Cholinesterase in Leprosy Nerves: A Preliminary Report

To the Editor:

Cholinesterases are ubiquitous enzymes which play a role in cholinergic transmission, neurogenesis, and are implicated in neurodegeneration and dementia (13). Cholinesterases of vertebrates fall into two categories, Acetylcholinesterase (AChE) and Butrylcholinesterase (BChE). These two enzymes differ in substrate specificities and inhibition by selective inhibitors. AChE preferentially hydrolyzes acetylcholine or acetyl-beta-methyl choline while BChE hydrolyzes butyrylcholine preferentially (4.10).

Work on AChE and BChE in leprosy skin (1,3), muscle (2-5), and serum (13,14) have been reported. However, to the best of our knowledge, there is no reported study on cholinesterases in the peripheral nerves of leprosy where the damage is pronounced. Hence, the present study was carried out to assess cholinesterase levels in leprosy nerve.

Leprosy nerves (tibial) were obtained from 12 patients who underwent lower limb amputations due to early squamous cell carcinoma of the foot. Normal peripheral nerves (tibial and sural) were obtained from amputated limbs where lower limb disarticulation was carried out in patients with osteogenic carcinoma without secondaries or soft tissue sarcomas. All the patients had long-standing leprosy ranging from 10-12 years and their clinical classification included lepromatous, borderline and tuberculoid leprosy. Nerves were traced within one hour of amputation and stored at -20°C until they were processed (usually within a month).

The processing of nerves was carried out at 4°C. The nerve sample was finely minced and homogenized in 20 mM Tris HCl buffer pH 7.6 (10 ml/gram of tissue) containing 1 mg% benzamidine hydrochloride, 200 µM phenylmethyl sulfonyl fluoride and 0.1% Triton-x-100 v/v. Phenylmethyl sulfonyl fluoride, benzamidine hydrochloride, Triton-x-100 and trishydroxymethylaminomethane were obtained from the Sigma-Aldrich Chemical Company, St. Louis, Missouri, U.S.A. The homogenate was centrifuged at 1000 x g for 20 min and the supernatant was used for cholinesterase studies.

Protein was estimated according to Lowry, et al. (6). AChE and BChE in 1000 x g supernatants were assayed by the method of Ellman, et al. (7) using acetylthiocholine and butyrylthiocholine, respectively, as substrates. Bis-(4-allyl dimethyl ammonium phenyl)-pentane-3-one dibromide (BW284C51), a selective inhibitor of AChE was included when BChE was estimated and tetraisopropyl pyrophosphoramide (iso-OMPA) a selective inhibitor for BChE was included when AChE was estimated (4). The reaction mixture consisted of 50-100 µg protein (which was in the linear range of enzyme activity), 100 mM phosphate buffer, pH 8 containing 3 mM of thiocholine substrate, 10 µM of selective inhibitor, 2 mM of either iso-OMPA or BW284C51 and 2 mM 5,5-dithiobis (2-nitrobenzoic acid) in a total volume of 500 µl. The reaction mixture was incubated at 37°C for 20 min and was measured spectrophotometrically at 412 nm. One unit of cholinesterase activity is the change in absorbance at 412 nm of 1 OD/min/mg protein under standard assay conditions. Statistical analysis was carried out using Students t test.

The mean BChE level in leprosy nerves was 17.25 U/mg (S.D. 7.37) and 8.35 U/mg (SD 6.21) in the normal nerves. The mean AChE level in leprosy nerves was 16.71 U/mg (S.D. 6.81) and 21.87 U/mg (S.D. 5.50) in the normal nerves (The Table). AChE was not significantly altered, but BChE activity was significantly elevated in leprosy nerves when compared to normal nerves (p<0.05).

Earlier studies on cholinesterases in serum have suggested its involvement in pathogenesis and also in genetic susceptibility to leprosy (13,14). However, the evidences were inconclusive. The report on leprosy skin cholinesterase had shown that in early lepromatous leprosy the Meissner’s corpuscles appeared to be almost normal and the cholinesterase reaction was not diminished, but, in advanced cases, cholinesterases was reduced both in the corpuscles and in the papillary ridges. In tuberculoid leprosy the papillary ridges were compressed, with resulting destruction of Meissner’s corpuscles. Cholinesterase activity was completely absent in all
### THE TABLE. Cholinesterases in peripheral nerve.

<table>
<thead>
<tr>
<th>Specimen no.</th>
<th>Classification</th>
<th>BuChE U/mg</th>
<th>AChE U/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>10.39</td>
<td>18.8</td>
</tr>
<tr>
<td>2</td>
<td>Normal</td>
<td>5.14</td>
<td>27.82</td>
</tr>
<tr>
<td>3</td>
<td>Normal</td>
<td>1.50</td>
<td>13.63</td>
</tr>
<tr>
<td>4</td>
<td>Normal</td>
<td>7.31</td>
<td>28.39</td>
</tr>
<tr>
<td>5</td>
<td>Normal</td>
<td>20.90</td>
<td>24.90</td>
</tr>
<tr>
<td>6</td>
<td>Normal</td>
<td>4.90</td>
<td>17.72</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>8.35 ± 6.21</td>
<td>21.87 ± 5.50</td>
<td></td>
</tr>
</tbody>
</table>

1 LL
2 NA 9.63 25.04
3 NA 9.80 16.18
4 BL 11.05 11.72
5 BL 10.92 23.91
6 BT 23.90 13.69
7 NA 28.80 13.99
8 LL 28.80 28.79
9 LL 23.10 24.00
10 LL 19.62 9.25
11 BT 13.14 16.56
12 LL 11.25 11.72

Mean ± S.D. 17.25 ± 7.37 16.71 ± 6.81

t value Significant Not significant

*: Not available.

parts of the skin. This study concluded that cholinesterase activity was found altered wherever the nerve endings were damaged (5). Extensive studies in Alzheimer’s patients brains have shown that neurofibrillary tangles and amyloid plaques express AChE and BChE (4), and when cholinesterase inhibitors are used, there is an improvement of clinical symptoms (5). BChE is known to possess non-cholinergic functions, such as peptidase activity of growth promotion and morphogenesis and, hence, could be important in the process of nerve damage (16).

Neuritis in leprosy is usually a subacute degenerating neuropathy and a recurring event involving cutaneous and nerve trunks. In the present study, peripheral nerves were from patients with long-term leprosy neuropathy where extensive nerve degeneration and regeneration are observed (4). Histopathological examination by hematoxylin and eosin (H&E), Fite, Solochrome Cyanine & Glees Marshland stain was possible in 6 of the leprosy nerves. All of them revealed end stage neuropathy with extensive demyelination and axonal damage (results not presented). In conclusion, this report shows that BChE activity is significantly elevated in leprosy nerves. Further studies on these lines with histopathological correlation, localization by immuno-histochemistry may give more insight on the role of cholinesterases in nerve damage.

**Acknowledgment.** LMS is grateful to the Council of Scientific and Industrial Research (CSIR), New Delhi for the award of Senior Research Associateship and to LEPRO for support.

—Lavanya M. Suneetha, M.Sc., Ph.D.  
C. D. Bahumathy, M.Sc., Ph.D.

Christian Medical College & Hospital  
Vellore, Tamilnadu 632 004, India

—Samuel S. Solomon, M.B.B.S., M.S.  
Sujai Suneetha, M.B.B.S., D.C.P., Ph.D.

Schieffelin Leprosy Research & Training Centre  
Karigiri, Vellore District, Tamilnadu 632 106, India

—Ravi J. Korula, M.B.B.S., M.S.  
A. S. Balasubramanian, M.Sc., Ph.D.

Christian Medical College & Hospital  
Vellore, Tamilnadu 632 004, India

—Lavanya M. Suneetha, M.Sc., Ph.D.  
Sujai Suneetha, M.B.B.S., D.C.P., Ph.D.

Presently: LEPRO India, Blue Peter  
Research Centre, Cherlapally  
Hyderabad - 501 301, AP, India

Corresponding author: Dr. Lavanya M. Suneetha, Group Leader, Molecular Biology & HIV, LEPRO India, Blue Peter Research Centre, Cherlapally, Hyderabad - 501 301, AP, India. E-mail: bprc@hd2.dot.net.in

**REFERENCES**


4. **JAYANTI, L. D., BALASUBRAMANIAN, N. and BALASUBRAMANIAN, A. S.** Cholinesterases exhibiting arylaclylamide activity in human amnionic fluid:

Hansen's Disease Mimicking Palmoplantar Keratoderma

TO THE EDITOR:

Leprosy is a chronic, infectious disease caused by Mycobacterium leprae. Widespread skin lesions mainly involving the extremities, abdomen and back are seen in borderline lepromatous leprosy and lepromatous leprosy. The axilla, groin, perineum and a narrow band of skin over the lumbar-sacral region of the back are considered to be "immune zones" because of their relative warmth (1). The palms and soles are included in immune or relatively-immune zones of leprosy by some authors because of the infrequent occurrence of lesions over these areas. We are reporting a case of borderline lepromatous leprosy that had bilateral lesions over the palms and soles mimicking keratoderma.

CASE REPORT

A twelve-year-old Hindu male presented with hyperkeratotic, fissured lesions over both the palms and soles. The patient also had bilaterally symmetrical well-defined plaques showing fine scaling over the extensor aspects of his arms, legs, thighs, chest, back, and he had a few lesions over his face resembling psoriasis. On examination, the lesions over his palms and soles were bilaterally symmetrical, thickened and fissured, extending as erythematous plaques over both of his forearms and his left foot. Touch, pain and temperature sensations were intact in most of the lesions, but small areas of sensory loss were present over his palms and soles. The ulnar and common peroneal nerves on both sides were thickened and tender. The auspitz sign was negative, and his nails were normal. Histopathological examination of the skin biopsy taken from scaly plaque over his right arm was consistent with borderline lepromatous leprosy. Slit-skin smears prepared from four sites (the earlobe, eyebrow, a lesion over his back, and a normal skin area) revealed numerous acid-fast bacilli with an average bacterial index of 4+.

He was put on multidrug therapy with dapsone, rifampin and clofazimine as recommended by WHO for multibacillary leprosy. Emollients were prescribed for the lesions on his palms and soles. After four weeks of treatment, the lesions started regressing except for palmoplantar lesions which regressed very slightly. Since the lesions over his palms and soles were atypical of Hansen's disease and not responding to