

## S-100 PROTEIN IN THE DIAGNOSIS OF TUBERCULOID/ BORDERLINE TUBERCULOID LEPROSY

Abdur Rauf Khan, MD, FCAP

**Background:** A definitive diagnosis of tuberculoid and borderline tuberculoid leprosy is based on a demonstration of either acid-fast bacilli or nerve elements within the granulomas. On routine hematoxylin and eosin stains, the nerve fibers are not easily identifiable. In this study, we used S-100 protein to highlight the nerve elements and to count their numbers in leprosy and non-leprosy granulomas.

**Materials and Methods:** Skin biopsy specimens from 15 cases of tuberculoid/borderline tuberculoid leprosy and 14 cases belonging to other granulomatous diseases of the skin were stained with S-100 protein. The surface area of all the biopsies was calculated and the number of nerve bundles stained with S-100 protein were counted in each specimen.

**Results:** The nerve bundles were 15 per cm<sup>2</sup> in leprosy cases, and 9.2 per cm<sup>2</sup> in non-leprosy cases. In addition, the leprosy cases showed longer nerve twigs that were perpendicularly oriented to the skin surface.

**Conclusion:** Immunostaining with S-100 facilitated detection of nerve elements in tuberculoid/borderline tuberculoid leprosy. Also, an increased number of nerve elements were found in leprosy granulomas when compared with non-leprosy granulomas ( $P < 0.05$ ).

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**Key Words:** Skin biopsy, S-100 protein, leprosy diagnosis, skin diseases, granuloma, leprosy pathology.

The hallmark of tuberculoid leprosy is the presence of nerve fibers within the granuloma. Histopathological diagnosis of tuberculoid leprosy is difficult because leprosy granulomas cannot be distinguished from other granulomas that are seen in such conditions as leishmaniasis, sarcoidosis and tuberculosis. Moreover, the acid-fast bacilli in leprosy granulomas are scanty and usually fragmented. A definitive diagnosis of leprosy will prove difficult without any nerve element present in the granulomas. Emphasis is placed on searching for residual nerve elements in acid-fast bacilli-negative sections, because this increases the certainty level of the diagnosis.<sup>1</sup> On routine hematoxylin and eosin stain, the nerve fibers do not stand out well from the background, therefore, different techniques, such as plastic embedding, osmium-hematoxylin staining on paraffin-embedded sections,<sup>2</sup> or antibody to S-100 protein,<sup>3,4</sup> are used in order to make the nerve easily identifiable. The other approach for the diagnosis of tuberculoid leprosy is to determine mycobacterial antigens in the nerves by immunochemical method,<sup>5</sup> or by polymerase chain reaction.<sup>6</sup> In this study we have used antibody to S-100 protein to highlight the

nerve elements, and to count their numbers in leprosy and non-leprosy granulomas.

### Materials and Methods

Skin biopsy specimens from 15 cases of tuberculoid or borderline tuberculoid leprosy for which glass slides and paraffin blocks were available were identified from the surgical pathology files and records of the Asir Central Hospital. In addition to routine hematoxylin-eosin stains, all these specimens had Fite stains which had demonstrated some fragmented or beaded acid-fast bacilli to confirm the diagnosis of leprosy. Paraffin sections were cut from the blocks, and the slides were then stained with antibody to S-100 antigen, using commercially available kits (Omnitag Streptavidin/Biotin immunoperoxidase detection system, Lipshaw, Immunon, Pittsburgh, PA). As a control, slides from 14 patients belonging to other granulomatous diseases of the skin, such as sarcoidosis, leishmaniasis, fungal infection or tuberculosis, were also

TABLE 1. Comparison of leprosy and non-leprosy granulomas by using antibody to S-100 protein.

	Surface area of biopsy (mean±SD)	Average no. of nerve elements per biopsy	No. of nerve elements per cm <sup>2</sup>
Leprosy	0.45±0.3 cm <sup>2</sup>	6.9	15
Non-leprosy*	0.13±0.2 cm <sup>2</sup>	1.2	9.2

\*Non-leprosy granulomas include sarcoidosis, leishmaniasis, fungal infection or tuberculosis;  $P$ -value= $<0.05$ .

From the Department of Pathology, College of Medicine, King Saud University, Abha, Saudi Arabia.

Address reprint requests and correspondence to Dr. Khan: Department of Pathology, College of Medicine, King Saud University, P.O. Box 641, Abha, Saudi Arabia.

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FIGURE 1. A case of tuberculoid leprosy. S-100 antibody immunoperoxidase stain highlights the nerve in the center of an epithelioid granuloma (original magnification, 250x).



FIGURE 2. Detail of Figure 1 (original magnification, 400x).

similarly stained. The surface area of all the biopsies was calculated by multiplying the width with the depth of the biopsy specimen under a light microscope equipped with micrometer. The number of nerve bundles stained with S-100 protein were counted in each specimen.

### Results

The results (Table 1) showed that the surface area of the biopsy specimen taken from the leprosy cases was  $0.45 \pm 0.3 \text{ cm}^2$  (mean  $\pm$  SD), and from the other granulomatous skin diseases was  $0.13 \pm 0.2 \text{ cm}^2$  (mean  $\pm$  SD). Also, the nerve bundles were 15 per  $\text{cm}^2$  in leprosy cases, and 9.2 per  $\text{cm}^2$  in non-leprosy cases. In addition, the leprosy cases showed longer nerve twigs that were perpendicularly oriented to the skin surface, and were embedded within the center of the granulomas (Figures 1 and 2).

### Discussion

The diagnosis of lepromatous leprosy is not usually a problem, either clinically or histologically. It is the diagnosis of tuberculoid or borderline tuberculoid leprosy which poses clinical, as well as histopathological, difficulties. The diagnosis should be considered in all patients who present with peripheral neuropathy and/or anesthetic skin lesions.<sup>7</sup> Tuberculoid leprosy is characterized by the presence of epithelioid granulomas, which may be well-developed or ill-defined. Emphasis is placed on identifying the acid-fast bacilli or nerve fibers in the center of the granulomas. These bacilli are seen with considerable difficulty and when seen they are usually fragmented. On routine hematoxylin and eosin stains the nerve fibers do not stand out from the background stain. S-100 immunostain has been shown to highlight the nerve better.<sup>3,4</sup>

In this study, we measured the overall surface area of

the skin biopsy specimens from leprosy cases as well as from non-leprosy cases. The surface area from leprosy cases was larger compared to that of non-leprosy cases (Table 1). The larger surface area in leprosy cases was because of larger incisional biopsies that were carried out in leprosy cases, rather than punch biopsies. The latter were most often performed in other granulomatous diseases of the skin. In all our cases, the nerve fibers were much better seen by S-100 immunostain than ordinary hematoxylin and eosin stain. An increased number of nerve bundles was seen (15 nerve elements per  $\text{cm}^2$  of the skin) in leprosy cases compared to other diseases (9.2 nerve elements per  $\text{cm}^2$  of the skin). Moreover, these nerve bundles were longer, embedded within the center of the granulomas, and were oriented perpendicularly to the skin surface. In the study reported by Singh et al., 12 cases of tuberculoid leprosy were stained with S-100 antibody.<sup>4</sup> Of these, seven cases had nerve elements within the center of granulomas, however, no detectable nerve elements were found in five cases. Nerve elements between the granulomas were seen in non-leprosy cases. They concluded that findings of nerve twigs within, but not between, granulomas were highly suggestive of leprosy.

There are two possible explanations for why an increased number of nerve elements were seen in the skin biopsies from tuberculoid and borderline tuberculoid leprosy, compared to other granulomatous diseases of the skin. One reason is that the granulomas were simply neurotropic. The other explanation is that the perineurium was damaged by mycobacterial inflammation and regenerating nerve fibers insinuated in asymmetrical patterns among collagen bundles. Miko et al.<sup>8</sup> have described large-scale functionally ineffective nerve regeneration in a histopathological study on posterior tibial nerves of 14 treated leprosy cases. Anand determined the levels of endogenous nerve growth factors in patients with nerve trauma, diabetes mellitus and leprosy.<sup>9</sup> The levels were decreased in the leprosy-affected nerve but were

raised in the initial phase of nerve trauma.

In conclusion, immunostaining with S-100 is a very useful method for identifying nerves in the center of granulomas in tuberculoid or borderline tuberculoid leprosy. Also, more nerve bundles seem to be present in skin biopsy specimens from tuberculoid leprosy cases when compared with other granulomatous skin diseases, as demonstrated by our study.

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