CORRELATION OF SERUM ZINC LEVEL WITH HISTOPATHOLOGICALLY DIAGNOSED DIFFERENT VARIANT OF LEPROSY

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ABSTRACT

Leprosy is a chronic infectious disease caused by \textit{Mycobacterium leprae}. Leprosy involves multiple organs (skin, nerves, liver and kidney), that leads to a wide range of biochemical as well as immunological changes in the body including trace elements such as zinc, cooper and magnesium. Low serum zinc concentrations are found in various physiological as well as pathological conditions such as in Pregnancy and lactation, amongst pure alcoholics, people suffering from gastrointestinal and liver disease and in cases of sickle cell disease. Low serum zinc level in leprosy patients also is shown by various workers. Present study included 63 newly diagnosed leprosy cases compared to matched controls. This study was undertaken to know the histopathological features of leprosy in skin biopsies of newly diagnosed patients, to categorize them into various subtypes based on histomorphological features, to correlate with clinical presentations wherever possible and to investigate the level of serum zinc in leprosy. In the present study serum zinc level was found to be significantly reduced (P value < 0.05) i.e. 0.001 in all the 63 patients of leprosy.

KEY WORDS : Zinc, Leprosy, Fite- Faraco stain, TNF-\textalpha, IL-2

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INTRODUCTION

Leprosy is a chronic granulomatous disease caused by Mycobacterium leprae, principally affecting peripheral nerves and skin. Mycobacterium leprae, the causative agent of leprosy, was discovered by G. H. Amiauer Hansen in Norway in 1873, making it the first bacterium to be identified as causing disease in humans. Hansen observed a number of non-refractile small rods in unstained tissue sections. In 1879 he was able to stain these organisms with Ziehl's method and the similarities with Koch's bacillus (Mycobacterium tuberculosis) were noted. There were three significant differences between these organisms: (1) the rods in the leprosy lesions were extremely numerous (2) they formed characteristic intracellular collections (globbi) and (3) the rods had a variety of shapes with branching and swelling. These differences suggested that leprosy was caused by an organism related but distinct from Mycobacterium tuberculosis. The spectrum of its clinical manifestation is broad. At one end is Tuberculoid (TT) leprosy, in which the clinical manifestations are localized to a single area of skin and the associated nerve supply. At the opposite end is Lepromatous leprosy (LL) in which there is massive infection of the dermis by Mycobacterium leprae as well as involvement of the nerves, nasopharynx, testes and lymphoreticular system. In between these polar forms are the intermediate forms of leprosy which display mixtures of the clinical, histopathologic and immunologic features typical of TT or LL disease. Zinc is an essential mineral. It is required for the catalytic activity of approximately 100 enzymes and it plays a role in immune function, protein synthesis, wound healing, DNA synthesis, and cell division. A daily intake of zinc is required to maintain a steady state because the body has no specialized zinc storage system. Zinc is very essential mineral for the expression of antioxidant enzymes SOD, low zinc level indicating the impairment of antioxidant mechanism leading to free radicals generation. Leprosy patients were classified according to the Ridle and Jopling classification and divided into two main groups as follows, Tuberculoid, which consisted mainly of borderline tuberculoid patients and Lepromatous which consisted of borderline lepromatous and true lepromatous patients. The lepromatous group was found to have significantly lower serum levels of zinc and elevated levels of copper. The mechanism due to a redistribution of these metals from the blood to various tissues; brought about by the release of leucocyte endogenous mediators by continuing phagocytosis of tissue macrophages in the lepromatous group of patients. Serum zinc levels were significantly decreased in lepromatous leprosy patients whereas magnesium remains normal. Serum zinc level were significantly low due to zinc deficiency may be because of the suppression of cell mediated immunity, zinc is required for normal keratinisation of skin in higher animals. Leprosy bacilli required zinc for their own metabolism hence hypozincamia observed due to bacterial load and lepromatous leprosy. Lepromatous leprosy patients showed spontaneous apoptosis when cultured in the absence of mitogen for 24 hrs, which was inhibited by antitumor necrosis factor-a (TNF-α) antibodies. Apoptosis was also inhibited by ionimycin and zinc, which also increased IL-2 and decreased TNF-α production. The increase in IL-2 production suggested a mechanism whereby dietary supplements with zinc might alter the cell mediated immunity response in lepromatous leprosy patients.

MATERIALS AND METHODS

The study was carried out in Tertiary Health Care Centre, Bhopal. This study was undertaken over a period of 1 year from June 2010 to June 2011. It includes 63 newly diagnosed cases of leprosy in the age group of 10-60 and 71 age, gender and socio-economic status matched controls.

Inclusion Criteria

• Newly diagnosed patients of leprosy (all the age group and of both sexes) on the basis of clinical presentation.
• **Leprosy** patients with past history of treatment presenting with reactional state.

**Exclusion Criteria**
• **Leprosy** patients suffering from diabetes mellitus, hepatitis, nephritis and other systemic disease or chronic illness like *Tuberculosis*.
• **Leprosy** patients undergoing treatment.
• Old treated cases without *Lepra* reaction.

**Control Group**
Blood sample from normal subjects coming to Blood Bank of Tertiary Health Care Centre, Bhopal for blood donation in the age group of 18 to 60 years were collected and classified in each decade equally for males and females for their serum zinc level. An informed consent was taken from the donors before collecting blood sample for serum zinc level.

**Collection of sample**

1. **Skin Biopsy**
Skin biopsies received in Tertiary Health Care Centre were fixed in 10% formalin. Paraffin blocks were made and sections were cut at 4-5 microns thickness. The slides were stained by Haematoxylin and Eosin stain for morphological analysis and Fite-Faraco stain for identifying the bacilli.

2. **Blood sample**
5 ml of venous blood of same patient was collected in EDTA vial and plain vial from the antecubital vein under aseptic conditions using sterile disposable syringe. Sample was centrifuged, serum separated and estimation of serum zinc level was done. Other laboratory investigations performed were complete hemogram, random blood sugar, blood urea and creatinine and total and direct bilirubin.

3. **Control blood sample**
2 ml of venous blood was collected in plain vial from the antecubital vein under aseptic condition using sterile disposable syringe. Sample is centrifuged, serum separated and estimation of serum zinc level was done. Serum, zinc level is estimated by Colorimetric Method using Semi-auto analyzer MICROLAB 200 from MERCK (Germany) and Zinc kit manufactured by Coral-Tulip by CREST ECOSYSTEMS (Goa, India).

**RESULTS**

<table>
<thead>
<tr>
<th>Type of Leprosy</th>
<th>TT</th>
<th>BT</th>
<th>BB</th>
<th>BL</th>
<th>LL</th>
<th>IL</th>
<th>PN</th>
<th>ENL</th>
<th>Total</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age Group</strong></td>
<td><strong>M</strong></td>
<td><strong>F</strong></td>
<td><strong>M</strong></td>
<td><strong>F</strong></td>
<td><strong>M</strong></td>
<td><strong>F</strong></td>
<td><strong>M</strong></td>
<td><strong>F</strong></td>
<td><strong>M</strong></td>
<td><strong>F</strong></td>
</tr>
<tr>
<td>(in yrs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10-19</td>
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<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>20-29</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>30-39</td>
<td>0</td>
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<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>40-49</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>50-59</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>&gt; 60</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1</td>
<td>2</td>
<td>10</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

**Table 1**

<table>
<thead>
<tr>
<th>Age and sex distribution of total cases</th>
<th>Total</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>F</td>
<td>M &amp; F</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>10</td>
</tr>
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</table>

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Table 2

<table>
<thead>
<tr>
<th>Types</th>
<th>TT</th>
<th>BT</th>
<th>BB</th>
<th>BL</th>
<th>LL</th>
<th>IL</th>
<th>ENL</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Cases</td>
<td>3</td>
<td>17</td>
<td>2</td>
<td>14</td>
<td>6</td>
<td>17</td>
<td>2</td>
<td>61</td>
</tr>
<tr>
<td>%</td>
<td>4.76</td>
<td>26.98</td>
<td>3.17</td>
<td>22.22</td>
<td>9.52</td>
<td>26.98</td>
<td>3.17</td>
<td>100</td>
</tr>
</tbody>
</table>

Distribution of cases according to Histopathological types of leprosy
Table 3

Statistical analysis of serum zinc level in different subtypes of leprosy

<table>
<thead>
<tr>
<th>Subtypes</th>
<th>Range</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>t-test (Score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control  (71)</td>
<td>83.0-127.8</td>
<td>110.58</td>
<td>10.80</td>
<td>-</td>
</tr>
<tr>
<td>IL (17)</td>
<td>42.4-108.3</td>
<td>93.14</td>
<td>14.11</td>
<td>5.54</td>
</tr>
<tr>
<td>TT (3)</td>
<td>89.0-93.3</td>
<td>91.26</td>
<td>2.15</td>
<td>9.9</td>
</tr>
<tr>
<td>BB (2)</td>
<td>78.0-103</td>
<td>90.5</td>
<td>17.67</td>
<td>13.11</td>
</tr>
<tr>
<td>PN (2)</td>
<td>72.5-98.25</td>
<td>91.26</td>
<td>2.15</td>
<td>9.9</td>
</tr>
<tr>
<td>BT (17)</td>
<td>60.0-88.7</td>
<td>91.26</td>
<td>2.15</td>
<td>9.9</td>
</tr>
<tr>
<td>BL (14)</td>
<td>42.4-90.2</td>
<td>67.88</td>
<td>11.96</td>
<td>2.5</td>
</tr>
<tr>
<td>LL (6)</td>
<td>49.5-62.0</td>
<td>57.08</td>
<td>4.19</td>
<td>3.13</td>
</tr>
<tr>
<td>ENL (2)</td>
<td>52.0-55.26</td>
<td>53.63</td>
<td>2.30</td>
<td>7.34</td>
</tr>
</tbody>
</table>

DISCUSSION

This is a histopathology based clinicopathological correlation study of different subtypes of Leprosy along with estimation of serum Zinc level among the different subtypes.

Comparison of age distribution by different workers

Of the 63 patients in the present study, the patients with age group of 20-29 years (3rd decade) were affected most and patients below 9 years were affected least. Similar observations were made by other authors also.16,17,18,19

Comparison of histopathological type by different workers

The most commonly encountered type of leprosy was IL and BT 17 biopsies (26.98% each), second common type was BL 14 biopsies (22.22%), BB 3 biopsies (3.17%) was the least encountered type. Borderline group constituted the major spectrum 33 biopsies (53.5%), which included BT, BB, BL, similar to findings of other authors.19,20,21,22,23

Comparison of serum zinc level by different workers

Pure tuberculoid and Indeterminate leprosy group showed minimum decrease and pure lepromatous and Erythema nodosum leprosum group showed maximum decrease.
in Serum Zinc level i.e. leprosy patients with increased bacterial load have decreased serum zinc levels. Results obtained from our study of serum zinc are in agreement with those of earlier researchers. Mathur NK, et al (1984b) tried oral zinc (220 mg of dietary supplemental zinc sulfate) in 15 cases of multibacillary leprosy as an immunostimulant in addition to conventional anti-leprosy drugs. Results were compared with those in ten similar cases treated with dapsone alone. The study was continued for 18 months. Cases treated with zinc showed faster clinical improvement, regrowth of eyebrows, rapid fall in bacterial index and in the bacterial index in the granuloma, early and greater influx in lymphocytes in the granuloma and neovascularisation upgrading occurred in 6 out of 15 patients taking zinc but in only 1 out of 10 patients in the control group. Five out of six patients who showed upgrading in the treated group became lepromin positive. Only one patient showed lepromin conversion in control group.

CONCLUSION

Zinc is an important antioxidant, immunostimulant trace elements with a great nutritional and metabolic value. In the present study fall in serum zinc level was significant and more pronounced. This suggests that there could be a correlation of serum zinc levels and the bacillary load. Sher et al suggested that mechanism of alteration in trace element levels could be due to redistribution of these metals from blood to various tissues, brought about by the release of leucocyte endogenous mediators by continuing phagocytoses by macrophages in leprosy patients. Leprosy patient treated with oral zinc in addition to conventional anti-leprosy drugs showed faster clinical improvement, regrowth of eyebrows, rapid fall in bacterial index and in the bacterial index of granuloma; early and greater influx of lymphocytes in the granuloma and neovascularization. Similar results were reported by other author.

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REFERENCES