Erythrocyte superoxide dismutase, catalase activities and hydrogen peroxide induced lipid peroxidation in leprosy

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Accepted for publication 18 December 2007

Summary

Objectives To assess erythrocyte superoxide dismutase (SOD) and catalase (CAT) activities and hydrogen peroxide induced lipid peroxidation in leprosy.

Design One hundred leprosy patients and 50 normal healthy controls were studied for the parameters. The data was analysed by grouping the patients into Ridley–Jopling (RJ) types [Tuberculoid leprosy (TT, n = 22), Borderline tuberculoid leprosy (BT, n = 28), Borderline leprosy (BB, n = 13), Borderline lepromatous leprosy (BL, n = 16) and Lepromatous leprosy (LL, n = 21)] and into different levels of Bacteriological Index (BI) [bacteriologically negative (n = 32), BI = 0·1 – 1 (n = 22), BI = 1·1 – 2 (n = 16), BI = 2·1 – 3 (n = 14), BI = 3·1 – 4 (n = 10) and BI = 4·1 – 6 (n = 06)].

Results The induced peroxidation was significantly high and the enzyme activities were significantly low in leprosy (total patients) as compared to controls. A progressive increase in peroxidation was detected along the leprosy spectrum from TT to LL and the increase was significant in BB, BL and LL groups as compared to controls. Induced peroxidation in LL group as compared to TT, BT and BB and in the BL group as compared to TT and BT were significantly different. A concomitant progressive decline in enzyme activity was detected along the leprosy spectrum from TT to LL. The SOD activity in BB, BL and LL and the CAT activity in BL and LL were significantly low as compared to controls. SOD activity in BB, BL and LL groups as compared to TT and in the LL group as compared to BT were significantly different. A progressive trend of increasing peroxidation and decreasing SOD and CAT activity were also detected along the leprosy groups with advancing level of BI.

Induced peroxidation and SOD activity were significantly different in bacteriologically positive groups as compared to controls and in the BI levels 1·1–2, 2·1–3, 3·1–4 and 4·1–6 as compared to bacteriologically negative group. The peroxidation...
was significantly different in BI levels 2·1–3, 3·1–4 and 4·1–6 as compared to BI level 0·1–1. The CAT activity was significantly different in BI levels 2·1–3, 3·1–4 and 4·1–6 as compared to controls.

Conclusion The study findings suggest oxidative stressful state associated with reduced antioxidant defence potential in erythrocytes of leprosy patients. The study implicates association of erythrocyte oxidative stress with bacterial load and type of leprosy.

Introduction

The microbicidal ability of phagocytes through reactive oxygen species (ROS) such as hydrogen peroxide (H$_2$O$_2$), superoxide anion (O$_2^-$) and hydroxyl radical (OH$^-$) is a basic defence mechanism of the human host against microbial infection. Such ROS could also play a significant role in an infection with *Mycobacterium leprae* in leprosy. The ROS can diffuse from the site of generation and damage the structural and functional integrity of cells causing tissue damage. Thus, the oxidant force that kills pathogen is also cytotoxic to the host tissue. The possible role of ROS in the causation of renal damage in experimental leprosy has been investigated in mice infected with *M. leprae*. The reactive species like nitric oxide (NO$^-$) and peroxynitrite (ONOO$^-$) that are produced by macrophages in the skin lesions are shown to be involved in the nerve damage in borderline leprosy. The peroxidation of lipid component of the cells by ROS generate toxic species like lipid peroxides, lipid hydroperoxides and aldehyde breakdown products. Antioxidant systems exist in the body to protect against ROS toxicity. Superoxide dismutase (SOD), one of the important intracellular antioxidant enzymes present in aerobic cells has an antitoxic effect against superoxide anion. The presence of SOD in various fractions such as cytosol (Cupro zinc-SOD), mitochondria (Mangano SOD) and plasma (Cupro-SOD) enables SOD to eliminate superoxide radicals immediately and protect the cell from oxidative damage. Catalase, the haeme containing antioxidant enzyme protect cells from accumulation of H$_2$O$_2$ by decomposing it to H$_2$O and O$_2$. Oxidative stress ensues when the prooxidant-antioxidant balance gets perturbed in favour of prooxidants. Oxidative stress has been reported in leprosy. In the present study, the erythrocyte antioxidant enzyme activities of SOD and CAT were estimated to assess the effect of oxidant-antioxidant disturbances in red blood cells during leprosy. Alterations in the activity of these erythrocyte enzymes have been reported in some diseases involving oxidative stress. Hydrogen peroxide induced erythrocyte lipid peroxidation was also estimated in the study which could suggest the effectiveness of antioxidant protective mechanism in the cells. The erythrocyte changes of induced peroxidation and antioxidant enzymes may reveal whether leprosy is associated with erythrocyte oxidative stress.

Materials and Methods

One hundred newly diagnosed cases of leprosy who had not received MDT (Multidrug Therapy), belonging to both sexes (66 males and 34 females) and ranging in age from 20 to 45 years, were selected for the study. The leprosy patients recruited from Civil Hospital, Belgaum were used in the study. Leprosy patients with reactions, ulceration, and history of smoking, co-infections, diabetes mellitus, any other systemic diseases or health problems were excluded to rule out their possible influence on the study parameters. The diagnosis was
done on clinical grounds and slit-skin smear test. The patients were categorised according to Ridley–Jopling (RJ) scale\(^{17}\) into five subtypes as Tuberculoid leprosy (TT, \(n = 22\)), Borderline tuberculoid leprosy (BT, \(n = 28\)), Borderline leprosy (BB, \(n = 13\)), Borderline lepromatous leprosy (BL, \(n = 16\)) and Lepromatous leprosy (LL, \(n = 21\)). The patients were also grouped into different levels of Bacteriological index (BI) as bacteriologically negative (\(n = 32\)), BI = 0·1–1 (\(n = 22\)), BI = 1·1–2 (\(n = 16\)), BI = 2·1–3 (\(n = 14\)), BI = 3·1–4 (\(n = 10\)) and BI = 4·1–6 (\(n = 06\)). Fifty, age and sex matched healthy individuals served as controls. The study was approved by the Institutional ethical committee and written informed consent was obtained from the participants of the study.

Blood samples were collected in heparinised tubes and analysed for the study parameters. The erythrocyte lipid peroxidation product, Malondialdehyde (MDA) was estimated as Thiobarbituric acid reactive substances (TBARS) in Hydrogen peroxide (9·9 mM) stressed red blood cells.\(^{16}\) The TBARS level was expressed in nanomoles per gram of Haemoglobin (nmol/g Hb). The antioxidant enzyme activities of SOD and CAT were measured in appropriately diluted haemolysates. To prepare the haemolysate, RBCs were isolated by passing the blood through \(\alpha\)-cellulose and microcrystalline cellulose column.\(^{18}\) The cells were re-suspended in a stabilising medium (comprising of 2·7 mM EDTA and 0·7 mM \(\beta\)-mercaptoethanol) and lysed by freeze–thaw technique.\(^{19}\) The SOD activity was measured in chloroform-ethanol extract of haemolysate based on its ability to inhibit the auto-oxidation of epinephrine to adrenochrome at pH 10·2.\(^{20}\) The CAT activity was determined spectrophotometrically by noting the decline in optical density at 230 nm of decomposition of Hydrogen peroxide (H\(_2\)O\(_2\)).\(^{21}\) The specific activity of enzymes was expressed in International unit per gram of Haemoglobin (IU/g Hb). The Haemoglobin level was estimated by using cyanometh reagent.\(^{22}\)

**STATISTICAL ANALYSIS**

Unpaired ‘t’ test was used to compare the leprosy group (total patients) with controls. Analysis of Variance (ANOVA) followed by Bonferroni multiple comparison tests was done to compare the data in RJ types and in different BI levels. The differences at \(P < 0\cdot05\) were considered significant.

**Results**

Tables 1 and 2 shows erythrocyte induced lipid peroxidation and antioxidant enzyme activities of superoxide dismutase and catalase in RJ subtypes and different BI levels in leprosy respectively.

**ERYTHROCYTE INDUCED LIPID PEROXIDATION**

The erythrocyte induced peroxidation was significantly elevated in leprosy (total patients, \(n = 100\)) as compared to controls (\(n = 50\)). The mean level was lowest in TT, highest in LL and was progressively increased along the leprosy subtypes from TT to LL (Table 1). The proportion of cases having values higher than the control limit were 9%, 18%, 31%, 56% and 67% in TT, BT, BB, BL and LL groups respectively. The increase was significant in BB, BL and LL groups as compared to controls (Table 1). Within RJ types, the changes were significantly different in LL group as compared to TT, BT and BB. The changes were also
Table 2. Erythrocyte induced lipid peroxidation and antioxidant enzyme activities in different levels of BI grading in leprosy

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Total subjects</th>
<th>TBARS nmol/g Hb</th>
<th>SOD IU/g Hb</th>
<th>CAT IU/g Hb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>50</td>
<td>144.29 ± 34.79</td>
<td>739.74 ± 154.88</td>
<td>8.50 ± 2.22</td>
</tr>
<tr>
<td>Leprosy</td>
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<td>587.22 ± 190.96</td>
<td>7.22 ± 1.86</td>
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<tr>
<td>TT</td>
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<td>162.03 ± 31.07</td>
<td>704.25 ± 186.84</td>
<td>8.09 ± 1.80</td>
</tr>
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<td>BT</td>
<td>28</td>
<td>170.81 ± 36.45</td>
<td>645.83 ± 194.32</td>
<td>7.42 ± 1.92</td>
</tr>
<tr>
<td>BB</td>
<td>13</td>
<td>194.70 ± 51.68</td>
<td>527.86 ± 122.39</td>
<td>7.01 ± 1.88</td>
</tr>
<tr>
<td>BL</td>
<td>16</td>
<td>216.75 ± 42.67</td>
<td>503.96 ± 168.07</td>
<td>6.71 ± 1.80</td>
</tr>
<tr>
<td>LL</td>
<td>21</td>
<td>239.04 ± 53.49</td>
<td>486.66 ± 156.16</td>
<td>6.57 ± 1.65</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD.
*Significant change vs. controls.
† Significant change vs. BI = 0 group.
‡ Significant change vs. BI = 0.1–1 group.
§ Significant change vs. BI = 0.1–6 group.

Table 1. Erythrocyte induced lipid peroxidation and antioxidant enzyme activities in RJ leprosy types

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Values are expressed as Mean ± SD.
*Significant change vs. controls.
† Significant change vs. TT group.
‡ Significant change vs. BT group.
§ Significant change vs. BB group.
significant in BL group as compared to TT and BT (Table 1). A progressive increase in induced peroxidation was detected along the leprosy groups with advancing level of bacteriological grading. The increase was significant in bacteriologically positive groups as compared to controls. The changes were significant in groups with BI level 1·1–2, 2·1–3, 3·1–4 and 4·1–6 as compared to bacteriologically negative group. As compared to BI level 0·1–1, the changes were significant in BI levels 2·1–3, 3·1–4 and 4·1–6 (Table 2).

**ERYTHROCYTE SOD ACTIVITY**

The erythrocyte SOD activity was significantly reduced in leprosy (total patients, \( n = 100 \)) as compared to controls (\( n = 50 \)). The mean enzyme activity was highest in TT, lowest in LL and was progressively worsened along the leprosy spectrum from TT to LL (Table 1). The proportion of cases having activity lower than the control limit were respectively 9%, 18%, 38%, 50% and 48% in TT, BT, BB, BL and LL groups. The decrease was significant in BB, BL and LL groups as compared to controls (Table 1). Between RJ subtypes, significant differences were noted in BB, BL and LL groups as compared to TT and in the LL group as compared to BT (Table 1). A progressive decline in SOD activity was detected along the leprosy groups with advancing level of bacteriological grading. The decrease was significant in bacteriologically positive groups as compared to controls. The changes were significant in groups with BI level 1·1–2, 2·1–3, 3·1–4 and 4·1–6 as compared to bacteriologically negative group (Table 2).

**ERYTHROCYTE CAT ACTIVITY**

The erythrocyte CAT activity was significantly reduced in leprosy (total patients, \( n = 100 \)) as compared to controls (\( n = 50 \)). The mean enzyme activity was highest in TT, lowest in LL and was progressively declined along the leprosy spectrum from TT to LL (Table 1). The proportion of cases having CAT activity lower than the control limit were respectively 0%, 18%, 23%, 25% and 24% in TT, BT, BB, BL and LL groups. The decrease was significant in BL and LL groups as compared to controls (Table 1). A progressive decrease in CAT activity was detected along the leprosy groups with advancing level of bacteriological grading. The decrease was significant in bacteriologically positive groups as compared to controls. The changes were significant in groups with BI level 2·1–3, 3·1–4 and 4·1–6 as compared to bacteriologically negative group (Table 2). Within RJ subtypes or between different BI levels, the enzyme activity was not significantly different (Tables 1 and 2).

**Discussion**

The ROS generated in respiratory burst have been implicated as major mechanism of antimicrobial effector function in leprosy. Increased susceptibility of erythrocytes to lipid peroxidation as observed in the present study could be in relation to accelerated generation of ROS and their possible toxic effects on host tissue leading to depletion of the antioxidant protective mechanism. The progressive increase of induced peroxidation in leprosy groups with advancing level of bacteriological grading as well as along the RJ spectrum from TT to LL suggests its nonflexible association with bacterial load and type of leprosy.

There is progressive worsening in the status of SOD and CAT activity in leprosy groups with advancing bacteriological grading and along the leprosy spectrum from TT to LL.
A significant moderate negative correlation was observed between rise in induced peroxidation and fall of the enzyme activities [SOD: $r = -0.59$, ($P < 0.001$); CAT: $r = -0.57$, ($P < 0.001$)]. Bhadwat and Borade\textsuperscript{11} suggested a hypothesis that some component of \textit{M. leprae} might be down regulating the SOD gene in tissues including erythrocytes and macrophages. They noted a progressive decline in erythrocyte SOD along the leprosy spectrum from TT to LL. Diminution of SOD and CAT activity in leprosy could be in relation to increased exposure to oxidant environment that can destabilise RBC membrane by lipid peroxidation and cause significant leakage of these intracellular enzymes. There are evidences for plasma membrane becoming leaky owing to extensive damage by peroxidative attack which allows leakage of cytosolic enzymes from whole cells.\textsuperscript{24} Oxidative inactivation of the enzymes is also possible.\textsuperscript{25} There is also possibility that the host tissue provide biometals to the survival and intracellular multiplication of bacilli.\textsuperscript{26} Decreased availability of biometals could adversely affect the activity of these metalloenzymes.

In the present study, the TT and BT groups did not show significant decrease in enzyme activity. Bhadwat and Borade\textsuperscript{11} reported a progressive increase in mean serum MDA levels along RJ subtypes with minimum increase at tuberculoid side. Reddy \textit{et al.}\textsuperscript{12} noted a significant increase in serum MDA levels in multibacillary (MB) leprosy but the changes were nonsignificant in paucibacillary (PB) group. Therefore, the lipid peroxidation and its possible deleterious effects may not be that severe in TT and BT groups to cause significant diminution of enzyme activity. Further, due to low bacterial load in these groups, uptake of trace elements for bacterial utilization may be minimal to adversely affect the enzyme activity. Since the mean enzyme activity in these groups show a decrease it could also possible that variability between the patients makes it not possible to demonstrate statistical significance. A bigger sample size may be required to attempt to demonstrate this. The TT and BT groups also show increased induced peroxidation with values not reaching to significant levels; a bigger sample size may be required to attempt to demonstrate statistical significance.

Vitamin E functions as chain breaking antioxidant in lipid phase and first line of defence against peroxidation of membrane lipids. A significant and progressive fall in serum vitamin E levels from BT to LL end of the leprosy spectrum has been reported.\textsuperscript{27} This finding may have a bearing with our observation since there is a progressive increase in susceptibility of erythrocytes to peroxidation along the leprosy spectrum from TT to LL.

### Conclusion

The study findings suggest oxidative stressful state associated with decreased antioxidant defence potential in RBCs of leprosy patients. The findings indicate direct association of red cell oxidative stress with bacterial load and type of leprosy. The findings may provide a theoretical basis for development of novel therapeutic tools like antioxidant supplementation for management of persisting oxidative stress in leprosy.

### Acknowledgements

We are grateful to Dr. V. D. Patil, Principal, Jawaharlal Nehru Medical College, Belgaum 590 010, India for giving us permission and facilities to carry out this research work. We would like to thank Mr. M. D. Mallapur for statistical analysis.
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References