untreated PD. It is needed to evaluate the function of the GI tract in patients with early-stage, untreated PD.

Recently, the ^{13}C-acetate breath test (^{13}C-ABT) has been widely recognized as useful for evaluating gastric emptying because it is less invasive than isotope or acetaminophen methods [10]. It was reported that the ^{13}C-ABT was a reliable and non-invasive tool for the analysis of gastric emptying rates of liquid phases without radiation exposure [11]. Though the ^{13}C-ABT is an isotopic method, it uses a stable isotope not emitting ionizing radiation and is feasible methods for PD patients [12].

The aim of this study is to compare the gastric emptying between patients with early-stage, untreated PD and patients with advanced-stage, treated PD, and healthy volunteers using the ^{13}C-ABT. We tested whether there is the delayed gastric emptying of patients with early-stage, untreated PD.

Methods

Patients

Our study population consisted of 60 patients with an initial diagnosis of PD on the basis of the UK Parkinson's Disease Society Brain Bank Clinical diagnostic criteria [13, 14] and 20 healthy volunteers (control group). The control group was ten men and ten women, median age 69.0 years (range 63–73 years). The patients were divided into two groups: 20 patients with early-stage, untreated PD [eight men and 12 women; median age 70.5 years (range 54–82 years); disease duration 0.9 years (range 0.3–2.5 years)] and 40 patients with advanced-stage, treated PD [14 men and 26 women; median age 67.0 years (range 42–86 years); disease duration 6.0 years (range 3.0–31.0 years)]. Each group of the PD patients was consecutively consulted at our hospital. Modified Hoehn and Yahr classification of the patients with early-stage, untreated PD was stage 1–2, according to the Unified Parkinson's Disease Rating Scale [15, 16]. Modified Hoehn and Yahr classification of the patients with advanced-stage, treated PD was stage 3–4. All PD patients with early-stage were not treated with any medications at first visit, and were followed up for at least 1 year after this study in order to rule out atypical parkinsonism. All PD patients with advanced-stage were being treated with antiparkinsonian medications (long-term L-dopa therapy). No patient was treated with drugs that might alter gastric emptying. None of the PD patients had basic diseases such as liver dysfunction, renal failure, cardiopulmonary disease, diabetes mellitus, GI disease or history of gastric surgery. Clinical characteristics (including age, gender, body mass index) were not significantly different among the PD groups with early-stage and with advanced-stage, or the control group. The results of blood examinations were within normal range. In addition, there were no differences between the PD groups in terms of pepsinogen I, II, and serum gastrin levels, hemoglobin A1c (HbA1c), which might affect gastric motility [17]. The positive ratio of immunoglobulin G anti-Helicobacter pylori antibody did not differ significantly between the PD groups and no patients had past history of peptic ulcer. The positive ratio of orthostatic hypotension (OH) [18] and coefficient of variation of R-R intervals ($CV_{R\text{-}R}$) [19], heart/mediastinum (H/M) ratio of I-[123]-metaiodo-benzylguanidine (MIBG) scintigraphy [20] were not significantly different between the PD groups.

Informed consent was obtained from each subject prior to participation in this study. The study protocol was approved by the Ethical Committee of Gifu University, and was carried out in accordance with the 1975 Declaration of Helsinki.

Gastric emptying examination

The GE examination was carried out using the ^{13}C-breath test according to Ghoos [10] with slight modifications. PD patients and healthy volunteers were tested after an overnight fast of 12 h. All PD patients did not take any antiparkinsonism drug over 24 h. Early in the morning, PD patients and healthy volunteers took the liquid test meal (Racol: TM, 200 kcal/200 ml; Otsuka Pharmaceuticals Co., Ltd., Tokyo, Japan) containing 100 mg ^{13}C-sodium acetate. Thereafter, an expiration breath sample was collected every 10 min for 4 h and analyzed for $^{13}CO_2$ using an IR spectrophotometer (UBiT-IR300; Otsuka Electronics Co., Ltd., Tokyo, Japan). During the examination, all subjects were in a sitting position.

The principle of ^{13}C-ABT is ingestion of a liquid test meal containing ^{13}C-acetate, gastric emptying, absorption from the digestive tract, metabolism in the liver (production of $^{13}CO_2$), expiration from the lung, and increase of $^{13}CO_2$ in expired breath.

Mathematical analysis

The data were used for mathematical curve fitting. A best fit curve of expired $^{13}CO_2$ was constructed for each subject. The %-$^{13}CO_2$ cumulative excretion in the breath was assessed using a non-linear regression formula [21, 22]: $y = m\ (1 - e^{-kt})^{\beta}$ to fit the curve of the cumulative ^{13}C recovery. The %-$^{13}CO_2$ excretion per hour was fitted to the formula $mkbe^{-kt}\ (1-e^{-kt})^{\beta-1}$. T is time and m, k, and β are constants. The value of m represents the total cumulative $^{13}CO_2$ recovery when the time is infinite. The half emptying time (HET) was calculated using the formula: $HET = -1/k\ \ln(1 - e^{-1/\beta})$. T_{max} is the peak time of the ^{13}C%–dose-excess curve (%–dose/h) based on a time

profile of the $^{13}CO_2$ excretion rate. The parameters were estimated with Excel software (Microsoft Co., Ltd., Redmond, WA).

Statistical analysis

Categorical variables were compared using Fisher's exact probability test. Other variables were expressed as median (range). Medians were compared using Mann–Whitney's *U* test. All analyses were carried out on StatView statistical software, version 5.0 (Abacus Concepts, Inc., Berkeley, CA). A *P* value less than 0.05 was considered significant.

Results

Controls and early-stage, untreated PD patients (Figs. 1, 2)

The examinations were safely carried out in all PD patients and controls. The T_{max} was significantly delayed in patients with early-stage, untreated PD (median 1.17 h, range 0.67–1.83 h) as compared with the controls (median 0.83 h, range 0.67–1.00 h) ($P < 0.001$). The HET was significantly delayed in patients with early-stage, untreated PD (median 2.04 h, range 1.75–3.07 h) as compared with the controls (median 1.44 h, range 1.30–1.64 h) ($P < 0.001$). There was absolutely no overlap in the range of the HET between controls and early-stage, untreated PD patients.

Controls and advanced-stage, treated PD patients (Figs. 1, 2)

In all patients with advanced-stage, treated PD, the blood concentration of L-dopa was below 25 ng/ml (below the detection level) at start of examination. The T_{max} of GE

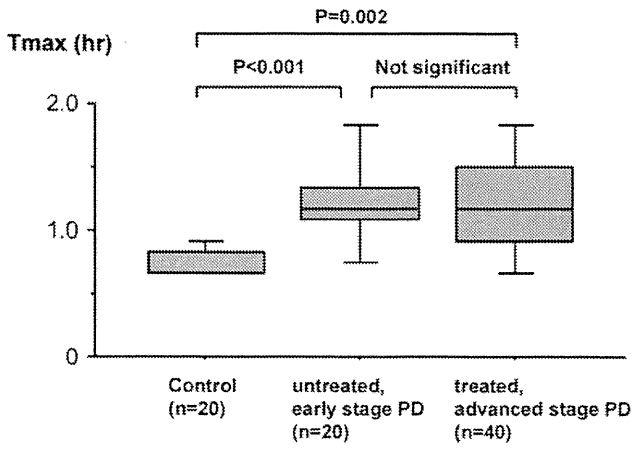

Fig. 1 T_{max} in control, PD patients with untreated, early-stage and those with treated, advanced-stage

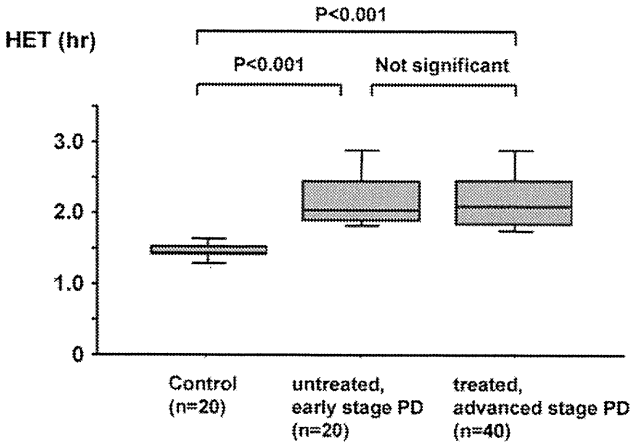

Fig. 2 HET in control, PD patients with untreated, early-stage and those with treated, advanced-stage

using the ^{13}C-ABT was significantly delayed in patients with advanced-stage, treated PD (median 1.17 h, range 0.50–2.17 h) as compared with the controls (median 0.83 h, range 0.67–1.00 h) ($P = 0.002$). The HET was significantly delayed in patients with advanced-stage, treated PD (median 2.09 h, range 1.60–3.30 h) as compared with the controls (median 1.44 h, range 1.30–1.64 h) ($P < 0.001$). There was virtually no overlap between controls and advanced-stage, treated PD patients.

PD patients with untreated early-stage and treated advanced-stage (Figs. 1, 2; Table 1)

The T_{max} was not significantly delayed in PD patients with early-stage (median 1.17 h, range 0.67–1.83 h) as compared with those with advanced-stage (median 1.17 h, range 0.50–2.17 h) ($P = 0.58$). The HET was not significantly delayed in PD patients with early-stage (median 2.04 h, range 1.75–3.07 h) as compared with those with advanced-stage (median 2.09 h, range 1.60–3.30 h) ($P = 0.77$).

Discussion

In the present study we have shown two novel important points. Firstly, we demonstrated that gastric emptying is significantly delayed in early-stage, untreated PD patients. Secondly, delayed gastric emptying does not differ between patients with early-stage, untreated PD as compared to those with advanced-stage, treated PD.

PD patients sometimes complain of upper GI symptoms such as heartburn, nausea, vomiting, and full abdomen sensation [1–3]. With respect to the pathological background of PD, during the pre-symptomatic stage of PD, the idiopathic PD related abnormal synuclein immunostaining

Table 1 Clinical characteristics of PD patients with untreated, early-stage and those with treated, advanced-stage

	Untreated, early-stage PD (n = 20)	Treated, advanced-stage PD (n = 40)	P value
Age (years)	70.5 (54–82)	67.0 (42–86)	0.49
Gender (male/female)	8/12	14/26	0.78*
BMI (kg m^{-2})	20.5 (17.8–27.9)	20.3 (16.8–27.1)	0.56
Duration (years)	0.9 (0.3–2.5)	6.0 (3.0–31.0)	<0.001
Duration with L-dopa (years)	0	5.5 (2.5–30.0)	<0.001
L-dopa (mg)	0	400 (100–750)	<0.001
Upper GI symptoms	6	11	0.99*
OH	9	21	0.78*
$CV_{R\text{-}R}$	1.70 (0.83–4.64)	1.71 (0.67–5.14)	0.43
MIBG (H/M ratio)	1.45 (1.13–2.56)	1.49 (1.27–1.95)	0.80

Variables expressed as median (range)

Medians were compared using Mann–Whitney's U test

PD Parkinson's disease, *BMI* body mass index, *GI* gastrointestinal tract, *OH* orthostatic hypotension, $CV_{R\text{-}R}$ coefficient of variation of R-R intervals, *MIBG* I-[123]-metaiodo-benzylguanidine scintigraphy, *H/M ratio* heart/mediastinum ratio

* Categorical variables were compared using Fisher's exact probability test

is confined to the medulla oblongata and olfactory bulb, according to Braak [6]. Neuronal degeneration occurring in the dorsal nucleus of the vagus may be responsible for the degeneration of the gastrointestinal myenteric plexuses. In the study of the enteric nervous system of PD, it has reported that Lewy bodies were found in the Auerbach's and Meissner's plexuses of the lower esophagus and stomach [23, 24]. Cytoplasmic inclusions similar to Lewy bodies were present in the ganglion cells of the colonic myenteric plexuses in PD patients [25]. The most likely causes of GI tract symptoms are degenerations of the dorsal vagal nucleus and the intramural plexus of the whole intestine [25]. These degenerations are likely to develop prior to the degeneration of dopaminergic neurons of the substantia nigra [7].

On the other hand, in lower GI symptoms, constipation has been identified as a rather frequent symptom in patients with PD ever since the first description of the disease [26–32]. Today, constipation is considered the most common manifestation of autonomic dysfunction in this disease occurring at a prevalence in the range of 70–80% [27, 30, 33, 34]. Constipation may precede development of PD [35]. It has been reported a prospective study which followed the bowel habits of 7,000 men for 24 years and reported that those with initial constipation (<1 bowel movement/day) had a threefold risk of developing PD after a mean interval of 10 years from initial constipation [35]. Involvement of the dorsal vagal nucleus, as would occur in Braak stage 1 may explain the pre-motor appearance of constipation [36]. It has been reported that motility of gut was controlled both by extrinsic inputs from the dorsal motor nucleus of the vagus and paravertebral sympathetic ganglia and by local reflexes mediated by intrinsic neurons of the enteric nervous system [37]. Both the enteric nervous system and the dorsal motor nucleus of the vagus are affected by Lewy body pathology at early stages of PD [37]. This early involvement provides insights into the pathophysiology of gastrointestinal dysmotility in GI disorder and may constitute an important step in the etiopathogenesis of Lewy body disease [37].

In the previous study, it was reported that delayed gastric emptying is common in patients with early-stage, treated PD [1, 2, 4]. However, because L-dopa therapy itself may worsen the symptoms of delayed gastric emptying [8, 9], their interpretation of the results of their study is limited. Gastric emptying of patients with early-stage, treated PD may be affected by L-dopa therapy. There is evidence to suggest that these complications in PD patients may be attributed to peripheral, pharmacokinetic mechanisms, mainly delayed gastric emptying as a side-effect of L-dopa [2, 38–40]. Dopaminergic agents also activate the vomiting center in the medulla via the chemoreceptor trigger zone, resulting in nausea, abdominal bloating, and vomiting [38].

In the present study, we tested whether there is the delayed gastric emptying of untreated patients with early-stage PD using ^{13}C-ABT. All PD patients were divided into two groups: those with untreated early-stage and those with treated advanced-stage. The patients with advanced-stage, treated PD were off drugs, including L-dopa, and the blood concentration of L-dopa was below the detection level at the start of examination. The two groups did not have significantly different blood parameters of gastric function such as pepsinogen I/II, serum gastrin, immunoglobulin G (IgG) anti-Helicobacter pylori antibody, hemoglobin A1c (HbA1c) [17] and neither was age of significant difference between the two groups [41]. The positive ratio of OH [18]

and $CV_{R\text{-}R}$ [19], H/M ratio of MIBG scintigraphy [20] were not significantly different between the PD groups. Under these conditions, there was not a difference in gastric emptying of PD patients with untreated, early-stage and those with treated, advanced-stage. And there is the delayed gastric emptying of untreated patients with early-stage PD. At the early-stage of PD, gastric emptying is already delayed similarly to the advanced-stage. Therefore, we speculate that delayed gastric emptying may be one of markers of the pre-clinical stage of PD.

In conclusion, our study demonstrated that gastric emptying is significantly delayed in untreated, early-stage PD patients as compared to controls, and that delayed gastric emptying does not differ between PD patients with untreated, early-stage and those with treated, advanced-stage. From a viewpoint of early diagnosis, it will be important to actually measure gastric emptying in PD patients in clinical settings. Our results demonstrated that from early-stage of PD, gastric emptying is already delayed similar to the advanced-stage. Delayed gastric emptying may be one of markers of the pre-clinical stage of PD.

Conflict of interest All authors report no disclosure.

References

1. Djaldetti R, Baron J, Ziv I, Melamed E (1996) Gastric emptying in Parkinson's disease: patients with and without response fluctuation. Neurology 46:1051–1054
2. Hardoff R, Sula M, Tamir A, Soil A, Front A, Badarna S, Honigman S, Giladi N (2001) Gastric emptying time and gastric motility in patients with Parkinson's disease. Mov Disord 16:1041–1047
3. Soykan I, Sarosiek I, Shifflett J, Wooten GF, McCallum RW (1997) Effects of chronic oral domperidone therapy on gastrointestinal symptoms and gastric emptying in patients with Parkinson's disease. Mov Disord 12:952–957
4. Djaldetti R, Koren M, Ziv I, Achiron A, Melamed E (1995) Effect of cisapride on response fluctuations in Parkinson's disease. Mov Disord 10:81–84
5. Tanaka Y, Kato T, Nishida H, Araki H, Murase M, Nagaki M, Moriwaki H, Inuzuka T (2009) Is there a difference in gastric emptying between Parkinson's disease patients under long-term L-dopa therapy with and without motor fluctuations? An analysis using the ^{13}C-acetate breath test. J Neurol 256:1972–1976
6. Braak H, Del Tredici K, Bratzke H, Hamm-Clement J, Sandmann-Keil D, Rüb U (2002) Staging of the intracerebral inclusion body pathology associated with idiopathic Parkinson's disease (preclinical and clinical stages). J Neurol 249(Suppl 3):III/1–III/5
7. Jost WH (2009) Gastrointestinal dysfunction in Parkinson's disease. J Neurol Sci 289:69–73
8. Bramble MG, Cunliffe J, Dellipiani AW (1978) Evidence for a change in neurotransmitter affecting oesophageal motility in Parkinson's disease. J Neurol Neurosurg Psychiatry 41:709–712
9. Kurlan R, Nutt JG, Woodward WR, Rothfield K, Lichter D, Miller C, Carter JH, Shoulson I (1988) Duodenal and gastric delivery of levodopa in parkinsonism. Ann Neurol 23:589–595
10. Ghoos YF, Maes BD, Geypens BJ, Mys G, Hiele MI, Rutgeerts PJ, Vantrappen G (1993) Measurement of gastric emptying rate of solids by means of a carbon-labeled octanoic acid breath test. Gastroenterology 104:1640–1647
11. Braden B, Adams S, Duan LP, Orth KH, Maul FD, Lembcke B, Hör G, Caspary WF (1995) The ^{13}C-acetate breath test accurately reflects gastric emptying of liquids in both liquid and semisolid test meals. Gastroenterology 108:1048–1055
12. Goetze O, Nikodem AB, Wiezcorek J, Banasch M, Przuntek H, Mueller T, Schmidt WE, Woitalla D (2006) Predictors of gastric emptying in Parkinson's disease. Neurogastroenterol Motil 18:369–375
13. Gibb WRG, Lees AJ (1988) The relevance of the Lewy body to pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 51:745–752
14. Hughes AJ, Daniel SE, Kilford L, Lees AJ (1992) Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 55:181–184
15. Fahn S, Elton RL, Members of the UPDRS Development Committee (1987) Unified Parkinson's disease rating scale. In: Fahn S, Marsden CD, Goldstein M, Calne DB (eds) Recent developments in Parkinson's disease, vol II. Macmillan Healthcare Information, Florham Park, pp 153–163, 293–304
16. Hoehn MM, Yahr MD (1967) Parkinsonism: onset, progression and mortality. Neurology 17:427–442
17. Yoshihara M, Sumii K, Haruma K, Kiyohira K, Hattori N, Kitadai Y, Komoto K, Tanaka S, Kajiyama G (1998) Correlation of ratio of serum pepsinogen I and II with prevalence of gastric cancer and adenoma in Japanese subjects. Am J Gastroenterol 93:1090–1096
18. Medow MS, Stewart JM, Sanyal S, Mumtaz A, Sica D, Frishman WH (2008) Pathophysiology, diagnosis, and treatment of orthostatic hypotension and vasovagal syncope. Cardiol Rev 16:4–20
19. Kuroiwa Y, Wada T, Tohgi H (1987) Measurement of blood pressure and heart-rate variation while resting supine and standing for the evaluation of autonomic dysfunction. J Neurol 235:65–68
20. Orimo S, Ozawa E, Nakade S, Sugimoto T, Mizusawa H (1999) (123)I-metaiodobenzylguanidine myocardial scintigraphy in Parkinson's disease. J Neurol Neurosurg Psychiatry 67:189–194
21. Gatti C, di Abriola FF, Dall'Oglio L, Villa M, Franchini F, Amarri S (2000) Is the ^{13}C-acetate breath test a valid procedure to analyse gastric emptying in children? J Pediatr Surg 35:62–65
22. González A, Mugueta C, Parra D, Labayen I, Martinez A, Varo N, Monreal I, Gil MJ (2000) Characterisation with stable isotopes of the presence of a lag phase in the gastric emptying of liquids. Eur J Nutr 39:224–228
23. Wakabayashi K, Takahashi H, Takeda S, Ohama E, Ikuta F (1988) Parkinson's disease: the presence of Lewy bodies in Auerbach's and Meissner's plexuses. Acta Neuropathol 76:217–221
24. Braak H, de Vos RA, Bohl J, Del Tredici K (2006) Gastric alpha-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. Neurosci Lett 396:67–72
25. Kupsky WJ, Grimes MM, Sweeting J, Bertsch R, Cote LJ (1987) Parkinson's disease and megacolon: concentric hyaline inclusions (Lewy bodies) in enteric ganglion cells. Neurology 37:1253–1255
26. Calne DB, Brennan J, Spiers AS, Stern GM (1970) Hypotension caused by L-dopa. Br Med J 1:474–475
27. Caplan LH, Jacobson HG, Rubinstein BM, Rotman MZ (1965) Megacolon and volvulus in Parkinson's disease. Radiology 85:73–78
28. Edwards LL, Pfeiffer RF, Quigley EMM, Hofman R, Balluff M (1991) Gastrointestinal symptoms in Parkinson's disease. Mov Disord 6:151–156

29. Klaue R (1940) Parkinsonsche Krankheit (Paralysis agitans) und postencephalitischer Parkinsonismus. Archiv für Psychiatrie 111:251–321
30. Kuroiwa Y, Shimada Y, Toyokura Y (1983) Postural hypotension and low R-R interval variability in parkinsonism, spino-cerebellar degeneration, and Shy-Drager syndrome. Neurology 33:463–467
31. Parkinson J (1817) An essay on the shaking palsy. Sherwood, Neely, and Jones, London, pp 1–66
32. Turkka JT, Juujärvi KK, Lapinlampi TO, Myllylä VV (1986) Serum noradrenaline response to standing up in patients with Parkinson's disease. Eur Neurol 25:355–361
33. Korczyn AD (1990) Autonomic nervous system disturbances in Parkinson's disease. Adv Neurol 53:463–468
34. Sakakibara R, Shinotoh H, Uchiyama T, Sakuma M, Kashiwado M, Yoshiyama M, Hattori T (2001) Questionnaire-based assessment of pelvic organ dysfunction in Parkinson's disease. Auton Neurosci 92:76–85
35. Abbott RD, Petrovitch H, White LR, Masaki KH, Tanner CM, Curb JD, Grandinetti A, Blanchette PL, Popper JS, Ross GW (2001) Frequency of bowel movements and the risk of Parkinson's disease. Neurology 57:456–462
36. Wolters ECh, Braak H (2006) Parkinson's disease: premotor clinico-pathological correlations. J Neural Transm Suppl 70:309–319
37. Cersosimo MG, Benarroch EE (2008) Neural control of the gastrointestinal system: implications for Parkinson's disease. Mov Disord 23:1065–1075
38. Edwards LL, Quigley EM, Pfeiffer RF (1992) Gastrointestinal dysfunction in Parkinson's disease: frequency and pathophysiology. Neurology 42:726–732
39. Jost WH (1997) Gastrointestinal motility problems in patients with Parkinson's disease. Effects of antiparkinsonian treatment and guidelines for management. Drugs Aging 10:249–258
40. Lanfranchi GA, Marzio L, Cortini C (1977) Effect of dopamine in gastric motility in man: evidence for specific receptors. In: Duthie HL (ed) Gastrointestinal motility in health and disease. MTP Publishers, Lancaster, pp 161–172
41. Urbain JL, Charkes ND (1995) Recent advances in gastric emptying scintigraphy. Semin Nucl Med 25:318–325

INTERNAL MEDICINE

□ CASE REPORT □

Diffuse Skeletal Muscles Uptake of [^{18}F] Fluorodeoxyglucose on Positron Emission Tomography in Primary Muscle Peripheral T-cell Lymphoma

Yuji Tanaka[1], Yuichi Hayashi[1], Jun'ichi Kato[1], Megumi Yamada[1], Akihiro Koumura[1], Takeo Sakurai[1], Akio Kimura[1], Isao Hozumi[1], Yuichiro Hatano[2], Yoshinobu Hirose[2], Tsuyoshi Takami[3], Hiroshi Nakamura[4], Senji Kasahara[4], Hisashi Tsurumi[4], Hisataka Moriwaki[4] and Takashi Inuzuka[1]

Abstract

A 40-year-old man presented with weakness of neck extensor muscles. Cervical magnetic resonance imaging showed high-intensity areas in muscles of the left lateral cervical region on T2-weighted images. Fluorodeoxyglucose-positron emission tomography scan demonstrated striking fluorodeoxyglucose uptake by multiple skeletal muscles of the neck, chest, and abdominal region. Muscle biopsy demonstrated peripheral T-cell lymphoma, unspecified. The diagnosis was primary skeletal muscle peripheral T-cell lymphoma. Primary skeletal muscle non-Hodgkin's lymphoma of T-cell immunophenotype is extremely rare and fluorodeoxyglucose-positron emission tomography demonstrated striking fluorodeoxyglucose uptake in multiple skeletal muscles and served as a quite useful modality for the diagnosis of this patient.

Key words: primary skeletal muscle lymphoma, non-Hodgkin lymphoma, peripheral T-cell lymphoma, fluorodeoxyglucose on positron emission tomography, skeletal muscle

(Intern Med 50: 2021-2024, 2011)
(DOI: 10.2169/internalmedicine.50.4992)

Introduction

Primary skeletal muscle non-Hodgkin's lymphoma is extremely uncommon and it is associated with a poor prognosis (1). Additionally, the majority of these lymphomas demonstrate B-cell immunophenotype (1). We report a case with primary skeletal muscle non-Hodgkin's lymphoma of T-cell immnophenotype, that showed slow progression, characterized by striking fluorodeoxyglucose uptake involving multiple skeletal muscles on positron emission tomography scans.

Case Presentation

In January 1996, a 27-year-old man had swelling of the right side of the labia and oral mucosa. In April 1996, the patient visited a hospital and was diagnosed with chronic lymphadenitis by a biopsy of the oral mucosa. Oral steroid therapy improved the symptoms. In July 1996, the patient had left ptosis, and left facial weakness. Steroid therapy again improved the symptoms. In September 1998, the patient had diplopia and bilateral facial weakness (manual muscle test (MMT): 1/1). The symptoms were not improved by steroid therapy and remained constant. In May 2000, the patient developed weakness of the neck extensor (MMT: 4/4). After several months, the symptoms disappeared spontaneously. Thereafter, the patient remained free of any new symptoms for the subsequent 9 years.

In May 2009, at the age of 40 years, weakness of neck extensor appeared again. The patient was admitted to our

[1]Department of Neurology and Geriatrics, Graduate School of Medicine, Gifu University, Japan, [2]Department of Pathology, Gifu University Hospital, Japan, [3]Department of Immunopathology, Graduate School of Medicine, Gifu University, Japan and [4]The First Department of Internal Medicine, Graduate School of Medicine, Gifu University, Japan
Received for publication December 13, 2010; Accepted for publication May 13, 2011
Correspondence to Dr. Yuji Tanaka, yutanaka-gif@umin.net

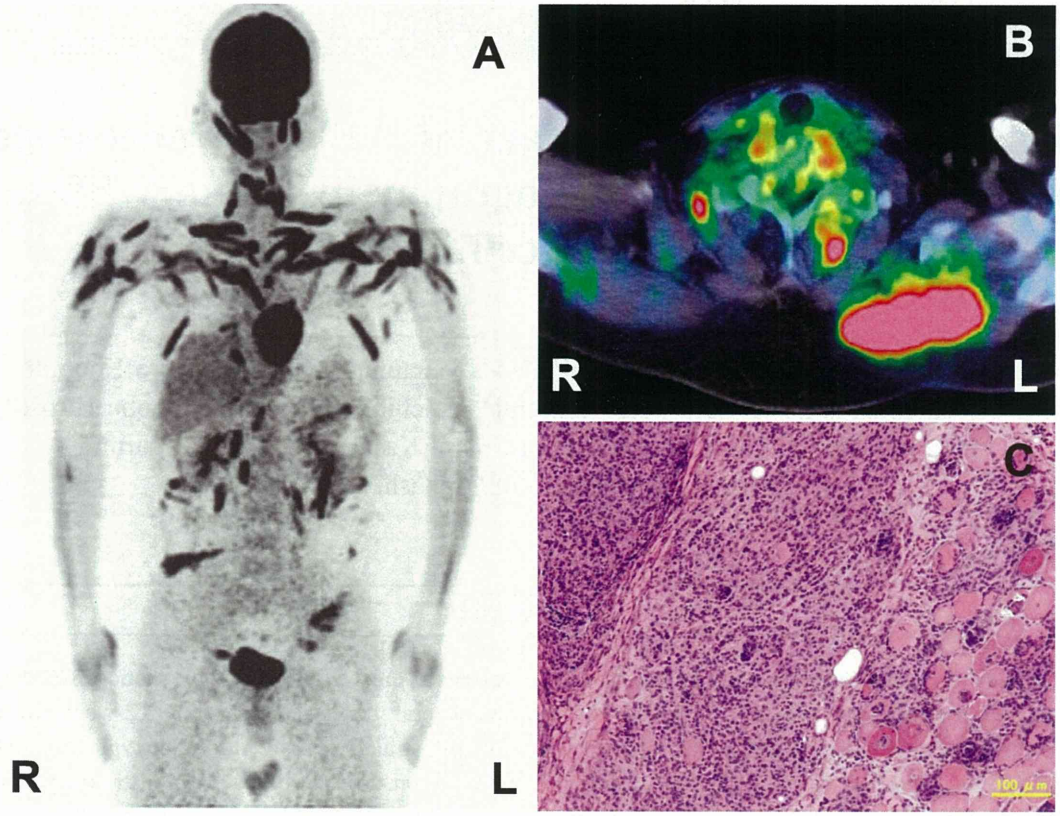

Figure 1. **Fluorodeoxyglucose-positron emission tomography (PET) and biopsy of the muscle. (A) (B) Fluorodeoxyglucose-positron emission tomography (PET): Fluorodeoxyglucose-positron emission tomography (PET) demonstrated striking fluorodeoxyglucose uptake involving the skeletal muscles of the neck, chest, and abdominal region. The maximum standardized uptake value of the left trapezius muscle was 10.32. (C) Biopsy specimen of the muscle: Muscle biopsy of the left trapezius muscle demonstrated massive infiltration of atypical lymphocytes of varying sizes and clusters of epithelioid histiocytes destroying muscle bundles.**

hospital. Neurologic evaluation showed weakness of the left pectoral girdle (MMT: 4), restricted bilateral eye movement, and facial weakness. However, no other muscle showed the weakness. The patient did not have myalgia. The patient did not appear to show a loss of weight, fever or sweating such as seen in B symptom. In the serum, creatine phosphokinase (CK) and soluble interleukin-2 receptor were high 311 IU/L (normal range, 40-200 IU/L) (CK-MM 95%) and 625 U/mL (normal range 145-519 U/mL), and continued to rise. Anti-human T-cell lymphotropic virus antibody and anti-human immunodeficiency virus antibody were negative. Cervical magnetic resonance imaging (MRI) showed high-intensity areas in the muscles of the left lateral cervical region on T2-weighted images. The muscles of other regions did not demonstrate abnormal findings on MRI. Fluorodeoxyglucose-positron emission tomography demonstrated striking fluorodeoxyglucose uptake by multiple skeletal muscles of the neck, chest, and abdominal region, but not of the lower extremities (Fig. 1A, 1B). Maximum standardized uptake value of the left trapezius muscle was 10.32. Ga scintigraphy showed no abnormal uptake. Computed tomography of the whole body revealed no evidence of lymphadenopathies, tumor infiltrations, or masses. There were no disease abnormalities involving the osseous structure. Bone marrow biopsy revealed a normocellular marrow with trilineage hemopoiesis and no evidence of lymphoma. Biopsy of the left trapezius muscle demonstrated massive infiltration of atypical lymphocytes which varied in size and clusters of epithelioid histiocytes destroying muscle bundles (Fig. 1C). Immunohistochemical staining revealed that the atypical lymphocytes were positive for CD3 and CD8, but negative for CD4, CD20, CD56, CD79a, and EBER. Histopathologic and immunocytochemical characteristics were consistent with those of peripheral T-cell lymphoma, unspecified (PTCL-U). The final diagnosis was established as primary skeletal muscle peripheral T-cell lymphoma. Chemotherapy with 6 cycles of cyclophosphamide, doxorubicin, vincristin and prednisone completely resolved the weakness of the left pectoral girdle, but it did not improve the eye movement or facial weakness. On serum analyses, CK and IL2R were decreased to the normal range. Fluorodeoxyglucose-positron emission tomography and magnetic resonance imaging improved after chemotherapy. The maximum standardized uptake value of the left trapezius muscle was reduced. After 6 months of chemotherapy, the symptoms remained the same.

Discussion

A 40-year-old man was diagnosed with primary skeletal muscle peripheral T-cell lymphoma. Multiple neurologic deficits had appeared for 13 years from the initial symptoms. The patient had been diagnosed with idiopathic or unknown disease because there was no specific finding. Although not definite, if those symptoms were related to lymphoma, this case showed very slow progression. Fluorodeoxyglucose-positron emission tomography demonstrated striking fluorodeoxyglucose uptake by multiple skeletal muscles and was useful in detecting disseminated lymphoma in the skeletal muscles.

Primary skeletal muscle non-Hodgkin's lymphoma is a rare manifestation of non-Hodgkin's lymphoma, accounting only for 0.1% of all lymphomas (1). The frequency of primary skeletal muscle non-Hodgkin's lymphoma (7%) is increased in patients with Acquired Immune Deficiency Syndrome (AIDS)-associated lymphomas (2). It is difficult to differentiate between primary skeletal muscle lymphoma and secondary muscle involvement from bone lymphoma. The following criteria is used to diagnose primary muscular lymphoma: 1) histopathology proven non-Hodgkin's lymphoma of the muscle; 2) the absence of systemic/nodal disease at initial presentation; and 3) the presence of a soft tissue mass with normal adjacent marrow or marrow disease much less extensive than soft tissue (3). The present case was diagnosed as primary skeletal muscle non-Hodgkin's lymphoma because these three 3 criteria were fulfilled. In cases of primary skeletal muscle non-Hodgkin's lymphoma the lower extremities and pelvic region are most commonly affected (4). Additionally, the majority of reported cases had B-cell immunophenotypes and behaved in an aggressive biologic manner (5). T-cell neoplasms constitute 10% to 12% of all non-Hodgkin's lymphoma (6). PTCL-U represents the most common subtype of T-cell lymphomas, manifesting most often as a nodal disease (7). Extranodal PTCL-U is a rare presentation among neoplasms. Some cases of primary skeletal muscle T-cell lymphomas have been described in the literature (8-12). Furthermore, in a few reported cases fluorodeoxyglucose-positron emission tomography demonstrated striking fluorodeoxyglucose uptake in multiple skeletal muscles.

Fluorodeoxyglucose-positron emission tomography scans demonstrate multiple sites of hypermetabolic activity within musculature. A recent study suggests that fluorodeoxyglucose-positron emission tomography scans are sensitive to detect T-cell lymphomas in all regions except for the skin (13). Fluorodeoxyglucose-positron emission tomography is useful in aiding the diagnosis and staging of lymphomas of aggressive subtypes, including PTCL-U (14). However, other reasons for skeletal muscle fluorodeoxyglucose uptake may stem from a variety of factors such as physical exertion, talking, and chewing as normal physiological phenomena (15). As for pathological phenomena, elevated fluorodeoxyglucose muscle may be associated with anxiety-induced neck muscle spasm (15), accessory muscle use in respiratory distress (16), intercostal and diaphragmatic activity in chronic obstructive pulmonary disease (17), abdominal muscle contraction in intractable vomiting (18), sepsis-induced shivering (19), hypoglycemia (20), infection, and inflammation of the muscle tissue that increases glycolytic activity (21, 22). In the present case, the patient did not satisfy these conditions. Skeletal muscle fluorodeoxyglucose uptake was induced by T-cell lymphoma, which histopathologically proved PTCL-U.

In conclusion, primary skeletal muscle non-Hodgkin's lymphoma of T-cell immunophenotype is extremely rare. Fluorodeoxyglucose-positron emission tomography demonstrated striking fluorodeoxyglucose uptake in multiple skeletal muscles, and served as a useful method for diagnosing this case.

Informed consent was obtained from this patient.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement

The authors are grateful thanks to Rie Tsuburaya, Yukiko K Hayashi, Ichizo Nishino, and Ikuya Nonaka, at the Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry for histopathological examination.

References

1. Travis WD, Banks PM, Reiman HM. Primary extranodal soft tissue lymphoma of the extremities. Am J Surg Pathol **11**: 359-366, 1987.
2. Raphael M, Gentilhomme O, Tulliez M, Byron PA, Diebold J. Histopathologic features of high-grade non-Hodgkin's lymphomas in acquired immunodeficiency syndrome. The French Study Group of Pathology for Human Immunodeficiency Virus-Associated Tumors. Arch Pathol Lab Med **115**: 15-20, 1991.
3. Lee VS, Martinez S, Coleman RE. Primary muscle lymphoma: clinical and imaging findings. Radiology **203**: 237-244, 1997.
4. Ueno A, Kuribayashi K, Iyama S, et al. Two cases of primary skeletal muscle lymphoma and a review of the literature. Rinsho Ketsueki **46**: 1141-1145, 2005 (in Japanese).
5. Jeffery GM, Golding PF, Mead GM. Non-Hodgkin's lymphoma arising in skeletal muscle. Ann Oncol **2**: 501-504, 1991.
6. Ralfkieer E, Muller-Hermelink HK, Jaffe ES. T-cell and NK-cell neoplasms. In: Pathology and Genetics of Tumours of the Haematopoietic and Lymphoid Tissues: World Health Organization Classification of Tumours. Jaffe E, Harris N, Stein H, Vardiman J, Eds. IARC Press, Lyon, France, 2001: 227-229.
7. Savage KJ, Chhanabhai M, Gascoyne RD, Connors JM. Characterization of peripheral T-cell lymphomas in a single North American institution by the WHO classification. Ann Oncol **15**: 1467-1475, 2004.
8. Lum GH, Cosgriff TM, Byrne R, Reddy V. Primary T-cell lymphoma of muscle in a patient infected with human immunodeficiency virus. Am J Med **95**: 545-546, 1993.
9. Chim CS, Choy C, Liang R. Primary anaplastic large cell lymphoma of skeletal muscle presenting with compartment syndrome. Leuk Lymphoma **33**: 601-605, 1999.
10. Chim CS, Loong F, Ooi GC, Srivastava G, Liang R. Primary skeletal muscle lymphoma. Am J Med **112**: 79-80, 2002.

11. Alekshun TJ, Rezania D, Ayala E, Cualing H, Frank GA. Skeletal muscle peripheral T-cell lymphoma. J Clin Oncol **26**: 501-503, 2008.

12. Broski SM, Bou-Assaly W, Gross MD, Fig LM. Diffuse skeletal muscle F-18 fluorodeoxyglucose uptake in advanced primary muscle non-Hodgkin's lymphoma. Clin Nucl Med **34**: 251-253, 2009.

13. Kako S, Izutsu K, Ota Y, et al. Fluorodeoxyglucose-PET in T-cell and NK-cell neoplasms. Ann Oncol **18**: 1685-1690, 2007.

14. Gill SI, Gibbs SD, Hicks RJ, Seymour JF. Primary skeletal muscle marginal zone lymphoma with persistent tissue tropism and PET-avidity. Leuk Lymphoma **47**: 117-120, 2006.

15. Cook GJ, Fogelman I, Maisey MN. Normal physiological and benign pathological variants of 18-fluoro-2-deoxyglucose positron-emission tomography scanning: potential for error in interpretation. Semin Nucl Med **26**: 308-314, 1996.

16. Bural GG, Mavi A, Kumar R, Alavi A. Fluorodeoxyglucose uptake in intercostal muscles is an indicator of severe respiratory disease. Clin Nucl Med **29**: 807-808, 2004.

17. Aydin A, Hickeson M, Yu JQ, Zhuang H, Alavi A. Demonstration of excessive metabolic activity of thoracic and abdominal muscles on fluorodeoxyglucose-PET in patients with chronic obstructive pulmonary disease. Clin Nucl Med **30**: 159-164, 2005.

18. Abdel-Dayem HM, Naddaf S, El-Zeftawy H. F-18 fluorodeoxyglucose gastric and anterior abdominal muscle uptake secondary to nausea and vomiting. Clin Nucl Med **23**: 769-770, 1998.

19. Ojha B, Muthukrishnan A, Raman AK, Satya R, Mahone T, Forero-Torres A. Diffuse F-18 fluorodeoxyglucose uptake in skeletal muscles secondary to chills resulting from sepsis: a cause for underestimation of viable tumor. Clin Nucl Med **29**: 600-601, 2004.

20. Shreve PD, Anzai Y, Wahl RL. Pitfalls in oncologic diagnosis with fluorodeoxyglucose PET imaging: physiologic and benign variants. Radiographics **19**: 61-77, 1999.

21. Bakheet SM, Powe J. Benign causes of 18-fluorodeoxyglucose uptake on whole body imaging. Semin Nucl Med **28**: 352-358, 1998.

22. Kaim AH, Weber B, Kurrer MO, et al. (18)F-fluorodeoxyglucose and (18)F-FET uptake in experimental soft tissue infection. Eur J Nucl Med Mol Imaging **29**: 648-654, 2002.

INTERNAL MEDICINE

□ CASE REPORT □

Neuromyelitis Optica in Japanese Sisters

Yuji Tanaka[1,2], Nobuaki Yoshikura[1], Naoko Harada[1], Megumi Yamada[1], Akihiro Koumura[1], Takeo Sakurai[1], Yuichi Hayashi[1], Akio Kimura[1], Isao Hozumi[1], Hisataka Moriwaki[2] and Takashi Inuzuka[1]

Abstract

We report cases of Japanese sisters with neuromyelitis optica (NMO). The elder sister was 25, when she was diagnosed with right optic neuritis. After 3 months, she developed left optic neuritis and myelitis. At age 27, she had the second relapse, but she has been free from episodes thereafter. The younger sister was 26, when she was diagnosed with optic neuritis. Thus far, she has 9 relapses, comprising both myelitis and optic neuritis. Both sisters had normal brain MRI scans, longitudinally extensive transverse myelitis over 3 vertebral segments, and positive results for anti-aquaporin-4 antibody (AQAP4Ab). They fulfilled the Wingerchuk criteria for definite NMO. Both sisters shared some immunogenetic factors, but they were not exposed to the same environmental factors after their early twenties. The final disability status was almost the same in both cases, and both showed a very benign course. These data suggest that genetic factors affect the age at onset and environmental factors may affect the frequency of relapse.

Key words: neuromyelitis optica, familial, HLA, anti aquaporin-4 antibody, environmental factor

(Intern Med 50: 2829-2832, 2011)
(DOI: 10.2169/internalmedicine.50.5613)

Introduction

Neuromyelitis optica (NMO) is a demyelinating disorder of the central nervous system that generally affects the optic nerves and spinal cord but less often the brain (1, 2). Opticospinal multiple sclerosis (OSMS) in Asians has been suggested to be the same entity as NMO, because anti-aquaporin-4 antibody (AQP4Ab) has been reported to be present in 30% to 60% of OSMS patients (3). The incidence of human leukocyte antigen (HLA)-*DPB1*0501* was significantly increased in anti-AQP4Ab-positive Japanese patients as compared with healthy controls, but not in anti-AQP4Ab-negative OSMS patients (4). Furthermore, cases of familial NMO have been reported suggesting genetic influence (5-9). The mechanism of NMO is speculated to be associated with humoral immunity, but still remains to be fully elucidated (4, 10, 11). Here, we report cases of Japanese sisters with NMO, in which the onset age was similar, but the frequency of relapse was different.

Case Report

Patient 1: Elder sister

Patient 1 was a 54-year-old woman. She did not have any family history of immunological disorders. When she was 25, right-sided blindness developed and she was diagnosed as having optic neuritis. Steroid therapy completely improved her vision. After 3 months, she lost her left sight and had paraparesis and dysuria. The neurologic evaluation revealed blindness in the left eye, weakness of bilateral lower extremities, positive bilateral Babinski reflexes, and bilateral loss of sensation below the Th10 level. Steroid therapy improved her symptoms within several months. At age 27, she had paraparesis and dysuria. Steroid therapy improved her symptoms again. Thereafter, she has been free from episodes of neurological dysfunction. At age 50, a test for AQP4Ab was found to be positive (12, 13). Her HLA type was *A*31, B*61, *51, DRB1*0802*, and *DPB1*0501*. At age

[1]Department of Neurology and Geriatrics, Graduate School of Medicine, Gifu University, Japan and [2]The First Department of Internal Medicine, Graduate School of Medicine, Gifu University, Japan
Received for publication April 5, 2011; Accepted for publication August 9, 2011
Correspondence to Dr. Yuji Tanaka, yutanaka-gif@umin.net

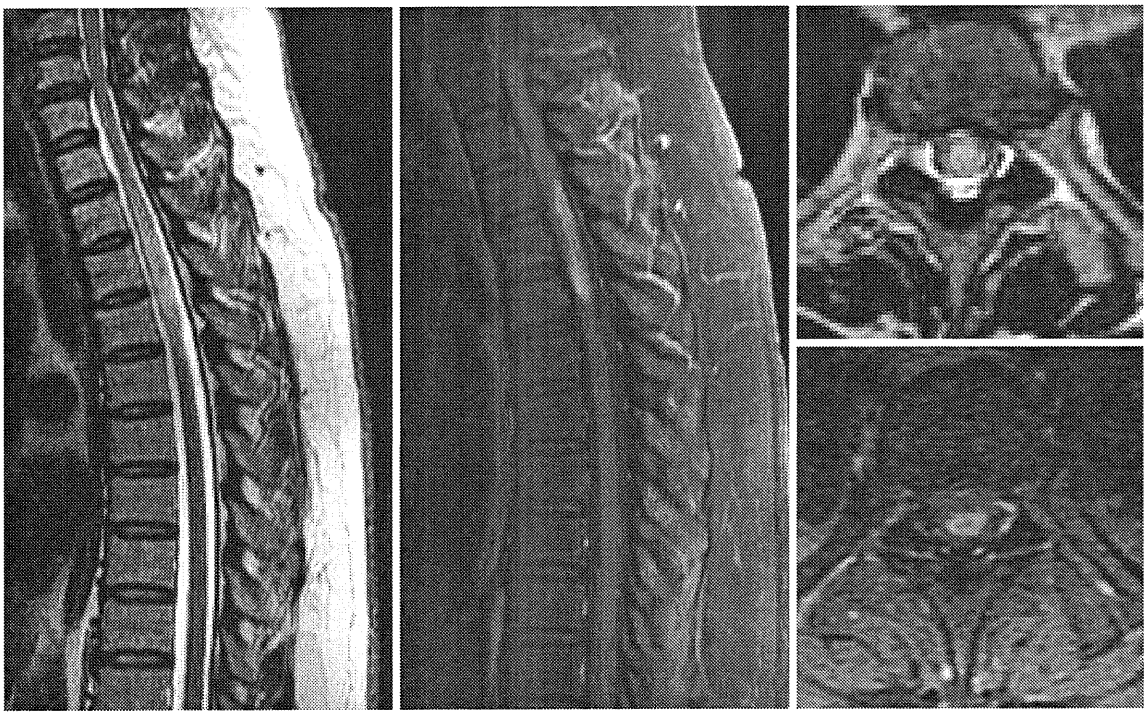

Figure 1. Spinal magnetic resonance imaging (MRI) of patient 2: T2-weighted image (1.5 T, repetition time [TR] 3000 ms, echo time [TE] 90 ms) and T1-weighted image with gadolinium enhancement (1.5 T, TR 400 ms, TE 9 ms). When patient 2 had her last relapse at age 49, the spinal MRI scan showed high-intensity areas at Th2-4 on T2-weighted images with swelling and gadolinium enhancement.

53, spinal magnetic resonance imaging (MRI) did not demonstrate apparent abnormality in parenchymal signal intensity, and her brain MRI was normal. There has been no medication for the prevention of relapse. The final Expanded Disability Status Scale of Kurtzke score was 1.0 (14) and the final visual function of both eyes was normal.

Patient 2: Younger sister

Patient 2 was a 53-year-old woman. When she was 26, right-sided blindness developed and she was diagnosed as having optic neuritis. Steroid therapy improved her vision completely. At age 27, she had paraparesis, loss of sensation below the L1 level and dysuria. The neurologic evaluation revealed weakness of bilateral lower extremities, positive bilateral Babinski reflexes, and bilateral loss of sensation below the L1 level. Steroid therapy improved her symptoms completely within several months. At age 29, she had loss of sensation below the Th5 level and steroid therapy improved her symptoms again. To date she has had 9 relapses, comprising of both myelitis and optic neuritis. At age 49, a test for AQP4Ab was found to be positive. Her HLA type was *A*31, B*61, *51, DRB1*0802*, and *DPB1*0501*, which is the same as that of her elder sister. At the last relapse, when she was 49, the MRI showed on T2-weighted images, and enlarged spinal cord lesions with gadolinium enhancement (Fig. 1). Her brain MRI was normal. Low-dose steroid treatment and immunosuppressant (azathioprine) treatment have been continued for the prevention of relapse. The final Expanded Disability Status Scale of Kurtzke score was 2.0 (14) and the final visual function of both eyes was normal.

Both patients fulfilled the criteria of NMO of Wingerchuk et al (15). The sisters had lived in the same area until the elder sister got married at age 23. The younger sister married at age 22 and moved to a northern rural area of Japan. Thus, they shared some immunogenetic factors but were not exposed to the same environmental factors after their early twenties.

Informed consents was obtained from both patients.

Discussion

These 2 cases of Japanese sisters met the criteria of NMO. Both sisters had positive results for AQP4Ab and shared some immunogenetic factors. Their onset ages of NMO were similar. The frequency of relapse was different. The final disability status was almost the same and both sisters showed a very benign course of the disease. These patients were the third cases of familial NMO in Japan (including that discussed in a Japanese language abstract) (7).

According to a literature search, several familial cases of NMO have been reported (5-9). Those cases included 7 cases of sisters, 2 mother-daughter pairs, 2 aunt-niece pairs, 2 brother-sister pairs, 1 father-daughter pair, 1 mother-son pair, and 1 family with 3 patients (mother-daughter-aunt). Ninety-three percent of these familial cases were fe-

male (11). The first report of familial NMO in 1938 described that identical twin women developed NMO at ages 24 and 26 years, respectively, but their HLA type was unknown (5). In the first report, 1 woman had 1 recurrence for 18 months; the other had 2 recurrences for 26 months (5). The second case of sisters in 1982 described 2 younger sisters who developed NMO at ages 3 years and 2 years 9 months. The HLA type of the elder sister was *A1, 2, BW35, W40*, and *BW622* and that of the younger sister was *A1, XBW35*, and *YBW622* (6). In another case of sisters, the women developed NMO at ages 62 and 59 years (7). The HLA type of the elder sister was *A2/33, B39/44, Cw7/-, DR 4/6*, and *DQ1/3* and that of the younger sister was *A26/33, B44/62, Cw3/-, DR6/12, DQ1/-*, and *DP1/-* (7). The onset ages in the forth case of sisters were 24 and 28 years (8). The HLA type of the elder sister was *A*24, B*07, *15, DRB1*01*, and **16 (DR2 positive)* and that of the younger sister was *A*02, 24, B*07, *40, DRB1*04*, and **08* (8). In the fifth report of 4 sibling cases, the onset ages were 29.2 and 28.1 years, 28.4 and 26.5 years, 33.9 and 32.1 years, and 40.7 and 25.1 years (9). There is little data regarding the long-term prognosis after onset. The fifth report described 4 sibling cases with recurrent episodes of optic neuritis and myelitis (1 and 6 times, 7 and 2 times, 5 and 13 times, and 3 and 7 times, respectively) (9). Interestingly, the initial episodes of NMO occurred at similar ages in these reports, except for the last pair of sisters. In the present cases, the onset ages of NMO were also similar (within 1 year). Such observation may suggest that there is a common trigger for the onset of NMO related to the immunogenetic background of NMO in sister cases.

In the present cases, although the frequency of relapse was different between the 2 sisters, the final disability status was almost the same and both sisters showed a very benign course of the disease. However, in previous reports on sisters with NMO, little data was provided on the final disability status. It would be interesting to study this aspect in the future. It is suggested that the clinical feature might be related to the immunogenetic background of NMO in sister cases.

The genetic contributions were supported by recent observations that have linked NMO to specific HLA loci, such as *DPB1*0501* in Japanese patients (10). The *HLA-DPB1*0501* allele was more frequent in 38 Japanese patients who were seropositive for NMO-IgG as compared to 52 patients with multiple sclerosis (MS) (4). Forty-five French Caucasian patients were compared to healthy controls and patients with MS for HLA class II A and B alleles; no association was found for *DRB1*1501* with NMO. *HLA-DRB1*03* was associated with NMO-IgG-seropositive NMO (16). The HLA type has been reported to be similar in sisters with NMO in 2 families (6-8). In the present cases, the sisters shared some immunogenetic factors because their HLA type was similar.

With regard to environmental factors, certain infections or cancers have been reported to complicate NMO (17, 18). However, there is no report on environmental or disease triggers that have a firm association with NMO (11). In the present cases, the sisters had lived in the same area just before the first episode of NMO, but 1 sister moved to a northern rural area and was then exposed to a different environment. The difference in environmental factors may have affected the frequency of relapse, although the precise factors are unknown.

These observations suggest that genetic factors affect the ages at onset and that environmental factors may affect the frequency of relapse in familial NMO.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement

The authors are grateful to Keiko Tanaka, M.D., Ph.D. (Department of Neurology, Brain Research Institute, Niigata University) for detection of AQP4Ab.

References

1. Pittock SJ, Lennon VA, Krecke K, Wingerchuk DM, Lucchinetti CF, Weinshenker BG. Brain abnormalities in neuromyelitis optica. Arch Neurol **63**: 390-396, 2006.
2. Cree BA, Goodin DS, Hauser SL. Neuromyelitis optica. Semin Neurol **22**: 105-122, 2002.
3. Kira J. Neuromyelitis optica and opticospinal multiple sclerosis: Mechanisms and pathogenesis. Pathophysiology **18**: 69-79, 2011.
4. Matsushita T, Matsuoka T, Isobe N, et al. Association of the HLA-DPB1*0501 allele with anti-aquaporin-4 antibody positivity in Japanese patients with idiopathic central nervous system demyelinating disorders. Tissue Antigens **73**: 171-176, 2009.
5. McAlpine D. Familial neuromyelitis optica: its occurrence in identical twins. Brain **61**: 430-448, 1938.
6. Ch'ien LT, Medieros MO, Belluomini JJ, Lemmi H, Whitaker JN. Neuromyelitis optica (Devic's syndrome) in two sisters. Clin Electroencephalogr **13**: 36-39, 1982.
7. Yamakawa K, Kuroda H, Fujihara K, et al. Familial neuromyelitis optica (Devic's syndrome) with late onset in Japan. Neurology **55**: 318-320, 2000.
8. Cabrera-Gómez JA, Ramón-Pérez L, Saiz A, et al. Neuromyelitis optica and multiple sclerosis in sisters. Mult Scler **15**: 269-271, 2009.
9. Matiello M, Kim HJ, Kim W, et al. Familial neuromyelitis optica. Neurology **75**: 310-315, 2010.
10. Yamasaki K, Horiuchi I, Minohara M, et al. HLA-DPB1*0501-associated opticospinal multiple sclerosis: clinical, neuroimaging and immunogenetic studies. Brain **122**: 1689-1696, 1999.
11. Wingerchuk DM. Neuromyelitis optica: effect of gender. J Neurol Sci **286**: 18-23, 2009.
12. Yanagawa K, Kawachi I, Toyoshima Y, et al. Pathologic and immunologic profiles of a limited form of neuromyelitis optica with myelitis. Neurology **73**: 1628-1637, 2009.
13. Tanaka K, Tani T, Tanaka M, et al. Anti-aquaporin 4 antibody in selected Japanese multiple sclerosis patients with long spinal cord lesions. Mult Scler **13**: 850-855, 2007.
14. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology **33**: 1444-1452, 1983.
15. Wingerchuk DM, Lennon VA, Pittock SJ, Lucchinetti CF, Weinshenker BG. Revised diagnostic criteria for neuromyelitis optica. Neurology **66**: 1485-1489, 2006.
16. Zéphir H, Fajardy I, Outteryck O, et al. Is neuromyelitis optica as-

sociated with human leukocyte antigen? Mult Scler **15**: 571-579, 2009.

17. Sellner J, Hemmer B, Muhlau M. The clinical spectrum and immunobiology of parainfectious neuromyelitis optica (Devic) syndromes. J Autoimmun **34**: 371-379, 2010.

18. Pittock SJ, Lennon VA. Aquaporin-4 autoantibodies in a paraneoplastic context. Arch Neurol **65**: 629-632, 2008.

Elevated Anti–Heat Shock Protein 60 Antibody Titer is Related to White Matter Hyperintensities

Akio Kimura, MD,* Takeo Sakurai, MD,* Megumi Yamada, MD,* Akihiro Koumura, MD,* Yuichi Hayashi, MD,* Yuji Tanaka, MD,* Isao Hozumi, MD,* Masao Takemura, PhD,† Mitsuru Seishima, MD,† and Takashi Inuzuka, MD*

Background: There are many reports that the antibody against heat shock protein 60 (Hsp60) is present in most patients with coronary artery disease and atherosclerosis, and that its titer correlates with disease severity. However, few reports have described the association between anti-Hsp60 antibody and cerebrovascular disease. *Methods:* We determined the anti-Hsp60 antibody titer in patients with neurologic diseases and healthy subjects using enzyme-linked immunosorbent assay (ELISA) and evaluated their findings of brain magnetic resonance imaging (MRI) of the white matter. White matter hyperintensities (WMHs) on T2-weighted and fluid-attenuated inversion recovery (FLAIR) images were classified into 2 categories: periventricular hyperintensity (PVH) and deep white matter hyperintensity (DWMH). The lesions in each category were then divided into 4 grades (grades 0-3) according to the Fazekas rating scale. *Results:* There were no significant differences in the titer between patients with neurologic diseases and healthy subjects. The mean grade of DWMHs (mean ± SD, 1.56 ± 0.70) was significantly higher in 18 subjects in the high-titer group (≥39.8 ng/mL; mean titer + 2 SD in sera from 23 healthy subjects) than in 86 subjects (mean ± SD, 0.09 ± 0.76) in the normal-titer group (<39.8 ng/mL; $P < .003$). The mean grade of PVHs (mean ± SD, 1.50 ± 0.71) was also significantly higher in the high-titer group than in the normal-titer group (mean ± SD, 1.17 ± 0.62; $P < .02$). *Conclusions:* A significant correlation was noted between anti-Hsp60 antibody titer and the severity of WMHs on brain MR images. We suggest that an elevated titer of the anti-Hsp60 antibody could be a risk factor for cerebral small-vessel disease. **Key Words:** Autoantibody—endothelial cell—enzyme-linked immunosorbent assay—heat shock protein 60—white matter hyperintensity.

Human heat shock protein 60 (Hsp60) is a molecular chaperone that participates in the folding of mitochondrial proteins and facilitates the proteolytic degradation of misfolded or denatured proteins.[1] It has been recently reported that Hsp60 was detected on the membrane surface of stressed human endothelial cells[2,3] and that the antibody against human Hsp60 induces endothelial cell cytotoxicity.[4,5] Moreover, it was also reported that Hsp60 is an important target of anti–endothelial cell antibodies (AECAs) and that such an interaction contributes to pathogenic effects, particularly in vasculitis-associated systemic autoimmune diseases.[6] The anti-Hsp60 antibody is present in most patients with coronary artery disease[7,8]

From the *Departments of Neurology and Geriatrics, and †Informative Clinical Medicine, Gifu University Graduate School of Medicine, Gifu City, Japan.

Received June 11, 2010; revision received August 28, 2010; accepted September 5, 2010.

Supported in part by a Japanese Health and Labour Sciences Research Grant for Research on Psychiatry and Neurological Diseases and Mental Health (H18-026).

Address correspondence to Akio Kimura, MD, Department of Neurology and Geriatrics, Gifu University Graduate School of Medicine, Gifu, 1-1 Yanagido, Gifu City, Gifu 501-1194, Japan. E-mail: kimura1@gifu-u.ac.jp.

1052-3057/$ - see front matter

doi:10.1016/j.jstrokecerebrovasdis.2010.09.003

and atherosclerosis,[9-11] and its titer correlates with disease severity.[8] However, few reports have described the association between anti-Hsp60 antibody and cerebrovascular disease.[12,13] Patients with systemic autoimmune diseases frequently show the abnormal findings of white matter lesions on brain magnetic resonance imaging (MRI) scans.[14-16] Previously, we reported the case of a patient with neuropsychiatric systemic lupus erythematosus (NPSLE) whose brain MRI scan revealed extended white matter hyperintensities (WMHs) on T2-weighted and fluid-attenuated inversion recovery (FLAIR) images that appeared to represent cerebral small-vessel disease (SVD).[17] The titer of the anti-Hsp60 antibody in serum from this patient was markedly higher than those from other NPSLE patients without WMHs and healthy controls. In this study, we aimed to determine whether an elevated titer of the anti-Hsp60 antibody is associated with cerebral SVD. We then determined the titer of the anti-Hsp60 antibodies in patients with neurologic diseases and evaluated the MRI findings of their white matter.

Methods

Study Population

Serum samples were obtained from 180 patients with neurologic diseases and 23 healthy subjects. Among the patients with neurologic diseases, 18 had multiple cerebral infarctions (MCIs), 69 had neurodegenerative diseases, 61 had neuroinflammatory diseases, and 32 had systemic autoimmune diseases complicated by neurologic symptoms. Among the patients with neurodegenerative diseases, 16 had Alzheimer disease (AD), 13 had amyotrophic lateral sclerosis (ALS), 22 had Parkinson disease (PD), and 18 had spinocerebellar degeneration (SCD). Among the patients with neuroinflammatory diseases, 11 had Guillain-Barré syndrome (GBS), 13 had meningitis, 26 had multiple sclerosis (MS), and 11 had neuromyelitis optica (NMO). Among the patients with systemic autoimmune diseases complicated by neurologic symptoms, 15 had systemic lupus erythematosus (SLE) and 17 had Sjögren syndrome (SS). The serum samples from the patients with neuroinflammatory diseases and patients with systemic autoimmune diseases complicated by neurologic symptoms were obtained in active phase, and the other samples were obtained in inactive phase. We evaluated the atherosclerotic risk factors (i.e., hypertension, diabetes mellitus, and hyperlipidemia) of these patients. The criteria for hypertension were a systolic blood pressure ≥140 mm Hg and/or a diastolic blood pressure ≥90 mm Hg, or current treatment with antihypertensives. The diabetes mellitus group included subjects who were diagnosed as having diabetes by the investigators on the basis of standard criteria or who were already treated with antidiabetic drugs. Hypercholesterolemia was defined as having a total serum cholesterol level ≥220 mg/dL (5.67 mmol/L) or current treatment with cholesterol-lowering drugs. This study was approved by the Institutional Review Board of the Gifu University Graduate School of Medicine, Gifu City, Japan.

Determination of Anti-Hsp60 Antibody Titer

Using enzyme-linked immunosorbent assay (ELISA), we determined the titer of anti-Hsp60 antibody in patients with neurologic diseases and healthy subjects. We carried out ELISA to analyze the reactivities of autoantibodies against human Hsp60, which were measured using an ELISA kit (Stressgen; Ann Arbor, MI). Sera diluted at 1:1000 in a dilution buffer were added to a precoated ready to use recombinant human Hsp60 immunoassay plate and then incubated for 2 hours at room temperature. After 4 washes, peroxidase-conjugated anti-human immunoglobulins G, A, or M were added to each well and then incubated for 1 hour at room temperature. After 4 washes, a stabilized tetrametylbenzidine substrate was added to each well and then incubated for 15 minutes at room temperature. The reaction was stopped by adding an acid stop solution, and the plate was read at 450 nm on a microplate reader. The Optical Density (OD) of control wells without Hsp60 was subtracted from the OD of Hsp60-coated wells. Serial dilutions of serum samples from healthy blood donors having high titers of the antibody against the tested Hsp60 were used as standards.

Assessment of MRI Scans

Ninety of the 180 patients with neurologic disease and 14 of 23 healthy subjects who showed no risk factors were investigated for the MRI findings of the white matter. MRI scans were analyzed by 2 experienced neurologists for white matter abnormalities. WMHs on T2-weighted and FLAIR images were classified into 2 categories: periventricular hyperintensity (PVH) and deep white matter hyperintensity (DWMH). The lesions in each category were then divided into 4 grades (grades 0-3) according to the Fazekas rating scale.[18,19] PVHs were rated as follows: grade 0, no PVHs; grade 1, "caps" or pencil-thin lining; grade 2, smooth "halo"; or grade 3, irregular PVH extending into the deep white matter. DWMHs were rated as follows: grade 0, no DWMHs; grade 1, punctate foci; grade 2, initial confluence of foci; or grade 3, large confluent areas.

Statistical Analysis

The Mann–Whitney *U* test and nonparametric analysis of variance (Kruskal–Wallis test) were used to assess the significance of differences in continuous variables between groups. $P < .05$ was considered statistically significant.

Results

Determination of Anti-Hsp60 Antibody Titer in Patients with Neurologic Diseases and Healthy Subjects

We determined the titer of the anti-Hsp60 antibody in sera from all the patients with neurologic disease and healthy subjects. The mean titers of the anti-Hsp60 antibody were 31.9 (± 36.0 SD) ng/mL in the MCI patients, 25.9 (± 14.6 SD) ng/mL in the AD patients, 38.9 (± 33.5 SD) ng/mL in the ALS patients, 26.2 (± 18.1 SD) ng/mL in the PD patients, 25.9 (± 19.8 SD) ng/mL in the SCD patients, 22.1 (± 20.1 SD) ng/mL in the GBS patients, 23.6 (± 13.2 SD) ng/mL in the meningitis patients, 33.2 (± 23.2 SD) ng/mL in the MS patients, 20.7 (± 12.1 SD) ng/mL in the NMO patients, 51.1 (± 83.8 SD) ng/mL in the SLE patients, and 46.0 (± 60.4: SD) ng/mL in the SS patients (Fig 1). The mean titer of anti-Hsp60 antibodies in healthy subjects was 20.0 (± 9.86 SD) ng/mL. The titers of anti-Hsp60 antibodies in patients with systemic autoimmune diseases (SLE and SS) complicated by neurologic symptoms tended to be higher than those in patients with other neurologic diseases and in healthy subjects, but this difference did not reach statistical significance. There were no significant differences in the titer between patients with neurologic diseases and healthy subjects (Fig 1).

To evaluate the association between anti-Hsp60 antibody titer and the findings of white matter on brain MRI scans, we defined that the mean titer +2 SD in the sera from 23 healthy subjects was the cut-off value (39.8 ng/mL). We divided 90 patients and 14 healthy subjects who showed no risk factors and underwent brain MRI scans in 2 groups. Eighteen patients whose ani-Hsp60 antibody titers were above the cut-off value were classified into the high-titer group, and the remaining 72 patients with neurologic diseases and 14 healthy subjects whose anti-Hsp60 antibody titers were below the cut-off value were classified into the normal-titer group (Fig 2).

Characterization of the High-Titer and Normal-Titer Groups

Among the 18 patients of the high-titer group, 1 had AD, 3 had ALS, 2 had PD, 1 had SCD, 1 had meningitis, 5 had MS, 3 had SLE, and 2 had SS. Among the 86 patients in the normal-titer group, 2 had MCI, 9 had AD, 2 had ALS, 9 had PD, 8 had SCD, 2 had GBS, 5 had meningitis, 13 had MS, 6 had NMO, 7 had SLE, and 9 had SS. There were 14 healthy subjects in the normal-titer group. The high-titer group had 9 females and 9 males, and the normal-titer group had 54 females and 32 males. The mean age of the high-titer group was 58.0 years (± 13.9 SD), and that of the normal-titer group was 57.9 years (± 14.2 SD). The mean titer of the anti-Hsp60 antibody of the high-titer group was 89.2 (± 78.4 SD) ng/mL, and that of the normal-titer group was 19.6 (± 8.56 SD) ng/mL (Table 1).

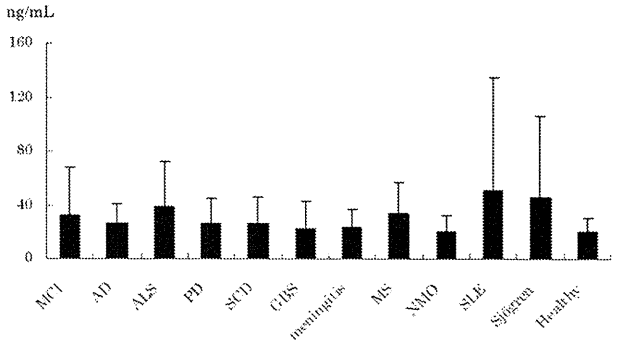

Figure 1. *Titers of anti–heat shock protein 60 antibody in patients with neurologic diseases and healthy subjects.*

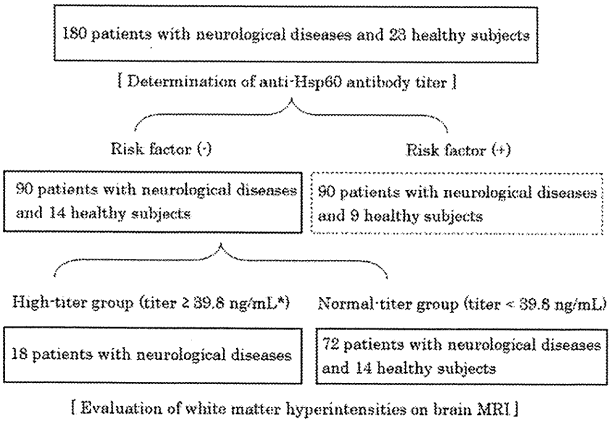

Figure 2. *Study design. The* asterisk *indicates the cut-off value (mean titer + 2 standard deviations in sera from 23 healthy subjects).*

MRI Findings of Patients and Healthy Subjects

According to the Fazekas rating scale, DWMHs of 90 patients and 14 healthy subjects were categorized as grade 0 (n = 24), grade 1 (n = 50), grade 2 (n = 27), or grade 3 (n = 3). In the high-titer group, DWMHs were rated as grade 3 in 1 patient, grade 2 in 9 patients, grade 1 in 7 patients, and grade 0 in 1 patient. In the normal-titer group, DWMHs were rated as grade 3 in 2 patients, grade 2 in 18 patients, grade 1 in 43 patients, and grade 0 in 23 patients. PVHs of 90 patients and 14 healthy subjects were categorized as grade 0 (n = 8), grade 1 (n = 68), grade 2 (n = 24), or grade 3 (n = 4). In the high-titer group, PVHs were rated as grade 3 in 1 patient, grade 2 in 8 patients, grade 1 in 8 patients, and grade 0 in 1 patient. In the normal-titer group, PVHs were rated as grade 3 in 3 patients, grade 2 in 16 patients, grade 1 in 60 patients, and grade 0 in 7 patients.

Relationship Between Anti-Hsp60 Antibody Titer and WMHs on Brain MRI

The mean grade of DWMHs (1.56 ± 0.70 SD) was significantly higher in the high-titer group than in the normal-titer group (0.09 ± 0.76 SD; $P < .003$). The mean grade of PVHs (1.50 ± 0.71 SD) was also significantly

Table 1. *Characteristics of the high- and normal-titer groups*

Characteristic	High-titer group (n = 18)	Normal-titer group (n = 86)
Sex (female:male)	9:9	54:32
Age, y (mean ± SD)	58.0 ± 13.9	57.9 ± 14.2
Anti-Hsp60 antibody titer (mean ± SD, ng/mL)	89.2 ± 78.4	19.6 ± 8.56
Grade of DWMH (mean ± SD)	1.56 ± 0.70†	0.99 ± 0.76
Grade of PVH (mean ± SD)	1.50 ± 0.71‡	1.17 ± 0.62

Abbreviations: DWMH, deep white matter hyperintensity; Hsp60, heat shock protein 60; PVH, periventricular hyperintensity; SD, standard deviation.

†$P < .003$, Comparison between the high- and normal-titer groups.

‡$P < .02$, Comparison between the high- and normal-titer groups.

higher in the high-titer group than in the normal-titer group (1.17 ± 0.62 SD; $P < .02$; Table 1).

Discussion

In this study, we determined the titer of the anti-Hsp60 antibody using ELISA and analyzed the brain MRI findings of the patients with high titers of this antibody. Our findings suggest a significant relationship between anti-Hsp60 antibody titer and the severity of WMHs on brain MRI scans. There is a previous report describing the association between anti-Hsp60 antibody and cerebrovascular disease.[12] However, our study showed the association between anti-Hsp60 antibody titer and WMHs on brain MRI scans for the first time. There is neuropathologic evidence that confluent MRI white matter lesions in the elderly reflect ischemic brain damage related to microangiopathy of cerebral small vessels.[20-22] Endothelial activation and dysfunction may play a causal role in the pathogenesis of cerebral SVD.[23] The abnormal WMHs on brain MRI scans may be related to the microangiopathy associated with vascular endothelial cell dysfunction of cerebral small vessels. Several risk factors have been identified for cerebral SVD.[24,25] Hypertension and age were the major risk factors for SVD.[26-28] It has been proposed that other risk factors are required to evaluate SVD. We suggest that an elevated anti-Hsp60 antibody titer could be a risk factor for cerebral SVD. Further studies using a large series of patients and controls are required to clarify the relation between anti-Hsp60 antibody titer and cerebral SVD.[5]

The pathogenicity of the anti-Hsp60 antibody remains unclear. Human Hsp60 is a molecular chaperone that participates in the folding of mitochondrial proteins and facilitates the proteolytic degradation of misfolded or denatured proteins.[1] However, it was reported that Hsp60 was detected on the membrane surface of stressed human endothelial cells,[2,3] and the antibody against human Hsp60 induces endothelial cell cytotoxicity.[4,5] Hsp60 is ubiquitous and structurally highly conserved molecules. Because of the strong homology between microbial and human Hsp60, an immune reaction against infectious microbes may lead to a cross-reactive autoimmune response against the native human Hsp60, which is increasingly produced and expressed cell membranes in stressed endothelial cells.[3,29,30] Recently, one of the antigens that reacted with AECAs in sera from SLE patients has been identified as human Hsp60.[2] The anti-Hsp60 antibody is present in most patients with coronary artery disease,[7,8] and its titer correlates with disease severity.[8] Moreover, the anti-Hsp60 antibody could contribute to the initiation or amplification of vascular endothelial cell damage, a crucial event in atherosclerosis[9-11] and Kawasaki disease.[31] As determined from these reports, it is possible that the anti-Hsp60 antibody could react with Hsp60 expressed on the surface membrane of stressed endothelial cells of cerebral small vessels and might be related to their dysfunction corresponding to cerebral WMHs on brain MRI scans. However, more studies are required to clarify whether the anti-Hsp60 antibody indeed play a role in the dysfunction of endothelial cells of cerebral small vessels.

References

1. Martin J, Horwich AL, Hartl FU. Prevention of protein denaturation under heat stress by the chaperonin Hsp60. Science 1992;258:995-998.
2. Dieudé M, Senécal JL, Raymond Y. Induction of endothelial cell apoptosis by heat-shock protein 60-reactive antibodies from anti-endothelial cell autoantibody-positive systemic lupus erythematosus patients. Arthritis Rheum 2004;50:3221-3231.
3. Pfister G, Stroh CM, Perschinka H, et al. Detection of HSP60 on the membrane surface of stressed human endothelial cells by atomic force and confocal microscopy. J Cell Sci 2005;118:1587-1594.
4. Schett G, Xu Q, Amberger A, et al. Autoantibodies against HSP60 mediate endothelial cytotoxicity. J Clin Invest 1995;96:2569-2577.
5. Alard JE, Dueymes M, Youinou P, et al. Modulation of endothelial cell damages by anti-Hsp60 autoantibodies in systemic autoimmune diseases. Autoimmun Rev 2007;6:438-443.
6. Jamin C, Dugué C, Alard JE, et al. Induction of endothelial cell apoptosis by the binding of anti-endothelial cell antibodies to Hsp60 in vasculitis-associated systemic autoimmune diseases. Arthritis Rheum 2005;52:4028-4038.

7. Burian K, Kis Z, Virok D, et al. Independent and joint effects of antibodies to human heat-shock protein 60 and *Chlamydia pneumoniae* infection in the development of coronary atherosclerosis. Circulation 2001;103:1503-1508.
8. Zhu J, Quyyumi AA, Rott D, et al. Antibodies to HSP60 are associated with the presence and severity of coronary artery disease: Evidence for an autoimmune component of atherogenesis. Circulation 2001;103:1071-1075.
9. Xu Q, Willeit J, Marosi M, et al. Association of serum antibodies to heat-shock protein 65 with carotid atherosclerosis. Lancet 1993;341:255-259.
10. Bason C, Corrocher R, Lunardi C, et al. Interaction of antibodies against cytomegalovirus with heat-shock protein 60 in pathogenesis of atherosclerosis. Lancet 2003; 362:1971-1977.
11. Mandal K, Foteinos G, Jahangiri M, et al. Role of antiheat shock protein 60 autoantibodies in atherosclerosis. Lupus 2005;14:742-746.
12. Gromadzka G, Zielińska J, Ryglewicz D, et al. Elevated levels of anti-heat shock protein antibodies in patients with cerebral ischemia. Cerebrovasc Dis 2001;12:235-239.
13. Kramer J, Harcos P, Prohászka Z, et al. Frequencies of certain complement protein alleles and serum levels of anti-heat-shock protein antibodies in cerebrovascular diseases. Stroke 2000;31:2648-2652.
14. Bell CL, Partington C, Robbins M, et al. Magnetic resonance imaging of central nervous system lesions in patients with lupus erythematosus. Correlation with clinical remission and antineurofilament and anticardiolipin antibody titers. Arthritis Rheum 1991;34:432-441.
15. Sanna G, Piga M, Terryberry JW, et al. Central nervous system involvement in systemic lupus erythematosus: Cerebral imaging and serological profile in patients with and without overt neuropsychiatric manifestations. Lupus 2000;9:573-583.
16. Hachulla E, Michon-Pasturel U, Leys D, et al. Cerebral magnetic resonance imaging in patients with or without antiphospholipid antibodies. Lupus 1998;7:124-131.
17. Kimura A, Sakurai T, Tanaka Y, et al. Proteomic analysis of autoantibodies in neuropsychiatric systemic lupus erythematosus patient with white matter hyperintensities on brain MRI. Lupus 2008;17:16-20.
18. Fazekas F, Chawluk JB, Alavi A, et al. MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. AJR Am J Roentgenol 1987;149:351-356.
19. Fazekas F, Kapeller P, Schmidt H, et al. MRI white matter hyperintensities: Three-year follow-up of the Austrian Stroke Prevention Study. Neurology 1999;53: 132-139.
20. Fazekas F, Kleinert R, Offenbacher H, et al. Pathologic correlates of incidental MRI white matter signal hyperintensities. Neurology 1993;43:1683-1689.
21. Schmidt R, Scheltens PH, Erkinjuntti T, et al. White matter lesion progression: A surrogate endpoint for trials in cerebral small-vessel disease. Neurology 2004;63:139-144.
22. Young VG, Halliday GM, Kril JJ. Neuropathologic correlates of white matter hyperintensities. Neurology 2008; 71:804-811.
23. Markus HS, Hunt B, Palmer K, et al. Markers of endothelial and hemostatic activation and progression of cerebral white matter hyperintensities. Longitudinal results of the Austrian stroke prevention study. Stroke 2005; 36:1410-1414.
24. Román GC, Erkinjuntti T, Wallin A, et al. Subcortical ischemic vascular dementia. Lancet Neurol 2002;1:426-436.
25. Alex M, Baron EK, Goldenberg S, et al. An autopsy study of cerebrovascular accident in diabetes mellitus. Circulation 1962;25:663-673.
26. Hiroki M, Miyashita K. Linear hyperintensity objects on magnetic resonance imaging related to hypertension. Cerebrovasc Dis 2001;11:164-168.
27. Manolio TA, Kronmal RA, Burke GL, et al. Magnetic resonance abnormalities and cardiovascular disease in older adults. The Cardiovascular Health Study. Stroke 1994; 25:318-327.
28. Khan U, Porteous L, Hassan A, et al. Risk factor profile of cerebral small vessel disease and its subtypes. J Neurol Neurosurg Psychiatry 2007;78:702-706.
29. Mayr M, Metzler B, Kiechl S, et al. Endothelial cytotoxicity mediated by serum antibodies to heat shock proteins of *Escherichia coli* and *Chlamydia pneumoniae*: Immune reactions to heat shock proteins as a possible link between infection and atherosclerosis. Circulation 1999; 99:1560-1566.
30. Wick G, Xu Q. Atherosclerosis—An autoimmune disease. Exp Gerontol 1999;34:559-566.
31. Nagata S, Yamashiro Y, Ohtsuka Y, et al. Heat shock proteins and superantigenic properties of bacteria from the gastrointestinal tract of patients with Kawasaki disease. Immunology 2009;128:511-520.

Journal of Alzheimer's Disease 29 (2012) 373–377
DOI 10.3233/JAD-2011-111343
IOS Press

Antibodies Against the Tom40 Subunit of the Translocase of the Outer Mitochondrial Membrane Complex and Cognitive Impairment in Alzheimer's Disease

Akio Kimura[a,*], Takeo Sakurai[a], Megumi Yamada[a], Akihiro Koumura[a], Yuichi Hayashi[a], Yuji Tanaka[a], Isao Hozumi[a], Hirofumi Ohtaki[b], Mitsuhiro Chousa[b], Masao Takemura[b], Mitsuru Seishima[b] and Takashi Inuzuka[a]

[a]*Departments of Neurology and Geriatrics, Gifu University Graduate School of Medicine, Gifu City, Japan*
[b]*Department of Informative Clinical Medicine, Gifu University Graduate School of Medicine, Gifu City, Japan*

Accepted 4 December 2011

Abstract. Recent studies suggest that microvascular abnormalities are involved in pathology and progression of Alzheimer's disease. The purpose of this study was to examine the presence of antibodies against cerebral microvascular endothelial cells specific for Alzheimer's disease, and to evaluate the association of these antibodies with cognitive impairment. The study included patients with Alzheimer's disease (age ≥60 years; 24 patients), control subjects without neurological diseases (age ≥60 years; 19 subjects), patients with multiple sclerosis (all ages; 17 patients), and healthy control subjects (age <40 years; 18 subjects). Serum was analyzed with 2-dimensional electrophoresis and Western blot, with cultured human brain microvascular endothelial cells as the antigen source. The anti-Tom40 antibody was identified significantly more frequently in patients with Alzheimer's disease than control subjects or patients with multiple sclerosis. In patients with Alzheimer's disease, the mean scores for the Mini-Mental State Examination were significantly lower for patients who were positive for anti-Tom40 antibody than those who were negative for anti-Tom40 antibody. In summary, the anti-Tom40 antibody is significantly associated with cognitive impairment in patients with Alzheimer's disease.

Keywords: Antigen, cognition, dementia, endothelial cell, mitochondrion, pathogenesis

INTRODUCTION

Regional microvascular abnormalities have been identified around blood vessels in the brain in autopsies of patients with Alzheimer's disease (AD), and these abnormalities may precede cognitive and neurodegenerative changes in AD and other cerebrovascular diseases with or without amyloid deposition [1, 2].

Inflammation of microvascular endothelial cells of the brain may contribute to disease processes in AD [3, 4]. Patients with AD have a high titer of antibrain autoantibodies [5, 6]. Autoantibodies to ecto-F1-ATPase, which could exert a pathogenetic role in AD, may be found in serum and cerebrospinal fluid from patients with AD [7].

We initially hypothesized that the antiendothelial cell antibodies may be a pathogenetic factor contributing to cognitive impairment in AD. We examined antiendothelial cell antibodies in the serum from patients with AD and target antigens by 2-dimensional electrophoresis, followed by Western blotting and

*Correspondence to: Dr. Akio Kimura, Department of Neurology and Geriatrics, Gifu University Graduate School of Medicine, Gifu, 1-1 Yanagido, 501-1194 Gifu, Japan. Tel.: +81 58 230 6253; Fax: +81 58 230 6256; E-mail: kimura1@gifu-u.ac.jp.

ISSN 1387-2877/12/$27.50

liquid chromatography with tandem mass spectrometry. The target antigen of our detected antibody in the serum from patients with AD was the translocase of the outer mitochondrial membrane 40 kDa subunit (Tom40), which was present in not only endothelial cells but also other tissues including neurons. Interestingly, a recent study showed that the gene polymorphism encoding this protein predicts the age of onset of late-onset AD [8]. Therefore we hypothesized that this antibody may be associated with cognitive impairment in AD, and we evaluated the relation between the presence of this antibody and cognitive dysfunction in patients with AD.

MATERIALS AND METHODS

Patients

From May 2006 to April 2010, there were 155 consecutive patients >60 years evaluated as outpatients at the Department of Neurology and Geriatrics, Gifu University Graduate School of Medicine because of memory loss. Of these patients, we randomly selected 24 patients with AD and 19 control subjects without neurological diseases for inclusion in the study. All subjects with AD had physical and neurological examinations, neuropsychological testing, laboratory studies, and brain magnetic resonance imaging to exclude other causes of dementia. All patients with AD met International Classification of Diseases (ICD)-10 criteria for dementia.

We also selected 17 consecutive patients with multiple sclerosis (all ages) who were evaluated at our institution and 18 other healthy volunteers (age <40 years). The study was approved by the institutional review board of the Gifu University Graduate School of Medicine.

Serum samples

Serum samples from all patients and control subjects were collected and stored at −30°C. All protein spots corresponding to target antigens that reacted with antiendothelial cell antibodies in the serum samples were detected with 2-dimensional electrophoresis after immunoblotting.

Primary human brain microvascular endothelial cell proteins

Primary cultured human brain microvascular endothelial cells (Applied Cell Biology Research Institute, Kirkland, WA, USA) were used as the antigen source for 2-dimensional immunoblotting. The cultured cells were homogenized in lysis buffer (5 M urea; 2 M thiourea; 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate [CHAPS], 2%; 0.1 M dithiothreitol [DTT]; Pharmalyte [pH, 3 to 10], 2%) containing proteinase inhibitor mixtures (Complete Mini, Roche Diagnostics GmbH, Mannheim, Germany) and centrifuged at 18 800 × *g* for 40 min, and the supernatant was used in all experiments. The protein concentration was determined by a protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA).

Electrophoresis, mass spectrometry, and immunoblotting

The primary cultured human brain microvascular endothelial cell sample was loaded onto an immobilized and rehydrated dry strip (pH, 3 to 10; length, 13 cm) (GE Healthcare, Buckinghamshire, UK). Extracted protein (35 to 50 μg) was applied to the dry strip for Western blotting. In the slip loading method, isoelectric focusing in the first dimension was performed according to a previously reported method [9]. The electrophoresis in the second dimension was done vertically, using an electrophoresis apparatus (ERICA-S, DRC, Tokyo, Japan) at a constant voltage of 300 V for 2 h. After the electrophoresis, the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels were stained (SYPRO Ruby, Bio-Rad Laboratories) or used for protein transfer onto polyvinylidene difluoride membranes. Separated proteins were electrophoretically transferred to a polyvinylidene difluoride membrane at a constant voltage of 32 V for 3 h using a buffer transfer tank with cool equipment (ERICA-S, DRC). Subsequently, this membrane was incubated in a blocking solution (5% skim milk in 1× TBST; 1× Tris-buffered saline containing 0.1% Tween 20) for 1.5 h at room temperature, and then reacted with a patient's serum diluted (1 : 2000), or commercially available rabbit anti-Tom40 polyclonal antibodies (Abnova, Taiwan) diluted (1 : 500), with 1% skim milk in 1× TBST overnight in a cold room. The polyvinylidene difluoride membrane was washed 5 times with 1× TBST and reacted with peroxidase-conjugated goat anti-human immunoglobulin (IgA, IgG, and IgM) antibodies (Life Technologies, Carlsbad, CA, USA) that had been diluted (1 : 2000), or peroxidase-conjugated goat anti-rabbit IgG antibodies (Santa Cruz Biotechnology, CA, USA) that had been diluted (1 : 8000), with 1% skim