Supplementary Online Content


eAppendix.

This supplementary material has been provided by the authors to give readers additional information about their work.
eAppendix

IMMUNOHISTOCHEMICAL STAINING, AND QUANTIFICATION OF EPIDERMAL INNERVATION

The procedure of immunohistochemistry and quantitation of epidermal innervation followed established protocol.\(^1\)

Briefly, sampled skin tissue was fixed in 2% paraformaldehyde-lysine-periodate in 0.1 M phosphate-buffered saline. Sections of 50 \(\mu\)m perpendicular to the dermis were cut on a sliding microtome. Each specimen yielded about 48–50 vertical 50 \(\mu\)m sections. All sections were sequentially labeled. The first and last few sections would not be used for nerve quantification because of possible artifacts during cutting and relatively small size compared with sections in the middle part of skin specimen.\(^1\) Sections were immunostained with antiserum to protein gene product 9.5 (PGP 9.5, 1: 1000; UltraClone, Isle of Wight, UK).

Epidermal innervation was quantified according to established criteria in a coded fashion by trained examiners who were blinded to the clinical information. PGP 9.5-immunoreactive nerve fibers in the epidermis of each section were counted at a magnification of \(\times\)40 with an Olympus BX40 microscope (Tokyo, Japan) through the depth of the entire section. Each individual nerve with branching points inside the epidermis was counted as one. For epidermal nerves with branching points in the dermis, each individual nerve was counted separately. The length of the epidermis along the upper margin of the stratum corneum in each section was measured with the Image-Pro PLUS (Media Cybernetics, Silver Spring, MD). The IENF density was thereby derived and expressed as the number of fibers/mm of epidermal length. For the normative data, normal subjects were recruited from the community and those visiting National Taiwan University Hospital, Taipei, Taiwan for a physical check-up. They were evaluated by detailed questionnaires, neurological examinations, nerve conduction studies, quantitative sensory testing, and laboratory tests including complete blood count, fasting blood glucose, hemoglobin A1c, liver and renal functions, serum protein electrophoresis, anti-nuclear antibody, and vitamin B12 level. There were 88 normal subjects (36 males and 52 females) aged 47.8 ± 13.5 years.\(^2\)

NERVE CONDUCTION STUDIES

NCS following standardized methods were performed with a Nicolet Viking IV Electromyographer (Nicolet, Madison, WI). Studied nerves included sural and peroneal nerves. In the present study, the age of patients was relatively high (60.7 ± 12.3 years) and the comorbidity of lumbosacral radiculopathy might become a confounder. Thus, the late responses were not included for analysis.\(^3\)\(^4\) The results of the NCS were compared to normative data in our laboratory.\(^5\)
An abnormal NCS was defined as having abnormalities in one or more nerves with reduced amplitude, prolonged distal latency, or slowed nerve conduction velocity.

AUTONOMIC FUNCTION TESTS

The R-R interval variability (RRIV) for cardiac-vagal function and the sympathetic skin response (SSR) for sudomotor function were determined following established protocols using a Nicolet Viking IV Electromyographer. The RRIV was obtained at rest and during forced deep breathing. Each test was repeated three times, and the mean value was compared with that for age-matched controls in our laboratory. The SSR was recorded in the palm and sole, and the results were interpreted as present or absent but were not evaluated quantitatively because of variations in the latencies and amplitudes of the SSR. Medications that interfered with sympathetic or parasympathetic functions were not administered before or during these tests.

STATISTICAL ANALYSIS

Data following Gaussian distribution were compared using t-tests and are expressed as mean ± SD. For data did not follow Gaussian distribution, were compared using a nonparametric test (Wilcoxon rank-sum test). Correlations between variables were graphically analyzed using the slope of the regression line, including the 95% confidence interval (CI). The correlation was further explored with a multiple linear regression analysis. All analyses were performed using Stata software (StataCorp LP, College Station, TX). Results were considered significant at \( p < 0.05 \).

References