The Relationship Among Pain, Sensory Loss, and Small Nerve Fibers in Diabetes

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OBJECTIVE — Many individuals with diabetes experience neuropathic pain, often without objective signs of large-fiber neuropathy. We examined intraepidermal nerve fibers (IENFs) to evaluate the role of small nerve fibers in the genesis of neuropathic pain.

RESEARCH DESIGN AND METHODS — Twenty-five diabetic subjects with neuropathic pain and 13 without were studied. The pain was present for at least 6 months for which no other cause could be found. Punch skin biopsies were obtained from the distal leg. IENFs were stained using antibody to protein gene product 9.5 and counted with confocal microscopy. Neuropathy was graded by vibration perception and cold detection thresholds and the Michigan Neuropathy Screening Instrument.

RESULTS — In the total cohort, IENF density was significantly lower in those with pain compared with those without (3 [1–6] vs. 10 [3–19], respectively, $P = 0.02$). There were significant inverse correlations between IENF and severity of neuropathy, with the pain group having a flatter gradient than their pain-free counterparts ($P < 0.02$). The difference in IENF density was greatest in subjects with less objective evidence of neuropathy ($P \leq 0.01$).

CONCLUSIONS — More severe loss of IENF is associated with the presence of neuropathic pain only in those with little or no objective sign of neuropathy. Thus, loss of IENF cannot explain pain in all cases, suggesting that different mechanisms underpin the genesis of pain at various stages of neuropathy.

Diabetes Care 29:883–887, 2006

Peripheral neuropathy is a common complication of both type 1 and type 2 diabetes (1). Most commonly, it manifests as sensory loss, which predisposes subjects to foot abnormalities and high risk of ulceration. However, it has been reported that between 4 and 33% of subjects with diabetes suffer from the painful type of neuropathy, which can become chronic and produce unremitting pain for which there is little satisfactory treatment (2–4).

It is known that pain transmission in peripheral nerves occurs along the small Aδ and C-type fibers (5,6). However, conventional clinical investigation of individuals with painful diabetic neuropathy is usually limited to nerve conduction studies that measure large–nerve fiber function. The results of these tests are often normal and unable to provide an explanation for the presence of pain (7,8). This conundrum reflects a common clinical observation that pain is often present in the absence of objective signs of neuropathy. Even in the presence of abnormal nerve conduction studies, some individuals may experience pain while others with the same degree of electrophysiological abnormalities are completely asymptomatic. It is commonly assumed that more specific testing of small-fiber function may better discriminate those with or without pain, but several studies, including our own, have shown this not to be the case (9–11). However, in view of the pivotal role played by small nerve fibers in the transmission of pain sensation, further studies are obviously of importance.

Direct examination of intraepidermal nerve fibers (IENF) using skin biopsy technique is a proven procedure to identify small-fiber abnormalities. Several studies using this technique have shown the density of IENF to be reduced in idiopathic and nondiabetic neuropathies (12–14). This technique has also shown that people with diabetes have reduced IENF and altered nerve morphology (14,15). However, to our knowledge, no studies have been specifically conducted to examine IENF in the context of its role in the genesis of pain in diabetes. Therefore, the current study was conducted to compare subjects with or without pain to determine the relationship among pain, sensory loss, and IENF density.

RESEARCH DESIGN AND METHODS

— Subjects were recruited from patients attending our Diabetes Centre. As the focus is on chronic neuropathic pain (rather than transient neurogenic pain, which may occur during acute fluctuation in glycemic control), for the purpose of this study patients were only recruited if they have had pain for >6 months. All patients had symmetrical foot pain. Patients were deemed to have neuropathic pain by obtaining a detailed history of the nature of the pain and performing a physical examination to exclude nociceptive pain such as arthritis or peripheral vascular disease. Blood was collected to eliminate other possible causes of pain such as vitamin B12 deficiency, hypothyroidism, and where clinically relevant, monoclonal gammopathy. Chest X-ray was performed if indicated to exclude paraneoplastic phenomena, and physical examination and history excluded spinal or hereditary causes. Where no other abnormality was found, the cause of pain was deemed to be diabetes. A total of 38 patients with diabetes were studied, composed of 13 without and 25 with painful neuropathy.

Apart from noting its presence, the severity of pain was recorded using a 10-cm visual analog scale. Subjects were

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Received for publication 8 November 2005 and accepted in revised form 22 December 2005.

Abbreviations: CDT, cold detection threshold; IENF, intraepidermal nerve fiber; MNSI, Michigan Neuropathy Screening Instrument; VPT, vibration perception threshold.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Demographic and clinical profiles of subjects with or without pain*

	No pain	Pain	Test statistic and *P* value
n	13	25	
Age (years)	57 (54–65)	59 (53–62)	$Z = 0.08$; $P = 0.9$
Duration of diabetes (years)	7 (2–13)	7 (5–12)	$Z = -0.5$; $P = 0.6$
Male (*n*)	13	15	Fisher's exact $P = 0.008$
Type 2 diabetes (*n*)	11	21	$\chi^2 = 0.002$; $P = 0.96$
Height (m)	1.8 (1.7–1.8)	1.8 (1.7–1.8)	$Z = 1.5$; $P = 0.1$
HbA_{1c} (%)	7.2 (6.6–8.9)	7.1 (6.4–8.9)	$Z = 0.5$; $P = 0.7$
VPT (volts)	33 (21–50)	35 (22–50)	$Z = -0.2$; $P = 0.9$
Absent ankle reflexes (%)	28.4	48	$\chi^2 = 0.5$; $P = 0.5$
MNSI	1.0 (0.5–5.5)	3.0 (1.0–6.0)	$Z = -0.8$; $P = 0.4$
CDT (°C)	−2.67	−3.42	$Z = -0.5$; $P = 0.6$
Pain score	0	8.0 (6.5–8.0)	$Z = -5.2$; $P < 0.001$
IENF (density/3 mm)	10 (3–19)	3 (1–6)	$Z = 2.4$; $P = 0.02$

Data are median (interquartile range), unless otherwise indicated.

asked to grade pain at the level they feel they experience most of the time. Control subjects were patients who were pain free, with similar age, duration of diabetes, and glycemic control. As we have shown previously, overall, subjects with pain have more sensory loss associated with neuropathy and the cohort was further characterized by measurement of large-fiber function with vibration perception threshold (VPT), small-fiber function with cold detection threshold (CDT), and neuropathy status with Michigan Neuropathy Screening Instrument (MNSI) (16). VPT was measured using a biothesiometer (Bio-medical Instrument, Newbury, OH) by placing the probe on the dorsum of the foot at the web of the first and second toes. The voltage is slowly increased from 0, and patients are asked to indicate when they can first feel the vibration. Three measurements are taken on each foot and the average of these recorded as the VPT. CDT was measured using the Computer-Aided Sensory Evaluator machine (CASE IV; Medical Electronics, Stillwater, MN). A series of cold stimuli of different temperatures is delivered with a sensor placed on the dorsum of the foot, and the patient indicates whether the stimulus is felt. Using a 4,2,1, stepping forced-choice algorithm, the smallest temperature differential from the baseline foot temperature that can be reliably detected is determined. For the MNSI, part B of the instrument was used.

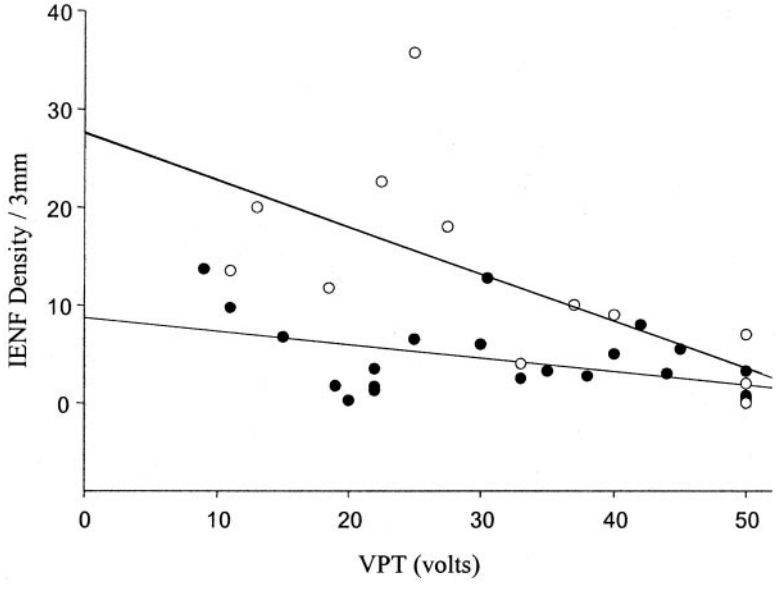

Figure 1—*Relationship between IENF density and VPT grouped by pain status.* ●, *pain;* r = *−0.5,* P = *0.009.* ○, *no pain;* r = *−0.8,* P = *0.01.* F = *9.4,* P = *0.0004 for ineteraction between groups.*

Five indicators were measured for each foot (appearance of foot, presence of ulceration, ankle reflexes, vibration perception, and ability to feel the 10-gm monofilament). Each indicator may be assigned a score of 0, which indicates normal, 0.5, or 1, respectively, for intermediate grade or gross abnormalities. Each foot was assessed independently, giving a possible total maximum score of 10.

Skin biopsies were obtained and analyzed according to the protocol developed by McArthur et al. (17). Skin specimens were obtained using a 3-mm punch biopsy from the leg 10 cm above the lateral malleolus. The skin sections were placed in 2% paraformaldehyde/lysine/periodate. Fifty-micron freezing microtome sections were immunostained with the panaxonal marker PGP 9.5. IENFs of the whole biopsy were counted using confocal microscopy at ×40 magnification in a standardized manner. Individual fibers were counted when they crossed the dermal-epidermal junction, and secondary fibers that branched from within the epidermis were excluded. The length of the epidermis was measured using a computerized tracing system, and the results were expressed as IENF number per 3 mm of the section. Due to the depletion of IENF in diabetic subjects, for convenience, the results were expressed as IENF number per 3 mm (rather than per millimeter). Four sections from each subject were counted and the mean IENF density calculated. Each section was counted by two observers blinded to the source of the specimens, and the mean interobserver variation was 6.1%.

Statistical analysis was performed using the NCSS 2004 statistical software package (Dr. J. Hintze, Kaysville, UT). Subjects were grouped according to pain and neuropathy status. Data were assessed for normality and if necessary normalized using logarithmic transformation. Continuous data were expressed as mean ± SD or median (interquartile range). Continuous data were compared using the Mann-Whitney test. The relationships between fiber count and neuropathy measurements grouped by pain status were calculated using correlation coefficient. Multiple regression was then used to assess for interaction among pain status, the grade of neuropathy as measured by the MNSI, and CDT for fiber count. VPT was stratified into categories (VPT <15, 15–30, and >30) because it was not possible to provide numerical data on VPT >50 volts. ANOVA was used to test for interac-

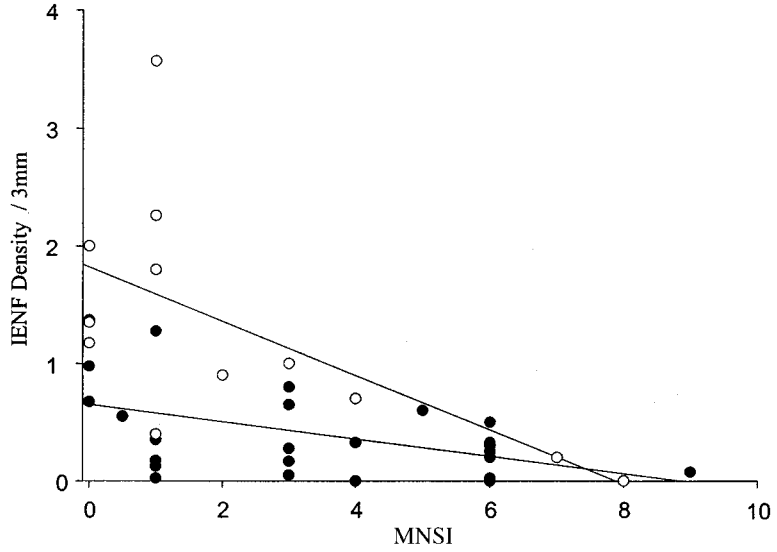

Figure 2—*Relationship between IENF density and MNSI grouped by pain status.* ●*, pain;* r = *−0.5,* P = *0.02.* ○*, no pain;* r = *−0.8,* P = *0.0003.* t = *−2.6,* P = *0.01 for interaction between groups.*

tion. Categorical data were analyzed by χ^2 test or Fisher's exact test. Statistical significance was accepted at *P* value of <0.05.

RESULTS — The demographic and clinical profiles of the subjects are shown in Table 1. There were a similar number of males in each group; however, the pain-free group was comprised solely of males. The majority of subjects have type 2 diabetes. There was no difference in the degree of neuropathy measured by VPT, CDT, or MNSI between the two groups. The median pain score reported by those subjects with pain was 8, and those without pain had a score of 0.

As shown in Table 1, in the whole group analysis, there was a significantly lower IENF density in the subjects with pain compared with those without, 3 (1–6) vs. 10 (3–19) fibers per 3-mm section, respectively ($Z = 2.4$; $P = 0.02$). Using VPT as a measurement of neuropathy, there was a significant negative relationship with fiber count in both the pain and no pain groups: $r = -0.5$, $P = 0.009$ and $r = -0.8$, $P = 0.01$, respectively (Fig. 1). Similarly for MNSI, a significant negative relationship with fiber count exists for the group with pain ($r = -0.5$, $P = 0.02$) or no pain ($r = -0.8$, $P = 0.0003$), as shown in Fig. 2. Using CDT as a measurement of neuropathy, a significant relationship with fiber count only exists in the no pain group ($r = 0.8$, $P = 0.006$) (Fig. 3). For all three modalities used to grade severity of neuropathy, there was statistically significant interaction between the groups with or without pain, indicating that the gradients of the slopes were significantly different ($P < 0.01$). There was no relationship between fiber count and heat as pain thresholds (no pain r = −0.7, $P = 0.1$ or pain $r = -0.1$, $P = 0.8$) nor between the degree of pain and fiber count ($r = -0.02$, $P = 0.9$)

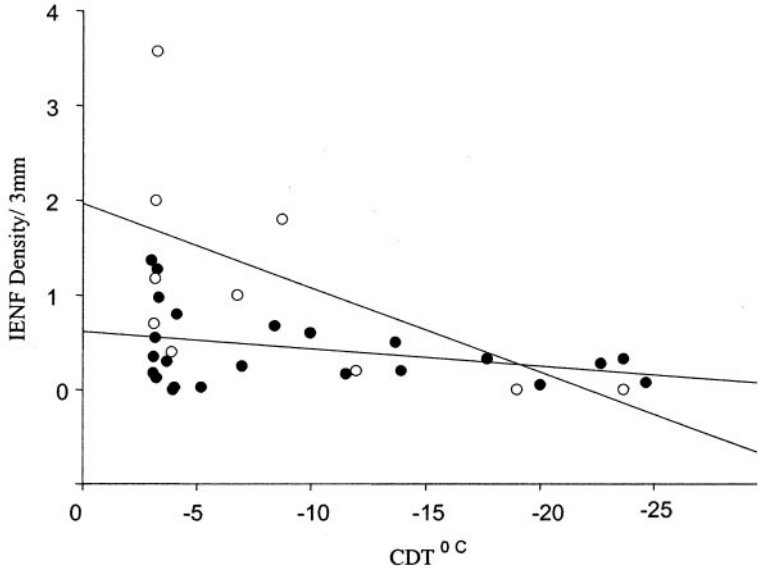

Figure 3—*Relationship between IENF density and CDT grouped by pain status.* ●*, pain; NS.* ○*, no pain;* r = *−0.8,* P = *0.0006.* t = *−2.6,* P = *0.006 for interaction between groups.*

CONCLUSIONS — Previous studies have shown that people with diabetes have fewer IENF than those without diabetes. Kennedy et al. (15) studied a group of young patients with type 1 diabetes who were awaiting pancreas transplant. He showed that in this group, the number of IENFs were decreased compared with age-matched nondiabetic healthy control subjects and that diabetic subjects had shorter nerves, which often ended bluntly at the dermal surface of the basement membrane. All of the diabetic subjects had neuropathy, and he found a negative correlation between fiber density associated with mild to moderate neuropathy and an absence of IENF in most patients with severe neuropathy. A recent study by Polydefkis et al. (18) also reported a similar reduction in IENF density between diabetic and normal control subjects, again a significantly lower density in those with diabetic neuropathy was demonstrated. Shun et al. (19) also showed in a group of 38 subjects with type 2 diabetes a reduced IENF density that negatively correlated with duration of diabetes but a variable relationship with severity of neuropathy as measured by quantitative sensory testing. Malik et al. (20) has shown an association between endoneurial angiography and reduced myelinated and unmyelinated fibers in the sural nerve of diabetic subjects. The above-mentioned studies did not focus specifically on painful diabetic neuropathy and did not compare with their nonpain counterparts (with or without neuropathy). One study by Lauria et al. did focus on painful neuropathy and found lower IENF than normal control subjects. However, there were only six subjects with diabetes (21).

Our findings are in substantial agreement with results published previously. However, we have identified a specific trend of IENF in painful diabetic neuropathy that was previously unreported. As a group, our diabetic subjects also have greatly reduced IENF compared with the normative mean values of 36 fibers/3 mm reported by McArthur et al. (17) and significantly different from those obtained by neurological colleagues at our hospital measured with the identical method (42 fibers/3 mm) (P. Spring, personal communication). Similar to previous descriptions, the fibers in our cohort often terminated abruptly at the dermal-epidermal junction and showed more nodularity (8,21). We also concur with the findings of Shun et al. in demonstrating that there is a progressive loss of IENF with decreasing ability to perceive vibration. However, our data showed the novel finding that in comparison with the pain-free group, patients with neuropathic pain have a much flatter gradient of relationship between IENF density and the severity of neuropathy, whether this was measured in terms of VPT, CDT, or the MNSI. As a result of this, the IENF counts are much more different between the pain and pain-free groups when patients have no severe objective signs of neuropathy. By contrast, the IENF density between the groups was very similar in the presence of severe neuropathy. In a previous study, we have shown that abnormalities of small-fiber function, as measured by CDT and heat as pain thresholds, are not predictive of pain (10). In this current study, a relationship exists between IENF and CDT but not with heat as pain threshold. This may be due to the fact that testing for heat as pain is more subjective and relies greatly on the individual's interpretation of pain.

To our knowledge, this is the first study that demonstrates that small-fiber dropout does not always parallel large-fiber function, and in fact differs between people with or without pain depending upon the degree of sensory loss. These observations suggest that in individuals with little objective sign of neuropathy, abnormalities of small nerve fibers are more likely to play a central role in the genesis of pain. In those with severe objective signs of neuropathy, a role of the small-fiber dysfunction in causing pain is still possible but less certain, as there is a great deal of overlap in IENF in those with or without pain. Studies in animals and humans have provided many theories as to how C-fiber damage leads to pain. These include, among others, increased ectopic and spontaneous firing of primary afferents, transmission of painful signals along large myelinated nerves that do not normally transmit pain (22), neurochemical and structural change in the dorsal root ganglion (23,24) and dorsal horn of the spinal cord (25), and altered brain processing and inhibition of painful sensation (26). At the current state of our knowledge, it is not clear whether these adaptive mechanisms are temporally or proportionally different in the three clinical categories of patients in our cohort, e.g., those with pain but no objective large-fiber neuropathy, those with both pain and large-fiber neuropathy, and those with no pain but objective large-fiber neuropathy. In assigning a role for IENF dropout in the genesis of pain, a couple of observations should be noted. First, even in our pain-free diabetic patients, the IENF density is very much reduced in comparison with normal patients. Second, in our cohort there were no individuals with pain but normal IENF, although a small number of these patients have been reported in nondiabetic forms of painful neuropathy. Third, our findings pertain only to subjects with chronic neuropathic pain at one point in time. The patterns of evolution of pain through its stages may be different.

While the mechanisms for the genesis of pain in the different stages of diabetic neuropathy remain uncertain, our findings have practical implications in designing studies to examine the role of small-fiber changes in this regard. If we select patients with long-standing diabetes and objective evidence of classical diabetic neuropathy, due to the overlap of the IENF density, the study is likely to lead to the conclusion that small-fiber pathologies play little role. On the other hand, if we were to select patients with little evidence of objective neuropathy, the opposite conclusion is likely to be reached. If we were to recruit a broad range of diabetic subjects, the results will be determined by the admixture of their clinical status.

Acknowledgments— We thank the Diabetes Australia Research Trust for financial support.

We also thank Dr. Dao Ping Liu and Dr. Sue McLennan for their assistance with immunohistochemistry and Dr Alex Birrell for her assistance with nerve fiber counting.

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