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STUDIES ON SURAL NERVE BIOPSIES IN ENDEMIC SKELETAL FLUOROSIS

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SUMMARY: Sural nerve biopsies from 13 patients with radiologically confirmed skeletal fluorosis were studied for myelinated fibre densities, frequency distribution of their diameters, and single teased nerve fibre preparations. It was observed that most of the biopsies showed a marked reduction in myelinated fibre densities with more than half of them involving the smaller fibres of less than 7 μm diameter. Teased fibre measurements of internodal lengths and internodal diameters point to myelinated fibre dropout being due to axonal degeneration with secondary demyelination. The selective loss of small fibres is unlikely to be due to an entrapment neuropathy alone, and possibility of primary toxic injury needs to be considered.

Key words: Fluorosis; Neuropathy; Sural nerve.

Introduction

Neurological changes associated with skeletal fluorosis have been attributed in part to compression radiculomyelopathy (1). There are also reports suggesting anterior horn cell involvement in fluorosis (2,3). Non-skeletal toxic effects in experimental fluorosis in various organs, especially skeletal muscle and spinal cord, have also been studied (4,5). Studies on peripheral nerves in patients with established skeletal fluorosis hardly exist, except one electrophysiological study (6) which confirmed the myopathy being secondary to compression myeloradiculopathy. We therefore felt it necessary to study the peripheral nerve (sural nerve) pathology in patients with radiologically confirmed skeletal fluorosis.

Materials and Methods

Thirteen patients (eleven men and two women) between 20 and 60 years of age, from endemic districts of Andhra Pradesh in Southern India, were included in this study. Their urinary fluoride levels ranged from 3 ppm to 7 ppm. All except one male patient had clinical evidence of cervical cord compression at about C_2-C_4 levels. Lower limbs were apparently unaffected. Minimal and patchy sensory deficits were observed in a few, and the levels corresponded to the levels of cervical cord compression. There was no evidence of pain or muscle weakness in the upper limbs. There was as such no evidence of peripheral nerve entrapment. Electrophysiological aspects unfortunately could not be studied in these patients.

An informed consent was obtained from these patients after the objectives of the study were explained. A sural nerve biopsy at level of the ankle was performed under local xylocaine infiltration anesthesia. There were no significant post biopsy sequelae apart from numbness over the little toe.

The excised nerve was processed in three different ways for study:

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1. Myelinated fibre densities and diameter frequency distribution.
2. Histological changes.
3. Internodal lengths and diameters on teased fibre preparations.

The methodologies used were standard established methods for studying myelinated fibres on paraffin section (7, 8) and single nerve fibre preparations (9). Routine histological stains like Haematoxylin and Eosin, Masson's trichrome, and Holme's stain for axons were used to study qualitative changes (10). Myelinated fibre densities and diameters were measured manually on photomicrographs. Areas were estimated by planimetry while internodal measurements were done with an ocular screw micrometer and mechanical stage vernier. Fibre densities were expressed as fibres per sq. mm cross-sectional endoneural area. Myelinated fibres were classed according to their size as < 1 µm, < 2 µm, *etc* as diameter measurements were made based on the concept of lesser fibre diameter (11). These were grouped into two categories: those with diameters less than 7 µm (small fibres), and those with diameters of 7 µm and more (large fibres). Functionally, these correspond to slow conducting and fast conducting myelinated fibres, respectively (12). The correlation coefficients (r values) were determined to establish the relationship (linear) between internodal lengths and internodal diameters (in 7 subjects).

Results

Myelinated fibre densities were compared with normal values for sural nerve reported in the literature (7), which were also determined by identical methodology (Table 1). This procedure was unavoidable because we could not obtain similar data owing to non-availability of suitable autopsy material.

TABLE 1. Myelinated fibre densities in normal sural nerves (7)

Age	Density in thousands/sq.mm Mean ± SD
17 to 39	6.13 ± 1.11
40 to 59	5.78 ± 0.90
60 to 80	4.78 ± 1.08

O'Sullivan and Swallow, 1968 (7)

Values of myelinated fibre densities in sural nerves as shown in Table 2 ranged from 2,494 to 5,433 fibres per sq. mm endoneural area, which were significantly lower (-2 SD) in 10 out of the 13 cases compared to normal values for age (Table 1). The diameter of these fibres, which normally show a bimodal distribution with equal number of small (<7 µm) and large (>7 µm) fibres, exhibited an unequal distribution with larger fibres constituting 60% or more in six out of thirteen nerves studied (Table 2 and Figure 1). This predominance was found to be statistically significant ($P<0.05$) using a students 't' test for differences in proportion. Only one subject, however, showed predominance of small myelinated fibres. Clusters of axons suggesting regenerating axonal clusters were seen in all cases.

Table 2. Myelinated fibre densities and diameters in sural nerve biopsies from cases of fluorosis.

Patient	Age in years	Mean fibre density**	C.V.	Sample size ***	Myelinated fibres : Percentage	
					< 7 µm	> 7 µm
MRIH	40	3451	7.40	433	79.5	20.5
MYSH	45	3427	8.44	518	51.7	48.3
SKMO	56	3422	0.99	397	35.3	64.7
JRMNA	50	2795	2.10	331	33.8	66.2
KSTR	50	4500	3.38	497	47.9	52.1
NRSR	20	3469	9.31	354	37.1	62.9
NRSH	45	2494	23.83	394	43.4	56.6
NRSL	60	3076	22.59	400	34.7	65.3
PPNA	38	2867	22.06	553	46.6	53.4
SBIH	57	5433	3.85	784	49.7	50.3
RMIH	35	3699	9.22	337	23.7	76.3
MLMA*	50	3461	19.88	471	44.4	55.6
MLMA*	40	4168	16.72	644	40.0	60.0

* Female C.V. Coefficient of variation
** Mean of three bundles
*** Number of fibres measured for diameters

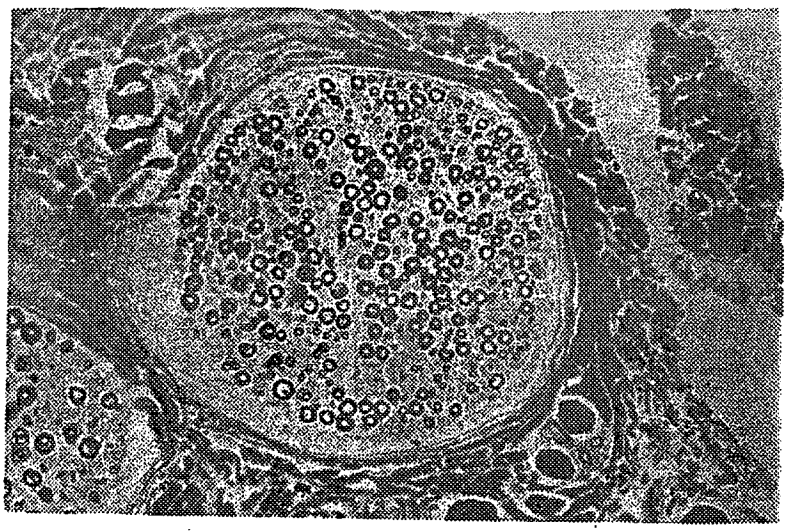

FIGURE 1. Photomicrograph of a sural nerve fascicle showing decreased density of myelinated fibres with prominent large diameter fibres. Kulthchitsky's Haematoxylin x 100.

Teased fibres

The correlation coefficient (r), which is an index of myelin/axon damage in single nerve fibre preparations, ranged between 0.35 and 0.54 which is well below the normal values for any age reported in the literature (9), and indicates damage and repair of myelin and axons over time. There was, in addition, a greater scatter of the individual values resulting in a loss of the linear relationship between internodal lengths and corresponding diameters. Evidence of paranodal demyelination and myelin wrinkling suggestive of axonal atrophy was also evident (Figure 2).

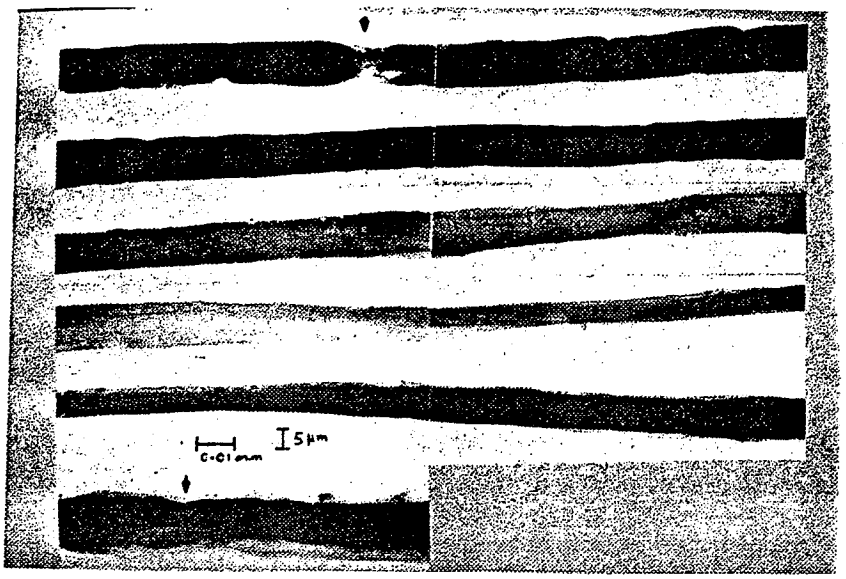

FIGURE 2. Single teased nerve fibre preparation. Arrows indicate Nodes of Ranvier. There is evidence of paranodal demyelination, wrinkling and demyelination in mid-segment.

Discussion

Myelinated fibre densities in the sural nerves were very much reduced, indicating a dropout, probably due to axonal degeneration or demyelination or both. It is, however, unusual that there was a relative sparing of larger fibres, which is not the case in most compression neuropathies.

It is also possible that due to a partial loss of fibres, especially the smaller and slow conducting myelinated fibres with intact larger and fast conducting fibres, no significant reduction in conduction velocities could be observed in earlier electrophysiological studies (6). The present data suggest a fairly selective damage to small myelinated fibres or their neurones due to fluorosis rather than compression neuropathy alone. Toxic neuronal injury due to excessive ingestion of fluoride needs to be considered.

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