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Thiamine-deficiency neuropathy in Japan

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Abstract

We reviewed the clinicopathologic features of patients with thiamine-deficiency neuropathy. Symmetric sensorimotor polyneurpathy predominantly involving the lower limbs was the typical presentation, while variations were seen in the severity and the distribution of weakness and sensory disturbance. Progression occurred over intervals varying from days to years. Some patients progressed rapidly in a manner resembling Guillain-Barre syndrome. Symptoms of heart failure and Wernicke-Korsakoff syndrome varied among individual patients. Major electrophysiologic findings were those of axonal neuropathy with more marked abnormalities in the lower limbs. Sural nerve biopsy specimens mainly showed predominant loss of large myalinated fibers and marked subperineurial edema. Major etiologies of thiamine deficiency were postgastrectomy state, chronic alcoholism, and dietary imbalance. Patients with chronic alcoholism showed slightly different clinical features in that they were sensory-dominant, slowly progressive, and predominantly impaired superficial sensation with pain or painful burning sensation compared to those of the other two major etiologies. Thiamine adnministration achieved substantial functional recovery within 6 months, irrespective of the initial deficit, but recovery of sensory impairment was incomplete. The variability of thiamine-deficiency neuropathy should be kept in mind, because early diagnosis and treatment facilitates recovery.

Introduction

Deficiency of thiamine (vitamin B1) causes peripheral neuropathy and heart failure, designated as beriberi. Wernicke-Korsakoff syndrome also may occur.[1] Deficiency may occur in individuals with decreased thiamine intake caused by insufficient consumption of appropriate food, due to high-

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carbohydrate or low-thiamine diets, chronic alcoholism,[2] anorexia nervosa,[3] or hyperemesis gravidarum,[4,5] or alternatively in persons with increased thiamine requirements such as laborers performing heavy outdoor work.[6] Historically, beriberi was an endemic disorder among Asians for whom milled white rice was a dietary staple in the late 19th century.[7] The number of patients with beriberi decreased markedly after the discovery of vitamin B1,[8] and public education regarding the need for a balanced diet. However, in Japan, an epidemic of beriberi occurred in the 1970s when instant foods with low vitamin contents became popular.[6,9,10] The epidemic was resolved by nutritional education and supplementation of food with thiamine as a countermeasure. Since that time, the disorder has been largely forgotten by many physicians, especially those in developed countries. However, recent reports suggest that neuropathy associated with thiamine deficiency is not uncommon among patients with a variety of background factors including chronic alcoholism[11] and prolonged parenteral nutrition.[12] Furthemore, we previously reported 17 patients with postgastrectomy polyneuropathy associated with thiamine deficiency, suggesting gastrectomy as a risk factor for thiamine deficiency.[13]

In this article, we review the clinicopathologic features of thiamine-deficiency neuropathy in Japan.

Causes of thiamine deficiency

We reviewed 66 patients with thiamine-deficiency neuropathy who were referred to Nagoya University Graduate School of Medicine and its affiliated institutions from 1990 to 2002. Major factors contributing to thiamine deficiency in these patients were gastrectomy, heavy drinking, and dietary imbalance. Among patients with dietary imbalance, the staple food was milled rice or noodles, without adequate meat and vegetables; also, food intake was irregular. A striking observation was that many of the patients had undergone gastrectomy. Gastrectomy was preformed to treat ulcers or neoplasms. Patients who had undergone gastric restriction surgery for morbid obesity were not present. Among these patients we reported 17 having postgastrectomy polyneuropathy with thiamine deficiency.[13] Gastrectomy had been performed in these patients 2 months to 39 years prior to the onset of neuropathy. Nine patients underwent gastrectomy for carcinoma, one for lymphoma, and 7 for gastric or duodenal ulcer. The method of reconstruction varied, and included Billroth I or Billroth II for subtotal gastrectomy and a Roux-en-Y method or a jejunal poutch interposition for total

gastrectomy. Interestingly, most patients took particular care of dietary balance after surgery. The presence of these patients supports the view that thaimne deficiency can occur in those who undergo gastrectomy. Wernicke's encephalopathy has been reported occasionally in association with operations performed to treat morbid obesity.[14,15] Neuropathy can also occur in some patients following gastric restriction surgery for morbid obesity.[15,16] Thiamine deficiency may occur in patients who undergo operations for morbid obesity, especially those who are prone to vomiting.[17] Roux-en-Y bypass to create a bypass or gastroplasty to produce stenosis of the gastrointestinal tract were the major procedures performed for morbid obesity. Patients undergoing such surgery show extensive weight loss, sometimes with protracted nausea and vomiting, as well as severe malnutrition. In our series, none of the gastric operations were performed for morbid obesity, and our patients underwent total or subtotal gastrectomy. Unlike patients who undergo surgery for morbid obesity, only one of our patients had vomiting and extensive weight loss. Some of our patients had carcinomas, but tumors did not show recurrence or metastasis at the time neuropathic symptoms appeared.

Thiamine is absorbed by the mucosa of the entire intestine, with the highest rate of absorption being in the duodenum.[18-20] That patients who underwent gastrectomy would develop thiamine deficiency is somewhat puzzling, because a large extent of the intestine presumably able to absorb thiamine was left intact. Recent reports of Wernicke's encephalopathy suggest that thiamine deficiency can occur in some, but not all, patients who undergo gastrectomy for cancer.[21-24] Some of these patients have shown electrophysiologic evidence of neuropathy, as well as Wernicke's encephalopathy.[21,24,25] Markkanen reported that erythrocyte transketolase activity decreased in 31% of patients who had operations involving the upper gastrointestinal tract, including partial gastrectomy.[25] These observations suggest that the relative abilities of different intestinal sites to absorb thiamine vary among individuals. Furthermore, only a few of the many patients who undergo gastrectomy develop neuropathy, so individual factors, possibly genetic in nature, may have an influence.

Most postgastrectomy patients in our series did not manifest neuropathic symptoms during the early period after operation, even though many of them had acute onset of symptoms. Thiamine is turned over relatively rapidly in the body, and is not stored in large amounts.

Patients who undergo gastrectomy could have a subclinical deficiency of thiamine, even with appropriate dietary intake. When patients experience increases in thiamine requirements due to fever, anorexia, heavy labor, or pregnancy, symptoms of thiamine deficiency may be precipitated. Increases in subclinical thiamine deficiency associated with aging also could account for the delayed appearance of neuropathic symptoms after gastrectomy in some patients.[26,27] Three patients with cancer received chemotherapy with either deoxifluridine or 5-fluorouracil. Fluoropyrimidines are reported to increase the cellular metabolism of thiamine.[28] This medication is considered to have contributed to the development of thiamine deficiency.

In addition to gastrectomy, chronic alcoholism is closely related to thiamine deficiency.[11] According to our previous study of consecutive patients with alcoholic neuropathy, 28 of 64 patients (44%) manifested thiamine deficiency.[29] In addition to dietary imbalance associated with chronic alcoholism, alcohol diminishes thiamine absorption in the intestine[30] and reduce hepatic storage of thiamine.[31] Decreased phoshporylation of thiamine to the active coenzyme thiamine pyrophosphate ascribed to alcohol may also contribute to the development of thiamine deficiency.[32-34] These observations support the view that some of the pathogenesis of alcoholic neuropathy are attributable to deficiency of thiamine absorption or metabolism.

Clinical features

We previously described the clinical features of 32 patients with thiamine-deficiency neuropathy.[29] All of the patients were nondrinkers. According to this report, all patients manifested symmetric polyneuropathy with more involvement in the lower than upper limbs, showing a centripetal pattern of progression. The initial symptom of neuropathy was variable; this was weakness in the lower extremities in 50% of patients and numbness in the distal lower limbs in 50%. Progression rate also varied; acute progression within 1 month was seen in 18 patients (56%), while slow progression over more than 1 year was seen in 19%. Impairment was usually motor-dominant, affecting 84% of patients. Some patients whose motor weakness progressed over days were initially thought to have Guillain-Barr_ syndrome. Motor symptoms were more predominant in the lower than upper extremities; even so, 81% showed weakness in the upper limbs. Sensory disturbance was present in the lower limbs in all

patients, and was also present in the upper limbs and the trunk in 78% and 28%, respectively. Varying degrees of numbness with or without painful sensations were noted in all patients, and painful sensations were reported by 22% of patients. Involvement of all sensory modalities was a common feature: superficial sensation was affected most in 9%; deep sensation was involved most in 28%; and, both modalities were affected equally in 63%. Biceps, patellar, and Achilles tendon reflexes were reduced or absent in most patients. Activities of daily living were impaired mainly because of the rapid progression of muscle weakness. Only 16% could walk unaided at the time of initial examination.

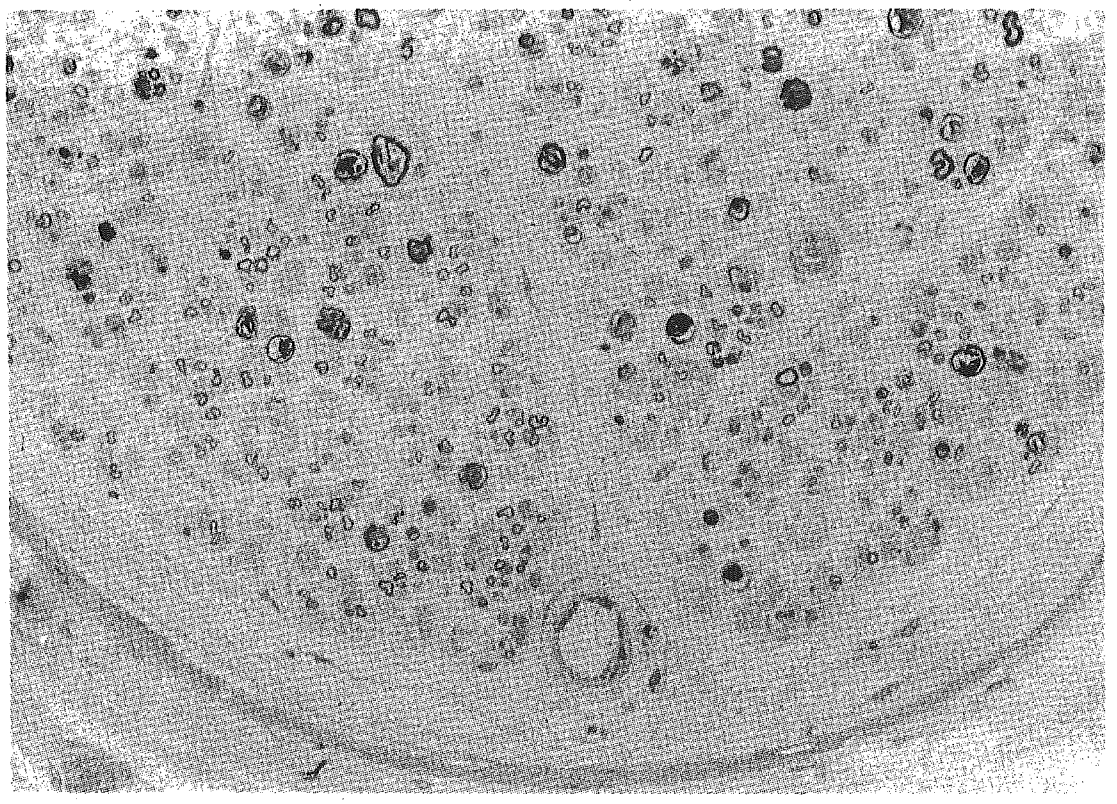
On the other hand, thiamine-deficiency neuropathy associated with chronic alcoholism show slightly different clinical features in the point that it is sensory-dominant, slowly progressive, and predominantly impairs superficial sensation with pain or painful burning sensation in comparison with nonalcoholic thiamine-deficiency neuropathy.[29,35] These differences are thought to be attributable to the toxic effects of ethanol. Recent studies indicate a direct neurotoxic effect of ethanol or its metabolites, involving ethanol-induced glutamate neruotoxicity,[36,37] decreased production of neurofilament protein or its phosphorylated form,[38,39] or impairment of fast axonal transport.[40] Axonal degeneration has been documented in animals receiving ethanol while maintaining a normal thiamine status.[41] Human studies also have suggested a direct toxic effect, because a dose-dependent relationship has been observed between severity of neuropathy and amount of ethanol consumed.[42]

Clinical features of polyneuropathy and associated symptoms such as Wernicke's encephalopathy and congestive heart failure varied among individual patients. The cause of the variations of clinical features has not been determined. Genetic factors may relate to variations and susceptibility to thiamine deficiency. For example, thiamine-responsive megaloblastic anemia associated with diabetes mellitus and deafness results from a mutation of the gene encoding the thiamine transporter protein; and this genetic defect preferentially involves hematopoietic, pancreatic islet, and auditory apparatus cells.[43-45] Other factors that could influence clinical features are deficiencies of nicotinic acid,[46] vitamin B2,[47] vitamin B6,[48] vitamin B12,[49,50] and folate.[51] However, characteristic symptoms associated with these individual vitamin deficiencies were not present. These clinical pictures include anorexia, diarrhea, erythematous and

hyperkeratotic dermatitis, and mental changes in pellagra (nicotinic acid deficiency); cheilosis, glossitis, keratoconjunctivitis, and dermatitis involving nasolabial folds, scrotum and labia in vitamin B2 deficiency; and myelopathy in vitamin B12 and folate deficiency. Thus, these vitamin deficiencies could modify thiamine-deficiency effects to some extent, but could not be the major factors for variations in our series, because these characteristic symptoms were not present.

Electrophysiologic and histopathologic features

According to previously reported data concerning nonalcoholic thiamine-deficiency neuropathy,[29] nerve conduction studies mainly showed evidence of axonal degeneration more predominantly in the lower



Transverse section of sural nerve in a patient with thiamine-deficiency neuropathy. Note myelin ovoids, significant reduction of the density of myelinated fibers. Large myelinated fibers are reduced more than small myelinated fibers. Endoneurial edema with enlargement of the subperineurial space is present.

extremities with some slowing of conduction velocities. Sural nerve biopsy specimens also indicated axonal neuropathy (Figure). Myelinated fiber density was significantly reduced. On average, the density of large myelinated fibers was 22% that of normal controls, while the density of small myelinated fibers was 33% that of normal controls. Axonal sprouting was scarce in all cases. Predominant loss of large myelinated fibers was a characteristic feature. Reduction of unmyelinated fibers was also seen especially in cases with severe myelinated fiber loss.

Findings of nerve conduction studies on thiamine-deficiency neuropathy with chronic alcoholism were similar to those on nonalcoholic thiamine-deficiency neuropathy.[29] Sural nerve biopsy specimen findings were variable, occupying a range between nonalcoholic thiamine-deficiency neuropathy and alcoholic neuropathy with normal thiamine status; the latter showed characteristically small fiber-predominant loss and scarce subperineurial edema and myelin irregularity.

Treatment and prognosis

Patients were treated with thiamine. Initially, a 100-mg daily intravenous dose of fursultiamine, a disulfide derivative of thiamine, was given. A 75-mg daily oral dose of fursultiamine was administered subsequently.[13] Cardiomegaly, edema of the legs, and arrhythmias improved dramatically within a few days after initiation of thiamine administration. Paresis of the bladder and intestine improved within a few weeks. Improvement of muscle strength was evident at one week. Recovery of sensation was less dramatic than motor recovery. Wernicke's encephalopathy improved substantially, but Korsakoff psychosis persisted in many patients.

Overall, complete recovery was very rare, and some residual deficit persisted in most patients, although functional status was favorable after thiamine administration. Substantial functional recovery, particulary in motor and autonomic involvement, was achieved by thiamine supplementation at 3 to 6 months, but sensory symptoms and Korsakoff psychosis were particularly likely to show residual deficits. Patients with rapid progression were more likely to respond well to thiamine supplementation than those with slow progression. Treatment should be initiated as early as possible. We believe that thiamine should assayed and administered early in patients with sensorimotor neuropathy and a history of gastrointestinal surgery, heavy alcohol intake, or dietary imbalance. Multivitamin supplementation is also recommended

because vitamins other than thiamine can be deficient as well, and modify the clinical features. Prophylactic supplementation of thiamine following gastrectomy also is recommended.

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CASE REPORT

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Widespread active inflammatory lesions in a case of HTLV-I-associated myelopathy lasting 29 years

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Abstract An autopsy case of human T-lymphotropic virus type I (HTLV-I)-associated myelopathy (HAM) of 29 years' duration is reported. The patient had no history of surgery or blood transfusion and likely contracted HTLV-I sexually while traveling in an endemic area. At age 45, the patient began to experience gait disturbance; he later developed spastic tetraparesis. Autopsy revealed marked gross spinal cord atrophy, particularly in the middle to lower thoracic levels. Myelin and axonal degeneration were identified predominantly in the middle to lower thoracic spinal cord, extending into the medulla oblongata and lumbar cord. Inflammatory infiltrates of mononuclear cells were diffuse in the white and gray matter of the spinal cord and medulla oblongata, particularly in perivascular areas. These infiltrates were also observed in perivascular areas of the pons, midbrain, cerebellum, and cerebrum. More than half of the infiltrating cells were positive for the pan-T cell marker UCHL-1, but some were positive for the B cell marker SL-26. There were far more CD8-positive cells than CD4-positive cells in the spinal parenchyma and perivascular areas. Neurons in the anterior horn, Clarke's column, and intermediolateral column were relatively well preserved. Active chronic inflammation was indicated. Despite the 29-year history of HAM, the presence of an active inflammatory reaction is surprising. We discuss possible modulation of the histopathological manifestations of HAM by corticosteroid therapy.

Keywords HTLV-I-associated myelopathy · Active inflammatory lesion · Myelopathy · Spastic tetraparesis · Corticosteroid therapy

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Introduction

Human T-lymphotropic virus type I (HTLV-I) retrovirus causes adult T cell leukemia and lymphoma and a chronic progressive myelopathy known as HTLV-I-associated myelopathy (HAM) [3, 8, 13]. HAM was first reported and established as a clinical entity in 1986 by Osame et al. [14]. It is now known that the pathogenesis of HAM involves both viral infection and host immune response [8]. Clinically, HAM is characterized by slow progression of spastic paraparesis, involving mainly the lower extremities, pyramidal signs, and mild sensory and sphincter disturbances [7, 11, 13, 14]. Akizuki et al. [1] reported the first autopsied case of HAM in 1987, and more than 30 autopsied cases have been reported since. Pathology studies of autopsied cases indicate several characteristic pathological changes associated with HAM: marked infiltration of lymphocytes and monocytes, presence of foamy macrophages, and demyelination and axonal loss in the spinal cord white and gray matter, particularly in the anterolateral column of the middle to lower thoracic segments [1, 5, 6, 8, 12]. Here, we report an autopsied case of HAM of 29 years' duration with widespread active chronic inflammatory

cell infiltration that amends the phenotypic spectrum of HAM.

Clinical summary

A Japanese man who was born and still lived in Aichi prefecture, Japan, a non-endemic area for HTLV-I infection, began to experience gait disturbance at the age of 45 years. He had no history of surgery or blood transfusion at that time or any family history of HTLV-I infection. Between the ages of 25 and 43 years, he traveled frequently to Southeast Asia (Taiwan, South Korea, and Thailand) and Kyushu, Japan, endemic areas for HTLV-I infection, and he had many opportunities for sexual transmission of the virus during his travels. The gait disturbance increased slowly but steadily, and the patient developed dysuria. At age 57, he was admitted to Aichi Medical University Hospital. Upon admission, neurological examination disclosed spastic gait, weakness and atrophy of the legs, and hyperreflexia in all extremities with bilateral Babinski's sign and ankle clonus. Cranial nerve function and superficial and deep sensation were normal. Cerebrospinal fluid (CSF) contained 2 mononuclear cells/mm^3, 23 mg/100 ml total protein, and 64 mg/100 ml sugar; pressure was normal. Serum and CSF were positive for anti-HTLV-I antibodies. A serological test for syphilis was negative. No abnormal lymphocytes were found in the blood or CSF. There was no other possible cause of immunodepression, such as HIV infection. The patient was diagnosed with HAM on the basis of the clinical symptoms and laboratory test results. Corticosteroid therapy was administered at this stage, and for a short time the symptoms and signs were slightly improved. However, the spastic paraparesis continued to worsen, and the patient became bed-ridden at age 70. Corticosteroid therapy was discontinued at this stage.

The patient visited Nagoya University Graduate School of Medicine for further evaluation at age 71. Examination revealed vertical gaze palsy, highly spastic tetraparesis with articular contracture of all four extremities, highly accentuated reflexes in all four extremities with bilateral Babinski's sign, and neurogenic bladder. Superficial sensation was normal, but the sense of vibration was mildly decreased in both legs. CSF contained 1 mononuclear cell/mm^3, 43 mg/100 ml total protein, and 63 mg/100 ml sugar. Serum and CSF were positive for HTLV-I antibodies at titers of 8,192× and 128×, respectively. Magnetic resonance imaging of the brain and spinal cord revealed mild frontotemporal atrophy, but no spinal cord lesions were identified. The articular contracture was diagnosed as HTLV-I-associated arthropathy on the basis of X-ray findings. Parkinsonism, including rigidity in all extremities and tremor in the upper extremities, was observed and treated with L-DOPA, which had an observable effect. The HAM diagnosis was confirmed, and Parkinson's disease was also diagnosed.

The patient was transferred to Higashi Nagoya National Hospital at age 72. He underwent gastrostomy at age 73 and tracheotomy at age 74. He suffered from frequent bronchopneumonia and died at age 74, 29 years after the onset of HAM and 4 years after the onset of Parkinson's disease.

The patient did not receive immunosuppressive treatment at any time during the 29 years of his illness or corticosteroid treatment during the 5 years prior to death.

Autopsy: neuropathological examination and findings

A thorough autopsy was performed 9 h after the patient's death. Central nervous system tissues were fixed in 20% neutral formalin for 4 weeks and embedded in paraffin, and 8-μm-thick sections were prepared. The sections were mounted, deparaffinized, hydrated, and stained. For routine neuropathological study, sections were subjected to hematoxylin-eosin, Klüver-Barrera, and Bodian staining. Immunohistochemical analysis was carried out with antibodies to pan-T cells (UCHL-1, mouse monoclonal diluted 1:100; Dako, Glostrup, Denmark), pan-B cells (SL-26, mouse monoclonal diluted 1:100; Dako), helper/inducer T cells (CD4, mouse monoclonal diluted 1:100; Nichirei, Tokyo, Japan), suppressor/cytotoxic T cells (CD8, mouse monoclonal diluted 1:100; Dako), macrophages (CD68, mouse monoclonal diluted 1:100; Dako), microglia (HLA-DR, mouse monoclonal diluted 1:200; Dako), and α-synuclein (mouse monoclonal diluted 1:100; Chemicon, Temecula, CA). Antibody binding was detected by the labeled streptavidin-biotin method (Dako LSAB kit; DAKO). Peroxidase-conjugated streptavidin was visualized with 3,3′-diaminobenzidine (DAB; Wako, Osaka, Japan) as the final chromogen. All immunostained sections were counterstained lightly with Mayer's hematoxylin.

Macroscopic findings

The brain weighed 1,480 g and showed moderate symmetric frontotemporal atrophy but no cerebellar or brainstem atrophy. Horizontal sections of brainstem showed depigmentation in the substantia nigra and locus ceruleus. The spinal cord showed marked atrophy and grayish discoloration of the anterolateral column, particularly in the thoracic region (Fig. 1A–C). Anterior and posterior nerve roots were well preserved, and the vessels of the brain and spinal cord showed no gross abnormalities.

Microscopic findings

Fibrous thickening of the pia mater with mononuclear cell infiltration was observed throughout the spinal cord. Inflammatory infiltrates consisting of many mononuclear cells were observed in perivascular areas and in the

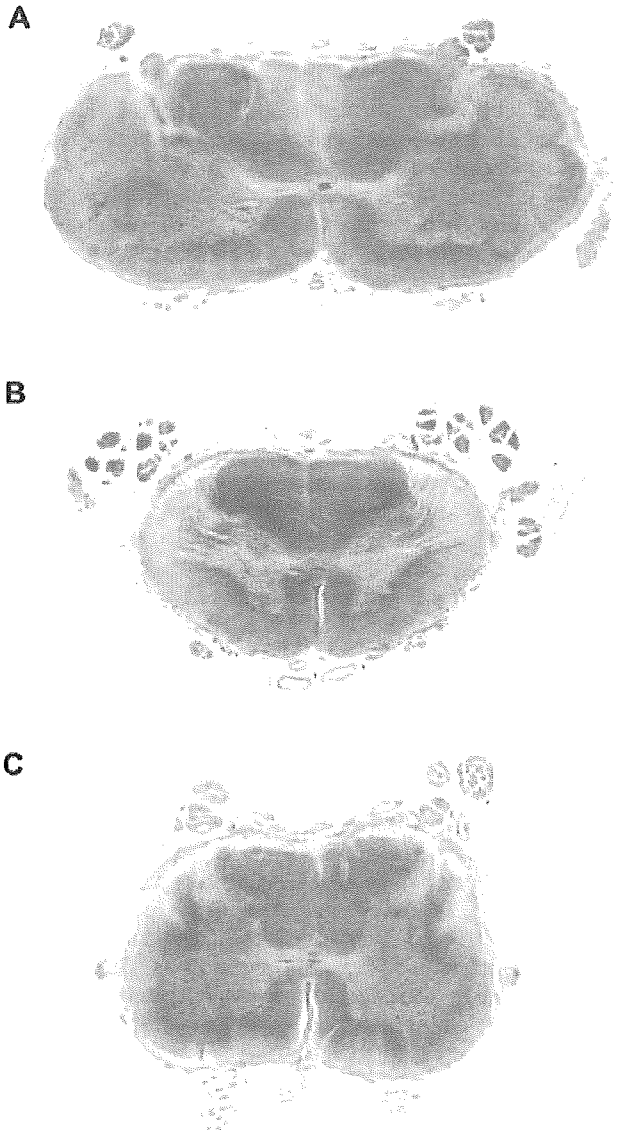

Fig. 1 Macroscopically, the spinal cord shows obvious bilateral atrophy of lateral and anterior columns with myelin pallor. A cervical cord, B lower thoracic cord, C lumbar cord. Klüver-Barrera stain

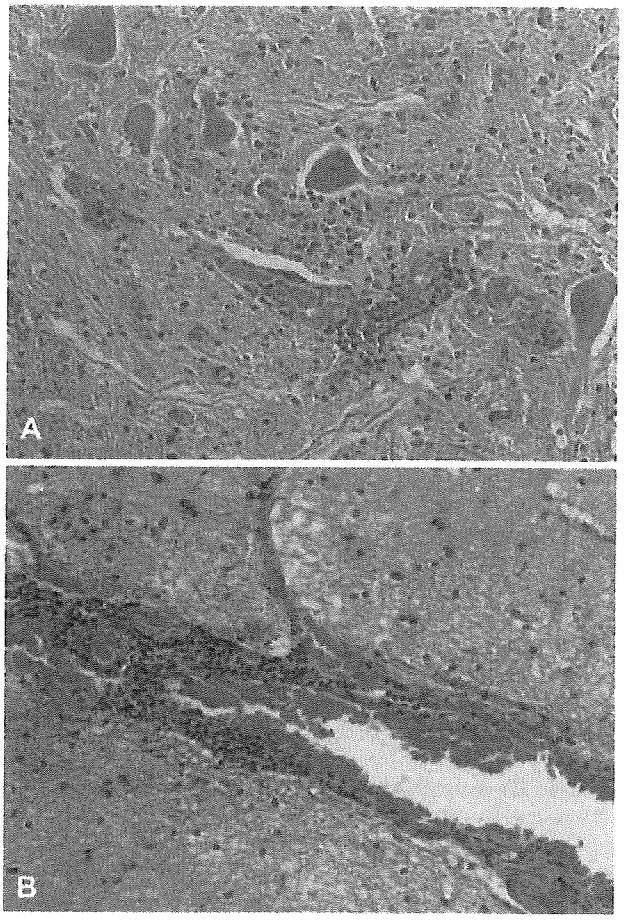

Fig. 2 Perivascular and parenchymal mononuclear cell infiltration indicates an active chronic lesion. A Lumbar cord, B temporal lobe. Hematoxylin and eosin stain, original magnification ×20

parenchyma of the spinal cord and medulla oblongata (Fig. 2A). Milder lesions composed of cuffs of perivascular cells were scattered in the pons, midbrain, cerebellum, and cerebrum, without adjacent tissue reaction or parenchymal lesions (Fig. 2B). The distribution of inflammatory lesions was horizontally symmetric at all spinal column levels, and the anterolateral column was the most severely affected.

Degeneration of the lateral corticospinal and spinocerebellar or spinothalamic tracts was evident. Demyelination and axonal degeneration appeared along the entire spinal cord, particularly in the middle to lower thoracic regions, and in the medulla oblongata. Demyelination was more prevalent than axonal degeneration. Axonal degeneration was more commonly found in small myelinated fibers than in large myelinated fibers. The posterior column was relatively free of degeneration and inflammatory infiltrates. Capillary proliferation was prominent in the spinal cord, particularly in the cervical and lumbar regions. Small-vessel hyalinization was visible in both the white and gray matter, particularly in the cervical and lumbar regions of the spinal cord, and was accompanied by gliosis in the surrounding tissues. In the vessels of the spinal cord, the endothelium showed no appreciable changes, and no obstructive changes were found. Neurons in the anterior horn, intermediolateral column, and Clarke's column were relatively well preserved. The number of large motor neurons in the anterior horn appeared normal. These findings indicated the presence of active chronic inflammatory cell infiltration. There was no evidence of malignancy.

Immunohistochemical findings

More than half of the infiltrated cells stained positively for the pan-T cell marker UCHL-1 (Fig. 3A). UCHL-1-positive cells were identified predominantly around perivascular areas and in the parenchyma of the spinal cord and medulla oblongata. In the pons, midbrain, cerebellum, and cerebrum, UCHL-1-positive cells were identified around perivascular areas and meninges. Cells that stained positively for the pan-B cell marker SL-26 were sparse in perivascular areas and in the parenchyma of the spinal cord and medulla oblongata. CD4-positive cells were rare; a few were identified exclusively in meningeal lesions and perivascular areas of the spinal cord, brainstem, cerebellum, and cerebrum. Few CD4-positive cells were identified in the spinal cord parenchyma. CD8-positive cells were present in perivascular areas of the spinal cord, brainstem, cerebellum, and cerebrum and scattered in the parenchyma of the spinal cord and medulla oblongata (Fig. 3B). Although a few CD68-positive cells were identified, predominantly in perivascular areas and in the parenchyma of the spinal cord and medulla oblongata, no HLA-DR-positive cells were identified in the parenchyma or perivascular areas of these regions.

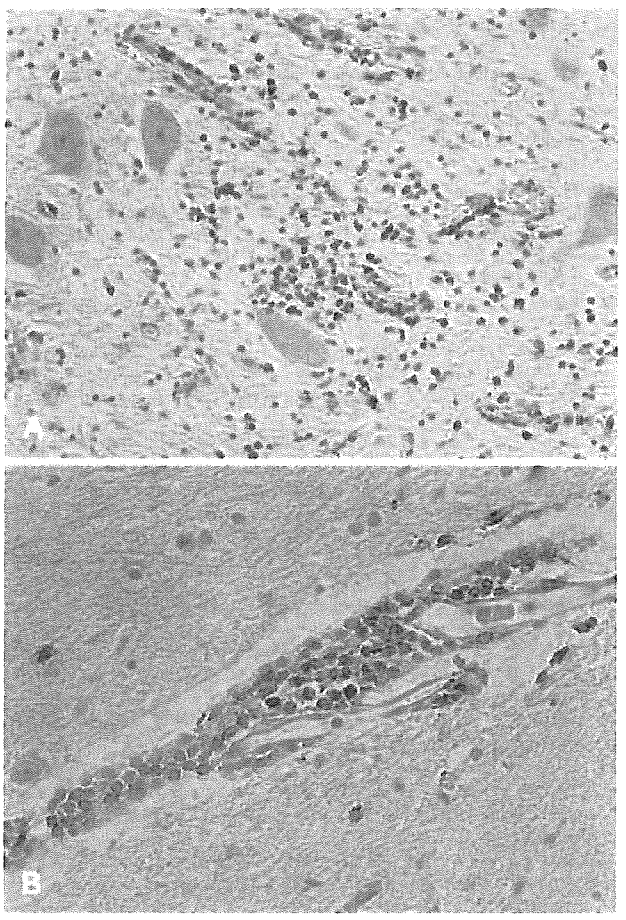

Fig. 3 Perivascular and parenchymal inflammatory infiltration in the spinal cord. **A** Lumbar cord, UCHL-1 stain; **B** medulla oblongata, CD8 stain. Original magnification **A** ×20, **B** ×40

Parkinson's disease lesions

Although neuronal loss was mild, many scattered free melanin granules were present in the substantia nigra and locus ceruleus. Lewy bodies were frequent in the substantia nigra, locus ceruleus, and sympathetic ganglia. A few cortical Lewy bodies were also identified in the cerebral cortex. These Lewy bodies immunolabeled with α-synuclein antibody. Interestingly, many residual neurons in the intermediolateral column of the thoracic region showed α-synuclein immunolabeling. No other significant age-related pathologies were identified.

Discussion

HTLV-I is transmitted primarily through breast milk, sexual intercourse, blood transfusion, or sharing of contaminated needles by drug users [8]. The incubation period varies, ranging in most cases from a few years to several decades [8]. In the present case, we believe that HTLV-I was transmitted sexually several years before the onset of symptoms. The incubation period depends to some extent on the route of infection: blood transfusion is not only a much more efficient means of transmitting infection than breast-feeding or sexual intercourse, but it also tends to be associated with a shorter incubation period before the development of disease [8]. HAM patients with a history of blood transfusion respond well to corticosteroid therapy, although the exact pharmacological mechanism is not known [12, 15].

The pathological findings of previously reported autopsy cases of HAM are similar and include spinal cord lesions consisting of marked perivascular and parenchymal infiltration of lymphocytes and foamy macrophages, demyelination, axonal loss, and gliosis [1, 6, 7, 8, 9]. The characteristic lesion distribution suggests a chronic inflammatory process that starts in the middle to lower thoracic spinal cord and extends gradually both rostrally and caudally [6, 7]. Izumo et al. [9] offer a detailed neuropathological description of HAM as follows: (1) T cell-dominant mononuclear cell infiltration; (2) diffuse and symmetric degeneration of the anterolateral columns and inner portion of the posterior columns, involving both myelin and axons; (3) presence of cytotoxic T cells and apoptosis of helper/inducer T cells; (4) in vivo localization of HTLV-I provirus in perivascular infiltrated T cells; and (5) accentuation of inflammatory lesions at sites of slow blood flow.

The present case showed active chronic inflammatory infiltration that is surprising despite the 29-year presence of HAM. Prior descriptions of HAM with clinical histories of more than 10 years include monotonic degen-

eration of the spinal cord and gliosis with little or no inflammatory cell infiltration in perivascular areas [9]. Perivascular accumulation of inflammatory cells and parenchymal exudation of T lymphocytes and monocytes apparently subside approximately 3 years after onset; perivascular cuffing then becomes less conspicuous, and inflammatory cells appear only sporadically in the spinal cord [4, 16]. The severity of spinal cord inflammation in HAM correlates roughly with the duration of the disease process [2]. Sasaki et al. [16] reported an autopsy case of HAM of 28 years' duration. Their case was purely degenerative, and the spinal cord showed no inflammatory reaction. Aye et al. [2] reported two autopsy cases of HAM of 15 and 21 years' duration. They observed very few inflammatory cells in the spinal cord and brain, although there was marked fibrosis of blood vessel walls in the spinal cord. However, in the reports of these cases, the treatment protocols were not indicated, and whether corticosteroid/immunosuppressive treatment was continued until death is not known [2, 16]. Our case did not resemble these cases; rather, our findings matched those reported for cases of HAM of shorter clinical duration [6, 7]. There was no other possible cause of immunodepression, including HIV infection in our case, and the CSF findings remained consistently normal, without pleocytosis.

A variety of treatments have been used for patients with HAM since the disease concept was proposed. Although long-term corticosteroid administration is a popular therapeutic option for HAM in Japan, our patient did not receive immunosuppressive treatment at any time during the 29 years of his illness or corticosteroid therapy during the 5 years prior to death. Possible modulation of the histopathological manifestations of HAM by corticosteroid therapy has been studied [4]. Immunosuppressive or corticosteroid treatment for HAM may reduce the intensity of the inflammatory reaction and consequent tissue damage, but it may also protract and retard the reparative process in damaged tissue [4]. Ohama et al. [12] reported an autopsied case of HAM with very mild infiltration of lymphocytes in the spinal cord lesions but also of short duration. Their pathological findings indicate a positive response to corticosteroid treatment and suggest that corticosteroids suppress the perivascular and parenchymal infiltration of lymphocytes characteristic of HAM [12]. There are two important potential causes of the active chronic inflammatory change in our case: (1) venereal transmission of HTLV-I, and (2) omission of any corticosteroid therapy during the 5 years prior to the patient's death.

Previously reported cases of long-term HAM showed very few UCHL-1- or CD8-positive lymphocytes in the spinal cord [2]. Although only a few CD4-positive inflammatory cells were found in the spinal cord and medulla oblongata in our case, many UCHL-1- and CD8-positive inflammatory cells were present in these areas. Umehara et al. [17] reported that CD4-positive cells are less dominant in chronic inactive lesions than in chronic active lesions. Immune responses in HAM spinal cord lesions change gradually over the course of the illness [8]. Infected CD4-positive lymphocytes are likely targets of the immune response; apoptotic CD4-positive lymphocytes are found within the inflamed spinal parenchyma, and an increase in the ratio of CD8- to CD4-positive cells in the spinal cord occurs during progression of the disease [17, 18]. Demyelination and axonal damage may be caused by the release of cytokines and other inflammatory mediators from infected and reactive lymphocytes, macrophages, and possibly astrocytes [11]. Alternatively, the cytotoxic and humoral response to infected lymphocytes may cause local autoimmune damage to the spinal cord parenchyma [10, 11]. Further examination of active chronic lesions is needed to clarify the mechanism underlying the development of inflammatory lesions and the pathogenesis of HAM.

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Age associated axonal features in HNPP with 17p11.2 deletion in Japan

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PAPER

Age associated axonal features in HNPP with 17p11.2 deletion in Japan

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Objective: To clarify age related changes in the clinicopathological features of hereditary neuropathy with liability to pressure palsy (HNPP) in Japanese patients with deletion of 17p11.2, particularly concerning axonal abnormalities.

Methods: Forty eight proband patients from 48 HNPP families were assessed as to clinical, electrophysiological, and histopathological features, including age associated changes beyond those in controls.

Results: Motor conduction studies showed age associated deterioration of compound muscle action potentials in nerves vulnerable to repetitive compression (median, ulnar, and peroneal nerves), but not in others such as the tibial nerve. Sensory conduction studies revealed more profound reduction of action potentials than motor studies with little age related change. Large myelinated fibre loss was seen in the sural nerve irrespective of age at examination.

Conclusions: Irreversible axonal damage may occur at entrapment sites in motor nerves in HNPP patients, progressing with aging. Sensory nerves may show more profound axonal abnormality, but without age association. The electrophysiological features of HNPP are presumed to be a mixture of abnormalities occurring from early in life and acquired features caused by repetitive insults at entrapment sites. Unlike Charcot-Marie-Tooth disease type 1A, age associated axonal damage may not occur unless the nerves are subjected to compression.

Hereditary neuropathy with liability to pressure palsy (HNPP) is an autosomal dominant disorder characterised by recurrent transient nerve palsies associated with compression at the typical anatomic sites of potential nerve entrapment.[1 2] Tomacula, which represent focal thickening of the myelin sheath, characteristically are seen in both sensory and motor nerves in HNPP.[3–6] This disorder usually is associated with a 1.5 Mb deletion of locus 17p11.2, which contains the gene for peripheral myelin protein 22 (PMP22).[7–9] HNPP therefore appears to represent a reciprocal product of Charcot-Marie-Tooth disease type 1A (CMT1A), which is associated with duplication of PMP22.[10] PMP22 is an important factor for regulation of Schwann cell proliferation and apotosis.[11] As the Schwann cell plays an important role in maintenance of the axon, axonal loss associated with demyelination has been reported to occur in patients with CMT1A.[12–15] Age associated reduction of compound muscle action potential (CMAP) amplitude resulting from large-axon loss has been reported in CMT1A[15] and is closely related to clinical manifestations and functional impairment.[14 15]

In Western countries, the clinical and electrophysiological features of HNPP have been described on a large scale.[16–20] Characteristic electrophysiological findings are multifocal slowing of conduction at sites of entrapment, prolonged distal latency (DL), mild slowing of motor nerve conduction velocity (MCV), and diffuse abnormality of sensory nerve conduction velocity (SCV).[16 18–20] However, there have been no similar large scale investigations of the clinical and electrophysiological features of HNPP in Asian subjects. Furthermore, it has not been clarified whether electrophysiological and histopathological abnormalities, particularly axonal features, worsen with aging in HNPP as happens in CMT1A.

The present investigation was carried out in Japan and we studied HNPP including its electrophysiological and histopathological features, especially in relation to aging.

METHODS

Patients and DNA diagnosis

An HNPP survey was conducted by the study group for hereditary neuropathy in Japan under the auspices of the Ministry of Health, Labor, and Welfare.[15 21] A total of 48 proband patients from 48 HNPP families, whose 17p11.2 deletion was confirmed, were investigated. The mean age (SD) of the patients at examination was 41.8 (18.5) years (table 1). All subjects underwent clinical examination by at least one neurologist. Patients with chronic alcoholism or vitamin deficiency were not included. Four patients manifested mild glucose intolerance. To confirm the diagnosis of HNPP, DNA analyses for the presence of a chromosome 17p11.2–12 deletion, which includes a 1.5 Mb region containing the PMP22 gene between CMT1A-REP repeats, were performed in all patients. For most patients these analyses were performed at the Department of Neurology at Nagoya University Graduate School of Medicine as described previously,[22] while DNA was analysed at other institutions for the rest. The characteristic deletion in HNPP was detected by Southern analysis, probing with PMP22 cDNA, and CMT1A-REP fragments as described previously.[22–24] Hybridisation with PMP22 cDNA and pNEA102, pHK1.0P, and pHK5.2 probes, which map within the CMT1A-REP, was carried out

Abbreviations: CMAP, compound muscle action potential; CMT1A, Charcot-Marie-Tooth disease type 1A; DL, distal latency; HNPP, hereditary neuropathy with liability to pressure palsy; MCV, motor nerve conduction velocity; PMP22, peripheral myelin protein 22; SCV, sensory nerve conduction velocity; SNAP, sensory nerve action potential

to determine the gene dose of the 1.5 Mb region containing PMP22. Deletion of one copy of the PMP22 gene, compared to the presence of two copies in normal controls, was genetically identified as HNPP. Informed consent was obtained in all patients, and the study as a whole was approved by the Ethics Committee of Nagoya University Graduate School of Medicine.

Electrophysiological study

Motor and sensory conduction was measured in the median, ulnar, tibial, peroneal, and sural nerves, using a standard method with surface electrodes for stimulation and recording.[25 26] Motor conduction was investigated in the median, ulnar, tibial, and peroneal nerves, recording from the abductor pollicis brevis, abductor digiti minimi, abductor hallucis brevis, and extensor digitorum brevis muscles, respectively. The following nerve segments were used for calculating MCV: wrist to elbow for the median nerve, wrist to distally at the elbow for the ulnar nerve, ankle to popliteal fossa for the tibial nerve, and ankle to distally at the fibular head for the peroneal nerve. Sensory conduction was investigated in the median, ulnar, and sural nerves, using antidromic recording from ring electrodes at the second and fifth digit for the median and ulnar nerves respectively, and bar electrodes at the ankle for the sural nerve. SCV was calculated for the distal segment. Amplitudes of CMAP and sensory nerve action potential (SNAP) were measured from the baseline to the first negative peak. Waveforms also were analysed to assess temporal dispersion. For motor nerves, we measured duration from the onset to the first crossing of the baseline in the CMAP.[27] For sensory nerves, duration from the onset of the SNAP to the first negative peak rather than to the first crossing of the baseline was measured to avoid artefacts from overlapping muscle action potentials.[25] This was necessary because some motor axons have thresholds similar to those of large myelinated sensory axons, resulting in superimposition on the SNAP that modifies the waveform, especially when abnormal nerves are examined.[28 29] Because of a delay at the neuromuscular junction, the initial phase of the waveform of SNAP is less likely to be affected by muscle action potentials than the later phase.[29]

Control values were obtained in 171 normal volunteers (51.0 (SD 16.3) years of age; male:female, 89:82) for the median nerve, 170 (51.2 (SD 16.4) years of age; male:female, 88:82) for the ulnar nerve, 161 (51.8 (SD 16.6) years of age; male:female, 85:76) for the tibial nerve, 171 (54.2 (SD 16.7) years of age; male:female, 92:79) for the peroneal nerve, and 163 (52.2 (SD 16.7) years of age; male:female, 85:78) for the sural nerve.

Table 1 Characteristics of 48 Japanese HNPP probands with deletion of 17p11.2–12

Clinical features	n (%)
Age at onset, years	33.1 (SD 19.3)
Age at examination, years	41.8 (SD 18.5)
Men/women	38/10
Family history	24 (50%)
Athletic impairment during childhood	1 (2%)
Pes cavus or hammer toe	2 (4%)
Atrophy in the legs	6 (13%)
Pattern of neuropathy	
Multiple mononeuropathy	41 (85%)
Symmetric polyneuropathy	7 (15%)
History of transient nerve palsy	
Median nerve	11 (23%)
Ulnar nerve	18 (38%)
Radial nerve	7 (15%)
Peroneal nerve	29 (60%)
Brachial plexus	10 (21%)
Activity of daily living	
Able to walk	46 (96%)
Unable to walk	2 (4%)
Bedridden	0

Age at onset, age at first awareness of neuropathic symptoms; Family history, obvious family history of recurrent transient nerve palsies.

Histopathological study

Sural nerve biopsy was performed in 14 patients as described previously.[30 31] Informed consent was obtained beforehand. Specimens were divided into two portions. The first portion was fixed in 2.5% glutaraldehyde in 0.125 M cacodylate buffer (pH 7.4) and embedded in epoxy resin for morphometric study. The density of myelinated fibres was assessed in toluidine blue stained semithin sections using a computer assisted image analyser (Luzex FS; Nikon, Tokyo, Japan) to calculate the densities of small and large myelinated fibres as described previously.[32–34] A fraction of the glutaraldehyde fixed sample was processed for a teased fibre study, in which at least 100 single fibres were isolated; their pathologic condition was assessed microscopically according to criteria described previously.[32 35] The second portion of the specimen was fixed in 10% formalin solution and embedded in paraffin. Sections were cut by routine methods and stained with haematoxylin and eosin as well as by the Klüver-Barrera and Masson trichrome methods. Control values were obtained from 13 autopsy cases in which patients died of non-neurologic diseases (48.5 (SD 23.5) years of age; male:female, 7:6). Specimens were processed in the same manner as for HNPP patients.

Statistical analysis

Quantitative data are presented as the mean (SD) and were compared with control values using the Mann-Whitney U test. To determine the relationship of electrophysiological and histopathological indices and age at examination, Pearson's correlation coefficient analysis was carried out. To determine whether worsening of these indices in HNPP patients with aging was significantly greater than in normal controls, regression slopes of patient and control groups were compared. Values of p less than 0.05 were considered to indicate significance.

RESULTS

Clinical features

The age at first awareness of neuropathic symptoms in the 48 probands was 33.1 (SD 19.3) years (table 1). The male:female ratio was 38:10. An obvious family history of recurrent transient nerve palsies was present for 24 patients (50%). Only one patient (2%) reported athletic impairment during childhood. Deformity in the distal part of the lower limbs such as hammer toe or pes cavus was present in two patients (4%). Atrophy was noted in the leg in six patients (13%). The pattern of neuropathic symptoms was multiple mononeuropathy associated with recurrent transient nerve palsies in 41 patients (85%), while the other seven (15%) manifested mainly a symmetric polyneuropathy pattern. A history of transient nerve palsy was noted in the median, ulnar, radial, and peroneal nerves in 11 (23%), 18 (38%), seven (15%), and 29 (60%) patients, respectively. Signs of brachial plexus palsy were reported in 10 (21%). With respect to the activities of daily living, all patients were non-disabled or only mildly disabled, except for two (4%) who became unable to walk.

Electrophysiological features

Motor conduction studies showed variable degrees of abnormality in individual nerves (table 2). For the median nerve, MCV was significantly slowed compared to normal controls ($p<0.0001$). This slowing of MCV was present regardless of age at examination, and there was no