Indian J Physiol Pharmacol 2005; 49 (2) : 187–192

LIPID PEROXIDATION AND ANTIOXIDANT STATUS IN PATIENTS WITH DIABETIC RETINOPATHY

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(Received on October 5, 2004)

Abstract : Non Insulin Dependent Diabetes Mellitus is responsible for 60% cases of retinopathy in the population and is one of the common cause of blindness. Oxidative stress as measured by the levels of malondialdehyde, superoxide dismutase (SOD), glutathione peroxidase (GPx) and vitamin C was measured in 50 normal controls, 40 diabetics without complications, 22 diabetics with proliferative and 20 with nonproliferative retinopathy respectively. Our finding suggests that lipid peroxidation increases ($P<0.001$) with the increase in severity and duration of diabetes. Antioxidants SOD and vitamin C decrease with the progression of the disease, however GPx tends to increase in the later part of the disease.

Key words : lipid peroxidation antioxidants diabetes retinopathy

INTRODUCTION

The relationship of diabetes and diabetic retinopathy is assuming clinical significance in the world scenario. Global literature survey from developed and developing countries highlights an intricate correlation between the type and duration of diabetes and occurrence of retinopathy. It is seen that diabetic retinopathy still remains a major cause of blindness despite the increased understanding of this disease and implementation of successful treatment modalities (1). The best predictor of diabetic retinopathy is the duration of the disease. The duration of diabetics have a strong correlation to the incidence and occurrence of diabetic retinopathy. A good control of the levels of blood sugar may delay the development of complications (2). It is known that 20–50% of long duration diabetics show proliferative diabetic retinopathy (PDR). The incidence of development of proliferative diabetes mellitus is higher in females as compared to males (3:2), though the rate of development does not differ.

Studies on patients with long term and poorly controlled diabetes suggest that free radicals are responsible for the development of diabetic macroangiopathy as well as microangiopathy (3). Oxygen free radicals liberated by metabolic processes can cause

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tissue damage. Endogenous oxidative damage to proteins, lipids and DNA is thought to be important etiologic factor in the pathophysiology of the complications of diabetes mellitus. This is likely to occur only after the production of reactive oxygen species has exceeded the body's or cell's capacity to protect itself and effectively repair oxidative damage. Normally the body has an abundant supply of antioxidants, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, vitamin A, vitamin C etc. which are naturally occurring substances that delay or inhibit oxidation and neutralize the oxygen free radicals. Free radicals in diabetes mellitus and increasing over time may play a role in the development of diabetic retinopathy which is an important complication of the disease (4). Little is known about the relationship in between oxidative stress and diabetic retinopathy in Central India.

Hence the study was undertaken to evaluate the role of oxidativc stress and its correlation with hyperglycemia and duration in patients of Non insulin dependent diabetes mellitus (NIDDM) with and without retinopathy.

MATERIALS AND METHODS

The control group comprised of 50 healthy volunteers (Group I). The controls were examined and were found to be free from disease. 40 cases of NIDDM (Group II) without any micro vascular or macrovascular complications attending the Diabetic Clinic of Indira Gandhi Medical College, Nagpur were included in the study. The criteria for the diagnosis of NIDDM was done by the National Diabetes Data Group of the National Institute of Health. 22 patients of diabetic proliferative retinopathy (Group III) and 20 patients of diabetic non proliferate retinopathy (Group IV) as diagnosed by direct ophthalmoscopy were included in the study group.

The groups as a whole were similar in age and sex distribution. Subjects suffering from renal, cardiac disease and any chronic or acute inflammatory illnesses were excluded from the study. All the diabetics were on hypoglycemic drugs and none of the study subjects were on antioxidant supplementation or lipid lowering drugs. Haemolysed samples were excluded from the study. The study was approved by the ethical committee of the college. The fasting blood samples were collected in an EDTA/ heparin/plain bulb vial for estimation from all the study subjects. Plasma glucose was measured using enzymatic kit method. Glycosylated Haemoglobin (HbA_{1c}) was measured by ion exchange resin method. Malondialdehyde (MDA), a marker of lipid peroxidation was estimated on the fact that lipid peroxides condense with 1 methyl 2 phenyl indole under acidic conditions resulting in the formation of a chromophore (Randox Laboratories, UK). Superoxide dismutase (SOD) estimation was based on the reaction between superoxide radicals and 2-4 iodophenyl-3-4 nitrophenol-5-phenyl tetrazolium chloride (5). Ascorbic acid was measured using phosphotungstic acid as colouring agent (6). Glutathione peroxidase (GPx) was measured by the method of Paglia and Valentine (7).

Statistical significance was analysed by students t test and correlation between variables were studied by using Pearson's correlation coefficient test.

RESULTS

As in Table I, all the diabetic patients (Group II, III and IV) had significantly higher (P<0.001) levels of plasma glucose and HbA_{1c} than in controls, indicating a poor control of diabetes.

As seen in Table II, the levels of MDA were significantly increased (P<0.001) where as levels of SOD, GPx and vitamin C were significantly decreased (P<0.001) in diabetics without complications (Group II) when compared with the controls.

The levels of MDA [P<0.001; P<0.05] and GPx [P<0.001; P<0.001] were significantly increased where as levels of SOD [P<0.001; P<0.05] and vitamin C [P<0.001; P<0.001] were significantly decreased in diabetics with proliferative retinopathy (Group III) when compared with the control and diabetic group without complications.

The levels of MDA [P<0.001; P<0.01] and GPx [P<0.01; P<0.001] were significantly increased where as levels of SOD [P<0.001; P<0.05] and vitamin C [P<0.001; P<0.001] were significantly decreased in diabetics

TABLE I: General data of controls and diabetics with and without retinopathy.

	Group I (n = 50)	*Group II (n = 40)*	*Group III (n = 22)*	*Group IV (n = 20)*
Age in years	46±8	0±6	46±5	47±6
Sex (M/F)	30/20	27/13	10/12	09/11
Weight in kg	58±7	57±10	58±10	58±12
Body mass index (kg/m^2)	23±3.9	26±4.2	25±3.8	26±4.1
Plasma glucose (mg/%)	79.89±12.30	173.22±37.86*	231.58±46.31*	226.7±44.7*
HbA_{1c} (%)	7.18±0.87	9.28±1.88*	12.28±1.21*	12.21±1.41*
Duration of diabetes in years		6.36±2.33	11.88±2.1	11.21±1.88

*P<0.001 when Group I compared with Group II, III and IV.

TABLE II: Prooxidant and antioxidant status in diabetics without complications and with retinopathy.

	Group I (n = 50)	*Group II (n = 40)*	*Group III (n = 22)*	*Group IV (n = 20)*
MDA (nmol/ml)	0.92±0.24	$1.72±0.27^{a}$	$1.93±0.25^{af}$	$1.98±0.2^{ae}$
SOD (U/gmHb)	6.83±0.7	$5.35±0.36^{a}$	$5.13±0.27^{af}$	$5.14±0.1^{af}$
GPx (U/gmHb)	14.64±1.43	$13.37±0.33^{a}$	$16.17±0.86^{ad}$	$15.94±1.0^{bd}$
Vit C (mg%)	1.13±0.33	$0.71±0.22^{a}$	$0.36±0.2^{ad}$	$0.31±0.17^{ad}$

P<0.001 = a, P<0.01 = b, P<0.05 = c; when group I compared with group II, III and group IV.
P<0.001 = d, P<0.01 = e, P<0.05 = f; when group II compared with group III and group IV.

with non-proliferative retinopathy (Group IV) when compared with the control and diabetic group without complications.

Also, correlation analysis revealed a significant positive correlation when levels of MDA were compared with plasma glucose [0.73, P<0.001; 0.84 P<0.001], HbA_{1c} [0.87 P<0.001; 0.87 P<0.001] and duration [0.67 P<0.001; 0.71 P<0.001] of proliferative and non proliferative diabetic retinopathy respectively.

However a significant negative correlation was found when the antioxidants SOD [–0.84, P<0.001–0.7, P<0.001] and Vitamin C [–0.7 P<0.001; –0.64, P<0.001] were compared with MDA in diabetics with proliferative and non-proliferative retinopathy. A significant positive correlation was found when the antioxidant GPx [0.79, P<0.001; 0.63 P<0.001] were compared with MDA in diabetics with proliferative and non-proliferative retinopathy.

A significant negative correlation was found when the anti-oxidant SOD [–0.77, P<0.001] was compared with plasma glucose levels in diabetic proliferative retinopathy. A significant positive correlation was found when the anti-oxidant GPx [0.55, P<0.01; 0.567 P<0.05] was compared with plasma glucose levels in diabetic proliferative and non proliferative retinopathy respectively. No correlation was found when vitamin C [–0.26, NS; –0.28, NS] was compared with plasma glucose levels in diabetic proliferative and non-proliferative retinopathy.

DISCUSSION

Reactive oxygen species intermediates include free radicals and singlet oxygen and they are often the by products of oxygen metabolism. The retina is particularly susceptible to oxidative stress because of its high consumption of oxygen, its high proportion of polyunsaturated fatty acids and its exposure to visible light In vitro 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 (8).

Oxidative stress in hyperglycemia is amplified by metabolic stress. In our study, the production of oxygen free radicals is directly related to hyperglycemia and duration of diabetes. The longer the duration of disease the higher is the lipid peroxide level. This is in agreement with that of Gurler B (4) and Kesavulu M (9), however Turk et al (10) reported that lipid peroxides were non-significantly increased in diabetics when compared with patients of diabetic retinopathy.

Decrease in the activities of antioxidant enzyme systems in diabetes is linked to the progressive glycation of enzymatic proteins. Numerous reports indicate variations in the levels of antioxidants in diabetic patients (4, 11, 12). In our study the levels of SOD, an enzyme responsible for the scavenging

of oxidant stress factors in the body is significantly decreased in diabetics with and without retinopathy. Superoxide anion, which is believed to be one of the initiators of free radical reaction plays an important role in the determination of the levels of antioxidant enzyme SOD. Products of membrane lipid peroxidation and other oxidants like H_2O_2 may react with SOD resulting in oxidative modification thereby causing loss of enzyme activity (13). Also, diabetic hyperglycemia leads to glycation and inactivation of SOD thus attributing to its decrease.

At physiological rates of hydrogen peroxide generation, the glutathione system is more important in catabolising H_2O_2. GPx is a selenium (Se) dependent enzyme and any alterations in the tissue levels of Se would alter GPx activity. Insulin deficiency promotes β oxidation of fatty acids with resulting increase in H_2O_2 formation. Thus with increase in the lipid peroxide levels, the paradoxical increase in the levels of GPx is an interesting finding in retinopathy and could be a compensatory mechanism by the body to prevent tissue damage. Our findings regarding antioxidant enzymes are in accordance with that of Kesavulu MM (9) who reported a decrease in the levels of GPx and SOD, however other authors have demonstrated either an increase (10) or no change (4) in the values of antioxidants.

Vitamin E and C are important components of cell defense against oxidative stress (14). These levels are significantly decreased in diabetics and more so in diabetic retinopathy (15). Our study also suggests similar findings. The uptake of ascorbic acid into the cell is mediated by processes related to glucose transport and it has been shown that the high extra cellular glucose concentration in diabetics may further impair cellular uptake and accentuate the problems associated with its deficiency (16). Also, therapeutic doses of vitamin C are associated with reversal of early signs of retinopathy in diabetics (17), confirming its protective role in the damage of blood vessels.

Thus the increase in lipid peroxides in blood coupled with weakness of the defense antioxidant system in diabetics without complications, probably serves as a background for the pathogenesis of endothelial dysfunction associated with diabetes.

In our study, correlation analysis reveals that the extent of oxidative stress ie an imbalance in the levels of oxidants and antioxidants, is thus related to the severity and duration of diabetes which justifies the aim of our study. In conclusion, free radical formation along with antioxidant deficiency in diabetes mellitus increases over time and may play an important role in the development of diabetic retinopathy, which is an important complication of the disease. The levels of antioxidants with the exception of GPx are significantly decreased in diabetics, particularly in those with retinopathy.

Further work is needed to confirm whether their exists an association in between antioxidant nutrient intake and reduction in the development of diabetic complications particularly retinopathy.

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