Skin Denervation in Vasculitic Neuropathy

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Background: Skin denervation in vasculitic neuropathy has rarely been documented despite frequent manifestations of small-fiber neuropathy including reduced sensitivity and neuropathic pain. Recently, skin biopsy has been established as a new approach to diagnose small-fiber sensory neuropathy.

Objectives: To investigate the pathologic features of cutaneous nerves and to evaluate inflammatory vasculopathy in the skin of patients with vasculitis.

Design: Case series.

Setting: National Taiwan University Hospital, Taipei.

Patients: Six patients with vasculitic neuropathy.

Interventions: Patients had 3-mm punch biopsy specimens taken from the distal part of the leg (without active vasculitic lesions) and a sural nerve biopsy specimen was taken in addition to detailed neurologic examinations, laboratory investigations, and nerve conduction studies.

Main Outcome Measures: Results of nerve conduction studies, epidermal nerve fiber density studies, and immunohistochemistry.

Results: All 6 patients had combined large- and small-nerve-fiber involvement on the neurologic examinations. Nerve conduction studies showed a pattern of axonal neuropathy or mononeuropathy multiplex. Epidermal nerve fiber densities were significantly reduced in the skin of all patients, consistent with concomitant small-fiber neuropathies. Perivascular infiltration by T cells and macrophages was demonstrated by immunohistochemistry. All patients experienced neurologic improvement in muscle strength and alleviation of sensory symptoms after immunotherapy with corticosteroids, plasma exchange, or cyclophosphamide.

Conclusions: Small-diameter sensory nerves are affected in vasculitis in addition to the well-known effect of vasculitis on large-diameter nerves. Significant inflammatory vasculopathy is present in the skin despite the absence of clinically active vasculitic lesions.

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VASCULITIS IS A RARE CAUSE of peripheral neuropathy. Clinical manifestations include reduced sensitivity and neuropathic pain in addition to motor weakness. The presence of these sensory symptoms and signs raises the possibility of concomitant involvement of small-diameter sensory nerve fibers. Traditionally, vasculitic neuropathies were diagnosed by means of nerve conduction studies, which evaluate only large-diameter nerves. It is not clear whether small-diameter sensory nerves are damaged in vasculitis, particularly the most vulnerable part of the sensory nerve terminals in the skin. Conventional examinations of small-diameter sensory nerves depend on ultrastructural studies of nerve biopsy specimens. Recently, several groups, including ours, have established skin biopsies as a new approach to diagnose small-fiber sensory neuropathies.1-7 In addition, the presence of a vascular network in the dermis offers the possibility of examining whether there is inflammatory vasculopathy in the skin with no active vasculitic lesions. In the present study, we attempted (1) to investigate the pathologic characteristics of cutaneous nerves in vasculitis and (2) to evaluate inflammatory vasculopathy of the skin.

METHODS

PATIENTS

Clinical data of patients were retrieved from the database of hospitalized patients at National Taiwan University Hospital, Taipei, from January 1, 2000, to December 31, 2003. All patients fulfilled 2 criteria: (1) clinical presentation of a mononeuropathy multiplex of acute or subacute onset and (2) pathological evidence of vasculitis on nerve biopsy specimens following the consensus criteria.8 All underwent detailed neurologic examinations and laboratory investigations to exclude metabolic and infectious diseases that affect the peripheral nerves, including...
plasma glucose level, and functional tests of the liver and kidneys. Specific autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis, were excluded by the following relevant laboratory tests: antinuclear antibody, antibody against double-stranded DNA, anti–Sjögren syndrome A antigen, anti–Sjögren syndrome B antigen, anti-Smith antigen, antinuclear factor, antinuclear ribonucleoprotein (anti-RNP), rheumatoid factor, C3 and C4 complement levels, cryoglobulin, hepatitis antibodies (hepatitis B surface antigen, anti–hepatitis B surface antibodies, and anti–hepatitis C virus antibodies), syphilis (VDRL/rapid plasma reagent test), anti–human immunoglobulin virus antibodies, lead levels in serum, immunoelectrophoresis of proteins in the serum and cerebrospinal fluid, and tumor markers (α-fetoprotein, carcinoembryonic antigen, CA125, and CA19-9).

**SKIN BIOPSY AND IMMUNOHISTOCHEMISTRY OF THE SKIN**

After informed consent was obtained, punch skin biopsy specimens (3 mm in diameter) were taken from the lateral side of the distal part of the leg and fixed in 4% paraformaldehyde overnight.10 No overt clinical vasculitis was noted at the biopsy sites. Sections of 50 μm perpendicular to the dermis were cut on a sliding microtome, quenched with 1% hydrogen peroxide in methanol, and blocked with 5% normal goat serum. Sections were incubated with rabbit antiserum to protein gene product 9.5 (PGP 9.5; UltraClone Ltd, Isle of Wight, England; 1:1000) overnight; PGP 9.5 is a ubiquitin carboxyl hydrolase that labels myelinated and unmyelinated nerve fibers in the peripheral nervous system. Sections were then incubated with biotinylated goat anti–rabbit IgG (Vector Laboratories, Burlingame, Calif) for 1 hour and the avidin-biotin complex (Vector Laboratories) for another hour. The reaction product was demonstrated by chromogen SG (Vector Laboratories, Burlingame, Calif) for 1 hour and the avidin-biotin complex (Vector Laboratories) for another hour. The reaction product was demonstrated by chromogen SG (Vector Laboratories). Skin biopsy specimens of control subjects were retrieved from the cohort described previously,10 and the project was approved by the Ethics Committee of National Taiwan University Hospital.

**IMMUNOHISTOCHEMISTRY OF INFILTRATING CELLS IN SKIN BIOPSY SPECIMENS**

Biopsy specimens of the skin were fixed with 4% paraformaldehyde and embedded in paraffin. Sections were stained with hematoxylin-eosin to search for pathological evidence of vasculitis. Additional sections were immunostained with various cellular markers, including CD68 (DAKO Corp, Glostrup, Denmark; 1:200) for macrophages, CD3 (Ventana Medical System, Tucson, Ariz; 1:100) for T cells, and CD20 (DAKO Corp; 1:100) for B cells. Sections were then incubated with biotinylated goat anti–rabbit IgG (Vector Laboratories) for 1 hour and the avidin-biotin complex (Vector Laboratories) for another hour. The reaction product was demonstrated by the chromogen 3,3′-diaminobenzidine (Sigma-Aldrich Corp, St Louis, Mo) and counterstained with hematoxylin.

**QUANTITATION OF EPIDERMAL INNERRATION**

Epidermal innervation was quantified according to the grading of the Medical Research Council (mean ± SD, fifth percentile, and first percentile) of epidermal nerve density. All patents had significantly reduced epidermal nerve density compared with the aforementioned normal values from our laboratory. Epidermal nerve density was therefore derived and expressed as the number of fibers per millimeter of epidermal length. In the distal part of the leg, normative values from our laboratory (mean ± SD, fifth percentile, and first percentile) of epidermal nerve density were 11.16±3.70, 3.88, and 4.2 fibers per millimeter, respectively, for subjects younger than 60 years and 7.69±3.08, 2.50, and 2.2 fibers per millimeter, respectively, for subjects 60 years or older.11

**NERVE CONDUCTION STUDIES**

Nerve conduction studies were performed with an electromyography machine (Viking IV; Nicolet Biomedical Inc, Madison, Wis) on all patients according to standardized methods.1,12 The temperature of the examination room was maintained at 32°C. Results of the nerve conduction study were classified according to the established criteria as demyelinating, axonal, inexcitable, equivocal, or normal.13

**RESULTS**

**CLINICAL PRESENTATIONS AND LABORATORY EXAMINATIONS**

The clinical features and laboratory findings of our 6 patients (4 men and 2 women) are listed in the Table. All patients had motor and sensory impairments with acute or subacute onset. Motor and sensory symptoms usually began focally or asymmetrically in one leg or thigh and then progressed to affect the contralateral side and the upper limbs. Three patients had simultaneous symptoms in the upper and lower limbs as the initial manifestation. Pain was noted in most patients (5 of 6). Elevated protein levels in the cerebrospinal fluid were noted in 3 patients. Nerve conduction studies showed a pattern of axonal neuropathy or mononeuropathy multiplex. After immunotherapy with corticosteroids, plasma exchange, or cyclophosphamide, there were significant improvements in muscle strength (at least 1 grade in the weakest limb according to the grading of the Medical Research Council) and reduction of neuropathic pain in all patients (Table).

**PATHOLOGICAL DEMONSTRATION OF VASCULITIS**

Inflammatory cells surrounding vessels were demonstrated in the sural nerves and dermal vessels of the skin (Figure, A), and cells were immunoreactive for markers of macrophages (CD68; Figure, B) and T cells (CD3; Figure, C), but not for B cells (CD20; Figure, D). In patients with vasculitis, the quantity of epidermal nerves was markedly reduced. The epidermis of some patients had even become completely denervated. We quantified epidermal innervation by measuring the epidermal nerve density. All patients had significantly reduced epidermal nerve fiber density (0-3.18 fibers per millimeter; Table) compared with the aforementioned normal values from our laboratory. In addition, dermal nerves showed a pattern of degeneration; sweat glands became denervated in some patients.
This report demonstrates 2 observations important for the evaluation and understanding of neuropathy in vasculitis. First, small-diameter cutaneous nerves were affected in patients with vasculitis in addition to the well-known influence of vasculitis on large-diameter nerves. Second, significant inflammatory vasculopathy was present in the skin despite the absence of active vasculitic lesions in the skin.
In patients with vasculitis, the present data from skin biopsy specimens demonstrate that degeneration of the small-diameter sensory nerves occurs as frequently as that of large-diameter nerves. Despite the frequent manifestations of sensory symptoms, studies on small-diameter sensory nerves in vasculitis have received less attention. 

This has mainly been due to the lack of objective and easily accessible methods for evaluating the degeneration of these nerves. Ischemia due to vasculitis can cause wallerian degeneration of both myelinated and unmyelinated fibers. 

A previous clinical study described 2 patients with predominant symptoms of small-fiber neuropathy and vasculitis in their nerve biopsy specimens. Small myelinated nerves were found to be more susceptible to ischemia than large myelinated nerves in an animal model of ischemic neuropathy. 

There is, however, a lack of direct evidence regarding the damage to cutaneous nerves with vasculitis. The present study clearly demonstrates the depletion of cutaneous nerves in vasculitis.

Skin biopsy with 3-mm punches and immunohistochemistry with anti–PGP 9.5 antiserum offer a diagnostic approach that is easily performed, minimally invasive, and potentially repeatable. 

As a complement to quantitative sensory testing, which is a psychophysical test, skin biopsy provides pathological evidence of cutaneous nerve degeneration in vasculitis.

The clinical manifestations of vasculitis may overlap with those of other rheumatic and nonrheumatic diseases, and the diagnosis of vasculitis can be evident from a simple skin biopsy specimen. The vessels affected in the current series are similar to those described by Dyck et al and Llewelyn et al in diabetic and nondiabetic patients with lumbosacral radiculoplexus neuropathy. Microvasculitis in distal cutaneous nerves is an important pathologic mechanism of the neurologic deficits. This is in contrast to clinically overt vasculitis involving large and medium-sized vessels, or arterioles in necrotizing vasculitis of multiple organs, such as polyarteritis nodosa, rheumatoid arthritis, Wegener granulomatosis, Churg-Strauss syndrome, and hypersensitivity angiitis. 

Affected vessels in microvasculitis are usually small, and prominent fibrinoid degeneration or necrosis of vascular walls is not evident.

All 6 patients in the present study showed infiltration of T cells and macrophages in the skin biopsy specimens from the sampling sites, which were “silent” for clinically overt vasculitis. These findings raise the possibility that patients with apparently isolated vasculitic neuropathy may in fact be affected by a milder form of microvasculitis of the cutaneous nerves. Taken together, these findings suggest that inflammatory vasculopathy may underlie cutaneous denervation in vasculitic neuropathy.

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REFERENCES


