Oxidative Stress Markers Regulating the Healing of Foot Ulcers in Patients With Type 2 Diabetes

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Abstract: Objective. This study was aimed at identifying factors that affect the healing of foot ulcers among patients with type 2 diabetes, focusing on the evaluation of oxidative stress—one marker of the inflammatory response. Methods. A cross sectional study comprised of 96 subjects who were divided into 6 groups (16 subjects in each group). The groups were classified as non-diabetic control (group I), diabetic subjects without foot ulcer (group II), diabetic subjects with foot ulcers were sub-divided as neuropathic ulcer-noninfected (group III), neuropathic ulcer-infected (group IV), neuroischemic ulcer-noninfected (group V), and neuroischemic ulcer-infected (group VI). Oxidative stress markers such as lipid peroxidation, thiobarbituric acid reactive substance (TBARS), superoxide dismutase (SOD), catalase, G-peroxidase, GS-peroxidase, and plasma total antioxidant status were assayed in the blood samples. Results. Lipid peroxidation increased progressively from group I to group VI subjects \((P < 0.001)\). The TBARS in erythrocyte membrane was higher than in plasma. A progressive decrease of the total antioxidant status in plasma from group III to group VI \((P < 0.01)\) was noted. There was a triggering increase in the antioxidative enzymes SOD and catalase in group V and group VI. Conclusion. There is a high level of lipid peroxidation with insufficient antioxidant enzymes and decreased total antioxidant status in plasma that leads to chronic ulceration and an extended inflammatory reaction. Thus, oxidative stress may be regarded as an important factor in nonhealing diabetic foot ulcers among patients with type 2 diabetes.

Oxidative damage due to the endogenous production of oxidative reactive oxygen species (ROS) or toxic oxygen free radicals by mitochondria is a common and ubiquitous form of oxidative stress in most mammalian cells. It is one of the classic events in the pathogenesis of diabetes and its complications. Intracellular hyperglycemia is the primary initiating event in the formation of both intracellular and extracellular advanced glycation end products (AGE). Plasma proteins modified by AGE precursors bind to AGE receptors on endothelial cells, mesangial cells, and...
macrophages, which induces receptor-mediated production of reactive oxygen species.

One of the most common complications of diabetes in the lower extremity is the diabetic foot ulcer. Diabetic foot infection is a common cause for hospital admission among patients with diabetes in India. Several studies have compared the oxidative stress between diabetic and nondiabetic subjects and among diabetic patients with and without complications. The present study was undertaken to assess the peroxidative damage due to secondary complications in diabetes, namely foot ulcers, in comparison with diabetic and nondiabetic controls. This study focuses on the Asian-Indian ethnic group for which there are very little data available.

The main focus of this study was to evaluate the biochemical and molecular markers of the inflammatory response by evaluating free radical production and the scavenging antioxidant status in blood, and to analyze whether neuropathy, ischemia, ulcer, and infection contribute to the reactive oxygen species production and indicate oxidative stress.

Methods

Study subjects and characteristics. A total of 96 subjects (59 male, 37 female) from M.V. Hospital for Diabetes and Diabetes Research Center (Royapuram, Chennai), a large referral center for diabetes in southern India, were categorized into 6 groups of 16 members each. Emphasis was placed on a comparative study of the nondiabetic control (group I) staff at the diabetic clinic or spouse of the diabetic patient; type 2 diabetic subjects grouped as diabetic without a foot ulcer (group II); diabetic with a foot ulcer—neuropathic ulcer-noninfected (group III); neuropathic ulcer-infected (group IV); neuroischemic ulcer-noninfected (group V); and neuroischemic ulcer-infected (group VI). The exclusion criteria were antioxidant supplementation, smoking, and alcoholism.

Details of individuals like gender, age, type and duration of diabetes, body mass index (BMI), glycemic control (HbA1c), hemoglobin concentration (Hb%), triglycerides, total cholesterol, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum albumin, the presence of the microflora, area and duration of the ulcer, microalbuminuria, proteinuria, retinopathy, hypertension, neuropathy and ischemia, were obtained from each patient’s medical record. Neuropathy was defined as vibration perception threshold (VPT) > 25 V using a biothesiometer (Biomedical Instruments Co., Newbury, Ohio). Peripheral vascular disease was diagnosed by history of intermittent claudication and/or ankle brachial index < 0.9 using a Doppler (Vaslab, Kody Lab, Chennai, India). Wounds of all subjects admitted to the clinic were examined by a team of expert wound care specialists and were defined as being infected or noninfected based on clinical signs of infection (swelling, redness, exudate, surrounding cellulitis, odor, tissue necrosis). Ulcer grade was determined using the Wagner classification system. Ulcer area was determined by multiplying the longest and widest diameters and was expressed in centimeters squared (cm²).

Sample collection. Biological samples were collected from human subjects after approval by the ethical committee at the hospital and after receiving informed consent from the patients. Fasting blood samples were collected from a peripheral vein and drawn into Vacutainers with and without heparin and processed to obtain plasma, red blood corpuscles (RBC), and serum, respectively, which were aliquoted and stored at -80°C for clinical and biochemical analysis.

Instruments. All biochemical assays to estimate the oxidative stress were carried out spectrophotometrically using Lambda-35 UV-visible spectrophotometer (PerkinElmer, Waltham, Mass) with a Peltier device attached to the instrument for temperature control.

Evaluation of Oxidative Stress Markers

RBC membrane and pellet hemolysate preparation. Two hundred µl of frozen RBC was added to 3 mL of phosphate buffer (pH 7.4), mixed well and centrifuged at 10,000 rpm for 10 minutes. The hemolysate was stored at 4°C for subsequent antioxidant assays. The RBC membrane pellet was washed twice with double distilled water (DDW) and centrifuged. The RBC membrane pellet was made up to 0.5 mL with DDW, and the thiobarbituric acid reactive substances (TBARS) assay was performed.

Protein estimation. Total protein content of all assay samples (plasma and hemolysate) were quantified by the method of Bradford. These values were expressed as mg/mL.

Lipid peroxide estimation. Lipid peroxidation in erythrocyte membrane was determined by estimation of thiobarbituric acid reactive substances (TBARS) by the Yagi method. These results were expressed as micromoles of MDA.

Enzymatic antioxidants. Superoxide dismutase
(SOD) was assayed using the Misra and Fridovich\textsuperscript{13} method. Superoxide dismutase activity was expressed as the amount of enzyme required to exhibit 50\% inhibition of epinephrine auto oxidation. Catalase was assayed using the Caliborne\textsuperscript{14} method, and the enzyme activity was expressed as micromoles of H\textsubscript{2}O\textsubscript{2} decomposed/min/mg protein. Glutathione peroxidase was assayed using the Rotruck et al\textsuperscript{15} method, and the activity was expressed as micromoles of glutathione oxidized/min/mg protein. Glutathione-S-transferase was estimated with the Habig et al\textsuperscript{16} method. The enzyme activity was expressed as micromoles of CDNB-GSH conjugated/min/mg protein.

Plasma total antioxidant status (TAS). Plasma TAS was determined with commercial kits (Calbiochem®, Merck Chemicals, Darmstadt, Germany) according to the method of Miller et al.\textsuperscript{17} The results are reported in millimoles (mM), and the sensitivity of the assay is for antioxidant levels up to 2.5 mM.

Statistical Analysis

The mean value, standard deviation, and standard error of mean were computed for all variables. The statistical significance was determined by ANOVA. The differences between groups and within groups were noted; \( P < 0.05 \) was considered significant.

Results

The clinical characteristics of the control patients and patients with type 2 diabetes are provided in Table 1. A total of 96 subjects were included in the study with 16 patients in each group. The age and duration of diabetes was comparable among the groups and was indicative of long standing diabetes. The body mass index (BMI) values of diabetic groups were similar to the nondiabetic control group. The glycosylated hemoglobin HbA1c (%) showed that type 2 diabetic patients in this study had values > 9\% representing poor glycemic control. The triglycerides and cholesterol of diabetic subjects were higher than the nondiabetic subjects. The liver function tests assessed by serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were within the normal range for groups I, II, III, IV, and was slightly greater for groups V and VI.

<table>
<thead>
<tr>
<th>Group</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. subjects (n)</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>54.9 ± 9.5</td>
<td>56 ± 8</td>
<td>56.2 ± 7</td>
<td>57.3 ± 8.9</td>
<td>57.4 ± 9.1</td>
<td>58 ± 8.4</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>-</td>
<td>12.9 ± 8.4</td>
<td>12.7 ± 6.8</td>
<td>11.8 ± 6.7</td>
<td>11.8 ± 7.2</td>
<td>11.9 ± 6.5</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>25.1 ± 3.3</td>
<td>25.7 ± 4.8</td>
<td>24.9 ± 4</td>
<td>24.5 ± 3.5</td>
<td>24.3 ± 4.6</td>
<td>24.2 ± 3.4</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>&lt; 5.6</td>
<td>4.6 ± 0.7</td>
<td>9.8 ± 3.2</td>
<td>10.2 ± 4.8</td>
<td>9.21 ± 1.6</td>
<td>9.8 ± 2.6</td>
</tr>
<tr>
<td>Hb (g%)</td>
<td>13–14</td>
<td>13 ± 0.9</td>
<td>11.8 ± 1.7</td>
<td>11.1 ± 2.1</td>
<td>10.3 ± 1.6</td>
<td>10.5 ± 2.1</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>50–200</td>
<td>130 ± 30.1</td>
<td>168 ± 80.6</td>
<td>184 ± 65.2</td>
<td>158 ± 64.2</td>
<td>176 ± 40.2</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>120–200</td>
<td>160 ± 41.9</td>
<td>196 ± 56.4</td>
<td>202 ± 80.2</td>
<td>186 ± 75.4</td>
<td>224 ± 82.1</td>
</tr>
<tr>
<td>SGOT (IU/L)</td>
<td>5–35</td>
<td>25 ± 5.2</td>
<td>23 ± 10.6</td>
<td>26 ± 7.2</td>
<td>22 ± 8.4</td>
<td>26 ± 12.8</td>
</tr>
<tr>
<td>SGPT (IU/L)</td>
<td>5–40</td>
<td>25 ± 4.3</td>
<td>27 ± 12.9</td>
<td>28 ± 6.8</td>
<td>27 ± 7.9</td>
<td>27 ± 15.8</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.5–5</td>
<td>4.2 ± 0.3</td>
<td>3.8 ± 0.5</td>
<td>3.6 ± 0.5</td>
<td>3.4 ± 0.5</td>
<td>3.4 ± 0.6</td>
</tr>
<tr>
<td>ABI (Doppler)</td>
<td>&gt; 0.8</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.4</td>
<td>1.1 ± 0.5</td>
<td>1.0 ± 0.5</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>VPT (biothesiometry)</td>
<td>&lt; 25 V</td>
<td>19 ± 2</td>
<td>20 ± 2.4</td>
<td>32 ± 4.6</td>
<td>32 ± 5.1</td>
<td>38 ± 4.6</td>
</tr>
<tr>
<td>Area (cm\textsuperscript{2})</td>
<td>-</td>
<td>-</td>
<td>8.0 ± 2.3</td>
<td>30.2 ± 11.5</td>
<td>9.1 ± 2.8</td>
<td>36.5 ± 15.7</td>
</tr>
<tr>
<td>Duration of ulcer (weeks)</td>
<td>-</td>
<td>-</td>
<td>3–5</td>
<td>12–20</td>
<td>3–5</td>
<td>12–20</td>
</tr>
<tr>
<td>Depth of ulcer (Wagner Grade)</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>No. subjects (%)</td>
<td>Microalbuminuria</td>
<td>-</td>
<td>2 (12.5)</td>
<td>1 (6.25)</td>
<td>1 (6.25)</td>
<td>2 (12.5)</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>-</td>
<td>2 (12.5)</td>
<td>1 (6.25)</td>
<td>1 (6.25)</td>
<td>2 (12.5)</td>
<td>3 (18.75)</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>-</td>
<td>7 (43.75)</td>
<td>6 (37.5)</td>
<td>6 (37.5)</td>
<td>4 (25)</td>
<td>4 (25)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>-</td>
<td>5 (37.5)</td>
<td>4 (25)</td>
<td>2 (12.5)</td>
<td>2 (12.5)</td>
<td>3 (18.75)</td>
</tr>
</tbody>
</table>

Table 1. Clinical characteristics of the study subjects.
Albumin decreased progressively from nondiabetic group to neuroischemic ulcer-infected group. The Doppler examination for evidence of peripheral vascular disease (PVD) showed an ankle brachial index (ABI) of < 0.8, which was considered as significant PVD. Thus, group V and VI had lower limb ischemia. A biothesiometer was used to measure vibration perception threshold (VPT) to signify neuropathy and indicated that patients with a VPT > 25 V had significant neuropathy. Thus, groups III, IV, V, and VI had neuropathy. Foot ulcer area was small in the noninfected group in comparison to the infected group. Duration of the ulcer ranges from 3 to 5 weeks in the noninfected group and from 12 to 20 weeks in the infected groups. The percentage of subjects with diabetic complications in terms of microalbuminuria, proteinuria, retinopathy, and hypertension are shown at the bottom of Table 1.

The levels of oxidative markers in different groups are shown in Table 2. Lipid peroxidation in erythrocyte membranes in terms of concentration of malondialdehyde was assessed by thiobarbituric acid reactive substance (TBARS) assay. The lipid peroxidation progressively increased from the nondiabetic (group I) to the neuroischemic ulcer-infected (group VI). There was high significance between nondiabetic groups with respect to the other groups (P < 0.001). The results were significant between neuropathic ulcer-infected (group IV) and neuroischemic ulcer-infected (group VI).

**Superoxide dismutase (SOD) activity.** The SOD activity in hemolysate was less in the diabetic groups when compared to the nondiabetic group. Interestingly, the SOD increased in diabetic groups with a foot ulcer. SOD activity of group I compared to the others (groups IV–VI) was very significant (P < 0.001) with the exception of group III (P < 0.01). Comparison of diabetic subjects without a foot ulcer with the other groups was also quite significant (P < 0.001). There was a significant increase in SOD activity of the neuropathic ulcer-infected (group IV) P < 0.001) with respect to the neuropathic ulcer-noninfected (group III). In the neuroischemic ulcer-noninfected and neuroischemic ulcer-infected groups there was a decrease in SOD activity when compared to the neuropathic ulcer-infected group but it was not significant (P < 0.671).

**Catalase activity.** There was a significant difference in catalase activity between the nondiabetic and diabetic subjects without an ulcer group (P < 0.001), with the exception of the neuroischemic ulcer-noninfected group (P < 0.1). The catalase activity decreased in the diabetic without an ulcer group when compared to nondiabetics, but the diabetic with an ulcer group showed an increase with respect to diabetics without an ulcer (P < 0.001).

### Table 2. Levels of oxidative markers.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
<th>Group VI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS Erythrocyte membrane (µM/MDA)</td>
<td>26.6 ± 2</td>
<td>40.2 ± 5.6</td>
<td>45.1 ± 5.8**</td>
<td>53.8 ± 5.2</td>
<td>57.6 ± 11.5#</td>
<td>68.6 ± 11</td>
<td>0.0001</td>
</tr>
<tr>
<td>SOD</td>
<td>20 ± 2.3</td>
<td>16.5 ± 3.2*</td>
<td>23 ± 3*</td>
<td>27.7 ± 4</td>
<td>28 ± 4</td>
<td>26 ± 3.2†,#‡</td>
<td>0.0001</td>
</tr>
<tr>
<td>Catalase (µM/H2O2 decomposed/min)</td>
<td>86.4 ± 3</td>
<td>61.4 ± 3.3</td>
<td>126.8 ± 4</td>
<td>133.9 ± 10.1†</td>
<td>84.4 ± 13.6*</td>
<td>98.1 ± 9.8</td>
<td>0.0001</td>
</tr>
<tr>
<td>G-peroxidase (µM of glutathione oxidized/min/mg protein)</td>
<td>14.8 ± 1.2</td>
<td>6.9 ± 0.7</td>
<td>9 ± 0.9</td>
<td>12.2 ± 0.9</td>
<td>13.9 ± 0.8*</td>
<td>12.8 ± 0.8#</td>
<td>0.0001</td>
</tr>
<tr>
<td>G-S-transferase (µM/cDNB conjugated/min/mg protein)</td>
<td>1.6 ± 0.6</td>
<td>1.1 ± 0.2*</td>
<td>1.7 ± 0.1*</td>
<td>2.4 ± 0.4</td>
<td>2.3 ± 0.7#</td>
<td>2.1 ± 0.5†,#‡</td>
<td>0.0001</td>
</tr>
<tr>
<td>TBARS (plasma) (µM/MDA)</td>
<td>2.4 ± 0.3</td>
<td>3.7 ± 0.2</td>
<td>4.4 ± 0.4</td>
<td>5.7 ± 0.5</td>
<td>9.7 ± 0.9</td>
<td>11.4 ± 0.4</td>
<td>0.0001</td>
</tr>
<tr>
<td>Total antioxidants (mM)</td>
<td>1.7 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>1.4 ± 0.1</td>
<td>1.3 ± 0.2</td>
<td>1.3 ± 0.1</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Non-significant groups: P ≥ 0.05
*versus group I
**versus group II
†versus group III
#versus group IV
‡versus group V

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Interestingly, the catalase activity decreased again in the neuroischemic ulcer group when compared to the neuropathic ulcer group. An increase in catalase activity was seen between the noninfected and infected groups.

**Glutathione peroxidase (GSH-Px).** Glutathione peroxidase activity in the homolysate of the various groups showed that the activity decreased in the diabetic subjects without a foot ulcer and progressively increased in the groups of diabetic subjects with a foot ulcer. Interestingly, there was a decrease in GSH-Px activity in the neuroischemic ulcer-infected group. There was a significant difference in GSH-Px activity between the nondiabetic and diabetic groups \((P < 0.001)\), with the exception of the neuroischemic ulcer-noninfected group \((P < 0.01)\).

**Glutathione-S-transferase.** Glutathione-S-transferase was significantly different between nondiabetic group I versus groups IV and V \((P < 0.0001)\), and between diabetic without a foot ulcer (group II) versus groups III, IV, V, VI. Group III was significantly different from groups II, IV, and V \((P < 0.05)\).

Lipid peroxidation in plasma, in terms of concentration of malondialdehyde assessed by thiobarbituric acid reactive substance (TBARS) assay, showed that the lipid peroxidation index increased progressively from the nondiabetic group I to the neuroischemic ulcer-infected group VI \((P < 0.0001)\). The neuroischemic ulcer-infected group had the maximum lipid peroxidation when compared to the other diabetic foot ulcer group.

Total antioxidant status assay in plasma revealed low antioxidant status in all the groups with respect to the nondiabetic group I \((P < 0.001)\). There was no significant inter-group differences among the diabetic patients \((P > 0.05)\).

**Discussion**

The results of the present study indicate that SOD, catalase, GSH-Px, and glutathione-S-transferase in the erythrocytes of the type 2 diabetic subjects are decreased when compared to nondiabetic controls due to increased free radical injury leading to increased lipid peroxidation. This study is unique because the diabetic subjects with a foot ulcer were classified as neuropathic foot ulcer and neuroischemic foot ulcer, and further subclassified as noninfected and infected for each category.

An ischemic index between blood pressure levels in the ankle and brachial arteries with Doppler technique is most commonly used despite the fact that some authors report an important limitation for the use of ankle pressure due to overestimation of the intra-arterial pressure due to calcification of the medial arterial wall. An index > 0.45 was necessary to heal minor amputations in some studies, but in another study an index > 0.6 was necessary to heal most foot ulcers. Our study subjects in the neuroischemic group had an ABI < 0.8 indicating vascular complication, which was in accordance with Vijay et al' who reported this value to be significant in peripheral vascular disease. An elevated vibration perception threshold (VPT) has been shown to be a sensitive indicator of neuropathy in adults. VPT values > 25 V of the diabetic subjects with foot ulcers justifies the classification of group III and IV as neuropathic. The neuroischemic group (groups V and VI) had both neuropathic and ischemic complications as visualized by the ABI and VPT measurements.

The markers of lipid peroxidation, plasma total antioxidant status, and specific antioxidant defence systems are normally used to evaluate oxidative stress. It has been reported that type 2 diabetic patients exhibited significantly higher plasma TBARS levels than type 1 diabetic patients. TBARS concentrations were higher in type 2 diabetic patients with HbA1c > 6.5%, and type 2 diabetic patients with angiopathy exhibited higher TBARS values than those without angiopathy. An enhanced lipid peroxidation has been shown in erythrocyte and leukocyte membranes of diabetic patients. Kesavulu et al reported that lipid peroxide concentrations were significantly increased in diabetic patients compared to control subjects, and that the diabetic patients with coronary heart disease (CHD) had higher levels of TBARS compared to diabetics without CHD with corresponding increased total cholesterol (hyperlipidemia).

The erythrocyte membrane TBARS and plasma TBARS in the present study was found to increase progressively from the nondiabetic control to the foot ulcer groups and reached a maximum in the neuroischemic ulcer-infected groups. Excessive lipid peroxidation in the plasma and cell membrane arise due to factors favoring the formation of reactive oxygen species. Moreover, the oxygenated free radicals and advanced protein glycation interplay because advanced glycation end products (AGEs) are able to produce free radicals that are involved in diabetic complications. The higher susceptibility of erythrocytes to lipid peroxidation arises out of its high levels of molecular oxygen and iron content.

Free radical scavenging enzymes such as catalase, superoxide dismutase, glutathione peroxidase, and glutathione S-transferase are the first line of cellular defense...
against oxidative injury. The equilibrium between the antioxidant enzymes is an important process for the effective removal of oxygen stress in intracellular organelles. The second line of defense consists of nonenzymatic scavengers, which scavenge residual free radicals escaping destruction caused by antioxidant enzymes.

Kedziora-Kornatowska et al. found that SOD and catalase activities were lower in erythrocytes of type 2 diabetic patients without nephropathy than in a control group and was the lowest in erythrocytes of type 2 diabetic patients with nephropathy. The decrease in activities of antioxidant systems in diabetes is linked to the progressive glycation of enzymatic proteins. Sekeroglu et al. have shown that dietary treatment will significantly decrease serum and erythrocyte lipid peroxidation and increase the erythrocyte antioxidant enzyme status of patients with type 2 diabetes.

Interestingly, in the present study the antioxidant enzymes increased with diabetic complications especially in the neuropathic ulcer-noninfected and infected groups and the neuroischemic ulcer noninfected and infected groups in comparison to diabetic subjects without foot ulceration. This could be due to an adaptive or compensatory mechanism to combat excessive oxidative damage due to neuropathy, ischemia, and ulceration of the foot. Sundaram et al. have reported on this adaptive mechanism. Hiramatsu et al. also have reported that oxygen radical scavenging enzymes can respond to conditions of increased oxidative stress with compensatory increases in activity. The catalase from bacteria breaks down the H$_2$O$_2$, so that H$_2$O$_2$ does not interact with the myeloperoxidase forming hypochlorite and the bacteria can live much longer, as the H$_2$O$_2$-myeloperoxidase halide killing system is the main oxygen dependent in the microbial killing mechanism. Neutrophils also have endogenous catalase, which contributes to the total catalase activity. Thus, the high levels of catalase in the neuropathic infected group may be due to the synergistic effect of catalase from erythrocytes, neutrophils, and some bacteria.

Another interesting observation in this study is the decrease in antioxidant enzymes present in the hemolysate of the patients in the neuroischemic ulcer noninfected group, which decreased even further in the neuroischemic ulcer-infected group in comparison with the neuropathic ulcer-infected group. This could be due to impaired blood flow characteristic of ischemia coupled with immunosuppressed activity leading to a decreased level of antioxidant enzymes not sufficient to neutralize the high levels of lipid peroxidation leading to greater oxidative stress.

Most studies conducted in type 1 or type 2 diabetic patients have shown a significant decrease in the plasma total antioxidant status. In accordance with the previous studies, the total antioxidant status in plasma in the present study decreased significantly in all of the diabetic groups, with and without an ulcer, when compared to the nondiabetic control. Once diabetes had set in there was significant decrease only in the neuroischemic foot ulcer groups, both infected and noninfected, in comparison to diabetic subjects—this may be due to poor superoxide dismutase activity and glutathione peroxidase in plasma. Albumin, the most abundant plasma protein and a powerful extracellular antioxidant that accounts for a significant part of total antioxidant status, was also found to be very low in the neuroischemic group when compared to nondiabetic and diabetic subjects.

**Conclusion**

A high level of lipid peroxidation accompanied by insufficient antioxidant enzymes and decreased total antioxidant status in plasma could contribute to the chronicity of an ulcer and an extended inflammatory reaction. Thus, oxidative stress may be regarded as an important factor for nonhealing diabetic foot ulcers among patients with type 2 diabetes.

**Acknowledgements**

The authors thank Dr. T. Ramasami, former Director, CLR for his encouragement and providing the necessary lab facilities to carry out this research and Ms. Priyanka Tilak, Research Associate, Diabetes Research Centre for rendering her valuable help in manuscript preparation. Dr. Vairam on thanks Dr. Annamma Philip, former Principal of Stella Maris College, and the University Grants Commission for providing the Faculty Development Programme Fellowship grant.

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