Does Oxidant Stress Play a Role in Diabetic Retinopathy?

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The role of oxidant stress in the causation of chronic tissue damage is being increasingly recognized. Oxidant stress is usually counteracted by abundant supply of antioxidants. If concomitant antioxidant deficiency occurs, oxidant stress may produce tissue damage. We took up a study on antioxidant status in non-insulin dependent diabetes mellitus (NIDDM) patients with and without retinopathy and compared them with a control non-diabetic group.

The levels of superoxide dismutase (SOD) were significantly reduced in all diabetic patients, i.e., those with and without retinopathy. However, the lowest levels were found in the diabetic patients with retinopathy. Vitamin E and vitamin C levels were also markedly lower in the diabetic patients. There was a paradoxical rise in the catalase and glutathione peroxidase (G-Px) in the diabetic patients with retinopathy. This may be a compensatory mechanism by the body to prevent tissue damage by increasing the levels of the two alternative antioxidant enzymes.

Key Words: Non-insulin dependent diabetes mellitus - Antioxidant status - Superoxide dismutase - Catalase - Vitamin E - Vitamin C.

Recently, there has been a great deal of interest in the role of oxidant stress in the causation of tissue damage in a number of diseases. Hypothetically, oxygen free radicals or "reactive oxygen species" liberated by metabolic processes can cause tissue damage. Normally the body has an abundant supply of "antioxidants" which are naturally occurring substances that delay or inhibit oxidation and neutralize the oxygen free radicals. In nature, therefore, when there is a balance of "oxidant stress" and the "antioxidant supply", there is perfect harmony and no tissue destruction occurs. However, if there is an imbalance, i.e., either an excess of free radicals and reactive oxygen species or a deficiency of antioxidant supply, tissue damage can occur.

The role of oxidant stress in the causation of diabetic retinopathy has not been adequately studied. In this paper, we report on the antioxidant status in patients with non-insulin dependent diabetes mellitus (NIDDM) with and without retinopathy.

MATERIALS AND METHODS

The groups of patients studied were (a) NIDDM patients with background diabetic retinopathy (n = 83); (b) NIDDM patients without retinopathy (n = 65); and (c) control non-diabetic group (n = 55). Healthy non-diabetic control subjects were selected mainly from spouses of patients to match for age, sex, socioeconomic status and nutritional status. All study subjects (controls and NIDDM patients) had normal renal and hepatic function and had no evidence of any acute infections or active inflammatory processes. None of the women in the study were on oral contraceptive agents or any hormones.

Laboratory Investigations

All study subjects had baseline investigations including a complete physical examination and height, weight and blood pressure readings. Fasting and postprandial plasma glucose levels were estimated by glucose oxidase method (Corning Plus Autonalyzer). Glycosylated haemoglobin was estimated by the method of Erwin et al.; serum cholesterol by the CHOD-PAP method and serum triglyceride levels by the GOD-PAP method (Boehringer Mannheim).

Antioxidant Estimations

The following assays were done:

Superoxide Dismutase (SOD): Superoxide dismutase
was assayed in the haemolysate according to the method of Misra and Fridovich. SOD levels were expressed as u mg/Hb.

Catalase: This enzyme was assayed in the erythrocyte membrane suspension according to the method of Sinha. Catalase activity was expressed as n moles of H₂O₂ liberated/min/mg protein.

Glutathione Peroxidase (GPx): Glutathione peroxidase was assayed in the haemolysate according to the method of Rotruch et al. GPx levels were expressed as GSH utilised/mg/Hb/min.

**TABLE 1**

<table>
<thead>
<tr>
<th>Clinical and Biochemical Data of the Study Population</th>
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<td>Healthy Controls</td>
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<td>(n = 56)</td>
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<td>------------------</td>
</tr>
<tr>
<td>Age (Yrs)</td>
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<tr>
<td>Weight (Kg)</td>
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<td>Body mass index (Kg/m²)</td>
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<td>Fasting plasma glucose (mg/dl)</td>
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<td>Glycosylated haemoglobin (%)</td>
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* p < 0.001 compared to diabetes without retinopathy.

Vitamin A: Vitamin A was estimated in the plasma by the method of Bayfield and Cole. Results were expressed as m moles/L plasma.

Vitamin E: Vitamin E was estimated in the plasma after extraction by the method of Kayden et al. After saponification and solvent extraction of lipids to remove interfering substances, alpha-tocopherol was estimated spectrophotometrically using haftophenanthrolone reagent. After extraction, Vitamin E was estimated according to the modified method of Emmerie and Engel. The Vitamin E values were expressed as m moles/L of plasma.

Vitamin C: Vitamin C (ascorbic acid) was estimated in blood according to the method of Omaye et al. Values were expressed as m moles of ascorbic acid/l of blood.

**Statistical Analysis:** Statistical analysis was done by one-way analysis of variance (ANOVA) to compare differences between groups and p < 0.05 was considered significant.

**RESULTS**

Table 1 shows the clinical and biochemical details of the study groups. There were no significant differences in the age, weight or body mass index between the three study groups. Patients with retinopathy had higher fasting plasma glucose levels (p < 0.001) and glycosylated haemoglobin levels (p < 0.002) compared to those without retinopathy.
Table 2 and Fig. 1 show the observations on the antiperoxidative enzyme status in the erythrocytes. The levels of SOD were significantly reduced in both groups of diabetic patients compared to the control group (p < 0.001). NIDDM patients with retinopathy had lower SOD levels compared to those without retinopathy (p < 0.01). Levels of catalase were paradoxically higher in diabetics compared to control (p < 0.001). Moreover, the levels were higher in those with retinopathy compared to those without retinopathy (p < 0.001). The levels of GPx were also paradoxically increased in diabetic patients with and without retinopathy compared to controls (p < 0.001). Again, the levels were higher in those with retinopathy compared to those without retinopathy (p < 0.001).

Table 2 and Fig. 2 show the non-enzymatic antioxidant levels in the plasma of NIDDM patients.

| TABLE 2 | Antiperoxidative Enzyme Status in the Erythrocytes of the Study Groups |
|---------|-----------------------------|-----------------------------|-----------------------------|
|         | Catalase (membrane) role   | GPx (lyase)                 |                             |
|         | SOD (lyase) (umg Hb) | H2O2 consuming             | \\S \text{GSH} \text{utilized | mg Hb/min)                   |
| Healthy controls (n = 56) | 3.6 ± 0.41 | 7.3 ± 0.49 | 5.6 ± 0.42 |
| Diabetics without retinopathy (n = 63) | 2.6 ± 0.29 | 18.4 ± 0.34 | 7.2 ± 0.60 |
| Diabetics with retinopathy (n = 83) | 2.4 ± 0.58 | 10.7 ± 1.69 | 8.7 ± 1.30 |

Values are expressed as Mean ± S.D.

Statistically significant differences between diabetics without retinopathy and healthy controls are denoted as *** p < 0.001. The difference between diabetics with retinopathy and healthy controls are denoted as ** p < 0.01. The differences between diabetics without retinopathy and diabetics with retinopathy as *** p < 0.001 and ** p < 0.01.

| TABLE 3 | Non-Enzymatic Antioxidant Levels in the Plasma of the Study Groups |
|---------|-----------------------------|-----------------------------|-----------------------------|
|         | Vitamin A (mmol/L) | Vitamin E (mmol/L) | Vitamin C (mmol/L) |
| Healthy controls (n = 56) | 7.3 ± 0.21 | 24.6 ± 3.49 | 62.8 ± 4.5 |
| Diabetics without retinopathy (n = 63) | 2.2 ± 0.06 | 21.0 ± 0.54 | 49.4 ± 5.10 |
| Diabetics with retinopathy (n = 83) | 2.1 ± 0.12 | 17.6 ± 3.70 | 53.2 ± 20.0 |

Statistically significant differences between diabetics without retinopathy and healthy controls are denoted as *** p < 0.001. The differences between diabetics with retinopathy and healthy controls are denoted as ** p < 0.01 and the differences between diabetics without retinopathy and diabetics with retinopathy are denoted as *** p < 0.001, ** p < 0.01.
with diabetic retinopathy, NIDDM patients without diabetic retinopathy and healthy controls.

There was no significant difference in vitamin A levels among the three study groups. Vitamin E levels were significantly lower in both the diabetic groups compared to the control group. Diabetics with retinopathy had lower values of vitamin E compared to diabetics without retinopathy (p < 0.001). Levels of vitamin C were markedly reduced in both diabetic groups compared to control group (p < 0.001). The differences between NIDDM subjects with and without retinopathy were not statistically significant.

DISCUSSION

The study has shown certain significant findings. The levels of superoxide dismutase (SOD), one of the key enzymes responsible for scavenging the active oxidant stress factors in the body is very much reduced in diabetics and more markedly so in those with retinopathy. This suggests that oxidant stress may be playing a role in the causation of diabetic retinopathy.

In further support of this is the observation of low levels of both vitamin E and vitamin C which are key substances needed for maintaining the levels of SOD in the body. Thus, the levels of antioxidants seem to be significantly depressed in diabetics, particularly in those with retinopathy.

The paradoxical increase in catalase and glutathione peroxidase is an interesting finding. The possible explanation for this phenomenon is that it could be a compensatory mechanism by the body to prevent tissue damage by increasing the levels of these two alternative antioxidant enzymes.

The levels of intermediate reduction products of oxygen metabolism are controlled by various enzymatic defence mechanisms consisting of superoxide dismutase, catalase and glutathione peroxidase.\(^{11}\)

Alterations in superoxide dismutase, peroxidase and catalase activities and tissue glutathione concentrations have been reported in diabetes.\(^{12}\) It has been shown that the antioxidant enzyme increases are possibly adaptive. Kaji et al.\(^{13}\) have also shown that the levels of lipid peroxidase and glutathione peroxidase are increased in the plasma of NIDDM patients.

Matkovic et al.\(^{14}\) reported that the activity of glutathione peroxidase is increased in the red blood cell haemolysates of diabetic patients. Glutathione peroxidase (GPx) is shown to be selenium depend-

ent. If the tissue levels of selenium are altered, GPx activity would also be changed.

Insulin deficiency promotes β-oxidation of fatty acids, with resulting increase in H₂O₂ formation. This fact when taken with an observation made by Wohlaeb and Bodin\(^{15}\) that insulin treatment prevents the increase in cardiac and pancreatic catalase activity, would support the suggestion that increases in RBC catalase and GPx in diabetes may be a compensatory response to an increase in endogenous H₂O₂ production.

The weakness of the antioxidant defense system may be the biochemical background for the pathogenesis of endothelial dysfunction associated with diabetes.\(^{16}\) The three essential nutrients that can directly scavenge free radicals are vitamins A, E and C. Alpha-tocopherol (vitamin E) is known to serve as the major lipid soluble chain breaking antioxidant in cells.\(^{17}\)

Ascorbic acid functions as an important component of cellular defense against oxygen toxicity and lipid peroxidation caused by free radical mechanism.\(^{18}\) Reduced levels and altered metabolic turnover of ascorbic acid has been reported in diabetic patients.\(^{19}\)

A decrease in the plasma concentration of ascorbic acid has been observed in diabetic patients. The uptake of ascorbic acid into the cell is mediated by processes related to glucose transport and it has been shown that the high extracellular glucose concentration in diabetes may further impair cellular uptake of ascorbic acid and accentuate the problems associated with its deficiency.\(^{20}\)

Reduced concentration of ascorbic acid and increased oxidative stress (as evidenced by elevated dehydroascorbic acid/ascorbic acid ratio) has been observed in NIDDM patients when compared with age-matched non-diabetic controls and the abnormalities were more pronounced in those patients with microangiopathy.\(^{21}\)

There are reports that high doses of vitamin C regimens are associated with reversal of early signs of retinopathy and normalisation of capillary strength in diabetes mellitus\(^{22}\) confirming the role of antioxidants against the development of the pathology in blood vessels.

In summary, antioxidant deficiency appears to be associated with a risk for diabetic retinopathy. Further longitudinal studies may help to throw more light on the aetiological link between antioxidant deficiency and diabetic retinopathy.
REFERENCES