive association between plasma MDA with both plasma glucose ($r=0.81$, $p < 0.001$; $r=0.92$, $p < 0.001$), whereas vitamin-E and vitamin-C registered significant negative correlation, pointing the contributory role of hyperglycemia towards oxidative stress. Similarly plasma MDA depicted significant positive relation ($p < 0.001$) with all lipid parameters except serum HDLc, expressing the role of dyslipidemia towards lipid peroxidation and free radical generation. The negative association of vitamin-E and vitamin-C with all these lipid parameters except HDL-Cholesterol (HDLc) strengthens this fact.

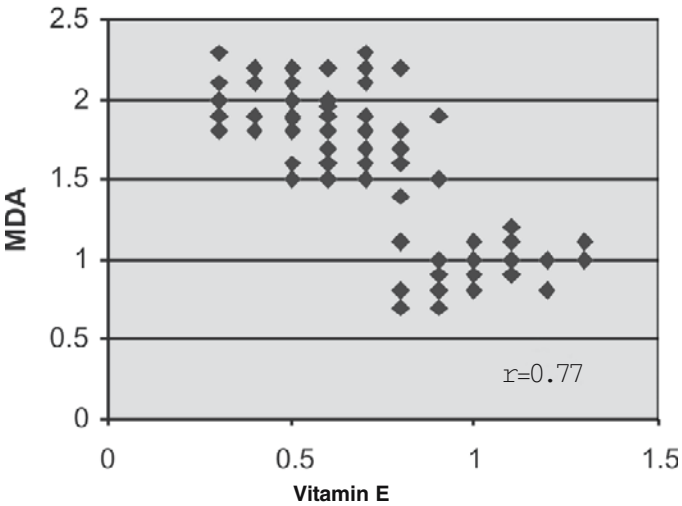
Graph –1 demonstrated the significant negative association of plasma MDA with both the vitamins ($p < 0.001$; $p < 0.001$) in the study group explaining their protective consumption in the oxidative process.

DISCUSSION

Hyperglycemia generates oxidative stress which is amplified by metabolic stress. The possible sources of oxidative stress include an increased production of reactive oxygen species especially from glucose autooxidation, glycoxidative activation of protein kinase C and increased polyol pathway with subsequent pseudohypoxia. Polyol pathway and subsequently raised levels of sorbitol leads to osmotic stress. Simultaneously the myoinositol level falls which in turn affects the Na^+-Ka^+ ATPase pump leading to hydration, prevailing in diabetic retinopathy. Glycation of protein leads to protein-protein cross link, which further undergoes molecular rearrangements to form Advanced Glycation End Product(AGE). Hydrogen peroxide with the aid of metal ions (Fe^{3+}) attack the carbohydrate moiety in the protein to form dicarbonyl derivatives, leading to AGE or maillard product formation. AGEs thus formed ,bind to Receptor for AGE (RAGE) on endothelial cells, pericytes and retinal pigment epithelial cells and initiates a wide range of cellular events and vascular homeostasis ,leading to retinal neovascularization in diabetic retinopathy(1).

Both dyslipidemia and lipoxidation also contribute to oxidative stress. Metabolically active Free Fatty Acids (FFAs) exert an inhibitory effect on the adenosine nucleotide translocator with a resultant decrease in available ADP. This decrease in ADP

Graph 1 : Correlation between Plasma MDA and Vitamin E



Graph 2 : Correlation between Plasma MDA and Vitamin C

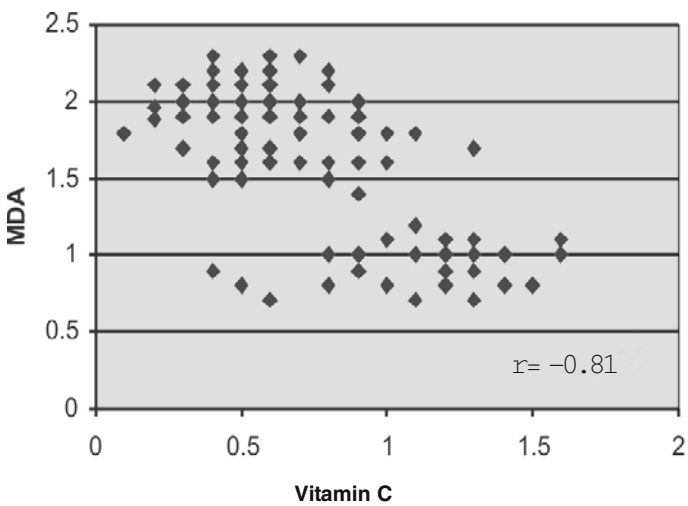


Table 4 : Correlation between Biochemical Parameters in Study Population

Parameters mean+SD	FPG (mg/dl)	2 hr PPPG (mg/dl)	Total chol. (mg/dl)	HDLc (mg/dl)	LDLc (mg/dl)	VLDLc (mg/dl)	TAG (mg/dl)
Plasma MDA (nmol/ml)	0.81**	0.92**	0.62**	-0.87**	0.65**	0.91**	0.89**
Serum vitamin-E (mg/dl)	-0.73**	-0.82**	-0.51*	0.80**	-0.52*	-0.79**	-0.78**
Serum vitamin-C (mg/dl)	-0.59*	-0.67**	-0.38*	0.68**	-0.42*	-0.67**	-0.65**

** $p < 0.001$; * $p < 0.01$

slows down the flow of electrons along the electron transfer chain and increases the possibility of having single unpaired electrons to create the superoxide anions (O_2^-) thereby increasing oxidative mitochondrial stress (FFA toxicity)(16). The lipid triad comprising of elevated VLDL/Triacylglycerol (TAG), atherogenic LDL and decreased HDL, along with the FFA toxicity reduce the natural antioxidant reserve. This combination supports an increase in redox stress that results in increased reactive oxygen species(17). Apart from hyperglycemia and dyslipidemia, the weakness of the antioxidant defense system may be the biochemical background for the pathogenesis of endothelial dysfunction associated with DM(18).

The retina is particularly susceptible to oxidative stress because of its high consumption of oxygen, its high proportion of PUFAs and its exposure to visible light. 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. Furthermore there is strong evidence that lipofuschin is derived at least in part, from oxidatively damaged photoreceptor, outer segments and that it itself is a photoreactive substance(19).

In the present study, the production of reactive oxygen species is directly related to hyperglycemia and dyslipidemia. The significant fall in both vitamin-E and vitamin-C in DM and more so in diabetic retinopathy explains their protective consumption in the scavenging process. Further the uptake of vitamin-C into the cell is mediated by the process related to glucose transport and the high extracellular glucose in DM may further impair cellular uptake and accentuate the problems associated with its deficiency. Therapeutic doses of vitamin-C have demonstrated the reversal of early signs of retinopathy in diabetics confirming its protective role in the damage of blood vessels as well as its therapeutic potential in diabetic retinopathy cases(6).

Thus the increase in lipid peroxidation product MDA in blood associated with weakness of the defense antioxidant system in diabetics probably serve as a background for the pathogenesis of endothelial dysfunction associated with diabetes and lead to both macrovascular as well as microvascular damage resulting in diabetic retinopathy. Further work may be undertaken to confirm the association between antioxidant nutrient intake and the reduction in the development of complications particularly retinopathy in diabetics.

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