6 infection, we determined the concentrations of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2), IL-4, IL-6, IL-10, and soluble TNF receptor 1 (sTNFR1) in the serum and cerebrospinal fluid (CSF) of affected infants, compared to infants with febrile seizures associated with HHV-6 infection. In addition, the relationships between the presence of HHV-6 DNA identified by the polymerase chain reaction (PCR) and cytokine levels in the CSF of infants with acute encephalopathy were investigated.

2. Patients and methods

2.1. Acute encephalopathy associated with HHV-6 infection

Informed consent was obtained from the parents of infants enrolled in this study. CSF and serum samples were obtained from 15 infants (seven males and eight females, aged from 3 to 19 months: median, 12 months) showing exanthema subitum clinically with HHV-6 encephalopathy on admission to our hospital and four collaborating research hospitals from January 2001 to June 2007 (Table 1). We divided the infants with the disease into two groups, i.e., those who had neurological sequelae (Group 1, $n = 9$), and those who survived (Group 2, $n = 6$). The criteria for the diagnosis of acute encephalopathy associated with HHV-6 infection were: (1) clinical symptoms and signs compatible with acute encephalopathy, which was defined as a febrile disorder with an alteration of consciousness and slow activity on electroencephalography lasting for more than 24 h after an acute onset, and no bacteria or fungi on CSF cultures, all other neurologic, vascular, metabolic, endocrine, toxic, and drug-induced disorders having been excluded; and (2) isolation of HHV-6 from the throat, and/or a 4-fold increase in the antibody titer determined by the fluorescence antibody test. The day of fever onset was considered as the first day of illness. Neurologic symptoms, including alternation of consciousness and/or seizures, occurred on days 1–2 of the illness. The duration of initial seizures in infants was 29 ± 18 min (mean ± SD; range, 5–60 min). The duration of consciousness disturbance of the infants was 3 to more than 60 days. CSF and serum samples were taken from the infants on days 1–6 of the illness. The neurologic prognoses of the affected infants were made by pediatric neurologists 6–24 months after the onset of encephalopathy.

2.2. Febrile seizures associated with HHV-6 infection

Febrile seizures associated with HHV-6 infection were defined as seizures with fever and impaired consciousness lasting less than 24 h without neurological sequelae, with HHV-6 infection being verified by the above mentioned method. Twelve infants were enrolled (six males and six females, aged from 6 to 25 months: median, 13 months), as shown in Table 1. The duration of febrile seizures of the infants was 12 ± 13 min (mean ± SD; range, 5–40 min). Serum and CSF were obtained from 10 and 9 of the 12 infants, respectively. CSF and serum samples were obtained from all infants on the onset day of febrile seizures. CSF cell counts were 1.9 ± 1.5/μl (mean ± SD; range, 0–4/μl), and CSF pro-

Table 1
Data from 15 infants with acute encephalopathy and febrile seizures associated with HHV-6 infection.

Patient no./age/sex	Day of onset[a]	Sampling day	Duration of initial seizures (min)	Duration of consciousness disturbance (days)	CSF findings		Neurological sequelae
					Cell (/μl)	Protein (mg/dl)	
Group 1, Infants with acute encephalopathy who had neurologic sequelae							
1/18 mo/F	2	2	40	14	1	11	Right hemiplegia
2/13 mo/F	2	2	15	40	1	14	MeR
3/11 mo/F	1	4	45	21	1	9	Left hemiplegia
4/13 mo/F	2	4	30	17	2	20	MeR
5/7 mo/M	1	4	30	30	1	13	MeR, Epi
6/9 mo/F	2	6	20	>60	1	17	Severe tetraplegia
7/12 mo/M	2	2	60	>60	1	10	Severe tetraplegia
8/6 mo/F	2	2	60	>60	1	21	Severe tetraplegia, Epi
9/9 mo/F	2	2	5	14	3	26	MeR, Epi
Group 2, Infants with acute encephalopathy who did not have neurologic sequelae							
10/15 mo/M	2	2	30	7	1	19	–
11/11 mo/M	1	2	40	5	1	28	–
12/12 mo/F	2	5	30	21	3	16	–
13/13 mo/M	1	1	20	7	2	22	–
14/3 mo/M	1	3	5	4	1	18	–
15/19 mo/M	1	4	10	3	2	14	–
Group 3, Infants with febrile seizures							
$N = 12$	1.4 ± 0.5	1.0	13 ± 12	–	1.9 ± 1.5	22 ± 5	–

The day of fever onset was considered the first day of illness. MeR, mental retardation; Epi, epilepsy.
[a] Day of onset of acute encephalopathy or febrile seizures.

tein levels were 22 ± 5 mg/dl (mean ± SD; range, 15–30 mg/dl).

2.3. Control subjects

The control subjects for the serum cytokine levels were 37 healthy children (17 males and 20 females, aged from 3 months to 3 years; median, 17 months). The control subjects for the CSF levels of cytokines were 17 afebrile and noninfectious children with neurological disorders, such as psychomotor delay, epilepsy, etc. (eight males and nine females, aged from 3 months to 3 years; median, 18 months). CSF samples were obtained from these subjects on routine analysis, and all exhibited normal CSF cell counts.

2.4. Determination of cytokine and sTNFR1 concentrations

The concentrations of serum and CSF IFN-γ, TNF-α, IL-2, IL-4, IL-6, and IL-10 were measured with a cytometric bead array (CBA) kit (BD PharMingen, San Diego, CA, USA) according to the manufacturer's instructions, as described previously [12–14]. Data analysis was performed using GraphPad Prism software (GraphPad Prism Software, San Diego, CA, USA). Briefly, a CBA comprises a series of beads exhibiting discrete fluorescence intensities at 670 nm. Each series of beads is coated with a monoclonal antibody against a single cytokine, and a mixture of six series of beads can detect six cytokines in one sample. A secondary phycoerythrin-conjugated monoclonal antibody stains the beads proportionally to the amount of bound cytokine. After fluorescence intensity calibration and electronic color compensation procedures, standard and test samples were analyzed with a FACScan flow cytometer equipped with CellQuest software (BD PharMingen). Data were transferred to GraphPad Prism for transformation and analysis. Starting with standard dilutions, the software performed log transformation of the data, and then fitted a curve to 10 discrete points using a four-parameter logistic model. A calibration curve generated for each cytokine was used to determine the cytokine concentrations of the samples. The lower detection limits for IFN-γ, TNF-α, IL-2, IL-4, IL-6, and IL-10 were 7.1, 2.8, 2.6, 2.6, 2.5, and 2.8 pg/mL, respectively.

The concentrations of sTNFR1 in serum and CSF were determined using an sTNFR1 ELISA kit (Bender Medsystems, Vienna, Austria), with a detection limit of 0.05 ng/mL.

2.5. Determination of HHV-6 DNA in the CSF

HHV-6 DNA in CSF samples from 15 infants with acute encephalopathy associated with HHV-6 infection was examined by the nested PCR method, as described previously [15].

2.6. Statistical analysis

All data were log-transformed to obtain an approximately normal distribution. The differences between groups were analyzed using the *t*-test. Correlations were analyzed using Pearson's correlation coefficient. The *p*-values less than 0.05 were considered significant. Analyses and calculations were performed using SPSS-12.0 (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Serum concentrations of cytokines

The serum IL-6 levels in infants with acute encephalopathy who had neurological sequelae were significantly higher than those without such sequelae, those with febrile seizures, and those in controls ($p = 0.043$, $p = 0.011$, and $p < 0.001$, respectively) (Fig. 1 and Table 2). The serum IL-6 levels in infants with acute encephalopathy who did not have neurological sequelae and those with febrile seizures were significantly higher than those in controls (both $p = 0.002$) (Fig. 1). The serum IL-10 levels in infants with acute encephalopathy who had neurological sequelae were significantly higher than those with febrile seizures and those in controls ($p = 0.043$ and $p < 0.001$, respectively) (Fig. 1). The serum IL-10 levels in infants with acute encephalopathy who did not have neurological sequelae were significantly higher than those in controls ($p < 0.001$) (Fig. 1). The serum sTNFR1 levels in infants with acute encephalopathy who had neurological sequelae were significantly higher than those without neurological sequelae, those with febrile seizures, and those in controls ($p = 0.026$, $p = 0.002$, and $p = 0.003$, respectively) (Fig. 1 and Table 2). There were significant correlations among serum IL-6, IL-10, and sTNFR1 levels in infants with HHV-6 infection, including acute encephalopathy and febrile seizures (IL-6 and IL-10, $p < 0.001$; IL-6, and sTNFR1, $p = 0.005$; IL-10, and sTNFR1, $p = 0.013$) (Fig. 2). There were no significant differences in serum IFN-γ, TNF-α, IL-2, or IL-4 levels among infants with acute encephalopathy with/without neurological sequelae, those with febrile seizures, and controls.

3.2. CSF concentrations of cytokines

The CSF IL-6 levels in infants with acute encephalopathy who had neurological sequelae were significantly higher than those without such sequelae, those with febrile seizures, and those in controls ($p = 0.029$, $p = 0.029$, and $p < 0.001$, respectively) (Fig. 3 and Table 2). The

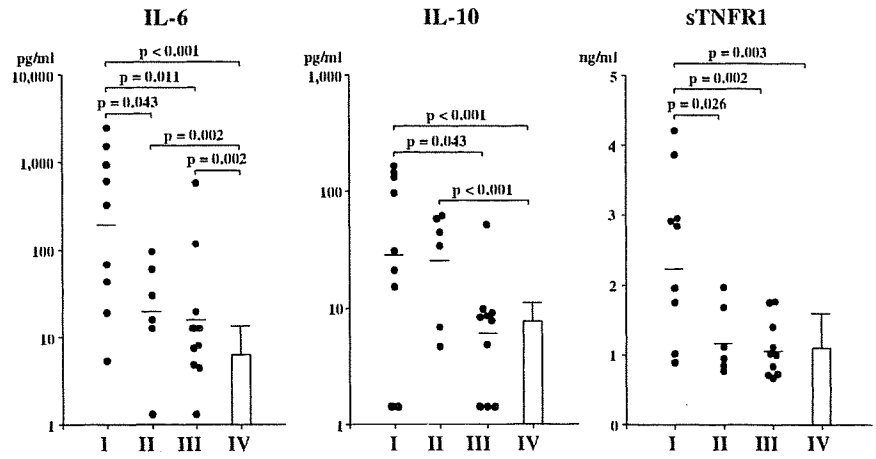

Fig. 1. Serum concentrations of IL-6, IL-10, and sTNFR1 in infants with acute encephalopathy and febrile seizures associated with HHV-6 infection, and controls. (I) Acute encephalopathy with neurological sequelae; (II) acute encephalopathy without neurological sequelae; (III) febrile seizures; (IV) controls. Horizontal lines show geometric means. Data of controls are presented as means + 1 SD. The shaded area indicates under the detection limits.

Table 2
Serum and CSF cytokine concentrations and CSF HHV-6 DNA in 15 infants with acute encephalopathy associated with HHV-6 infection.

Patient no.	Serum			CSF			HHV-6 DNA in the CSF (copies/ml)
	IL-6 (pg/ml)	IL-10 (pg/ml)	sTNFR1 (ng/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)	sTNFR1 (ng/ml)	
Group 1, Infants with neurologic sequelae							
1	5.3	<2.8	1.75	15.1	<2.8	1.27	–
2	923.6	30.7	2.91	25.6	<2.8	1.88	168
3	18.9	<2.8	1.95	116.0	<2.8	1.22	–
4	42.5	15.2	1.01	49.0	<2.8	0.98	1240
5	67.6	20.8	0.88	73.1	<2.8	1.33	56
6	320.0	146.0	2.95	59.8	16.4	1.49	–
7	2461.3	131.0	2.84	27.4	18.3	1.94	102
8	597.4	97.1	4.21	15.9	6.8	0.49	–
9	1498.1	166.5	3.87	22.7	4.4	1.66	–
Group 2, Infants without neurologic sequelae							
10	59.1	58.0	0.94	39.4	9.2	0.81	–
11	94.7	44.0	0.85	10.6	<2.8	0.91	–
12	12.4	61.3	1.96	8.6	<2.8	1.15	–
13	<2.5	4.6	1.67	<2.5	4.6	1.15	52
14	15.9	34.0	1.11	18.9	<2.8	0.98	–
15	29.4	6.8	0.76	19.4	<2.8	0.85	1600

CSF IL-6 levels in infants with acute encephalopathy who did not have neurological sequelae and those with febrile seizures were significantly higher than those in controls ($p = 0.002$ and $p = 0.005$, respectively) (Fig. 3). The CSF sTNFR1 levels in infants with acute encephalopathy who had neurological sequelae were significantly higher than those with febrile seizures, and those in controls ($p = 0.005$ and $p = 0.048$, respectively) (Fig. 3). There were no correlations among CSF cytokine levels in infants with HHV-6 infection, including acute encephalopathy and febrile seizures. There were no significant differences in serum IFN-γ, TNF-α, IL-2, IL-4, or IL-10 levels among infants with acute encephalopathy with/without neurological sequelae, those with febrile seizures, and controls.

3.3. Relationships between serum and CSF concentrations of cytokines and clinical data

All five infants in whom all of the serum IL-6, sTNFR1, and CSF IL-6 levels were elevated, had neurologic sequelae (Table 2). Especially, three infants with severe tetraplegia (Patients 6, 7, and 8) exhibited high levels of serum IL-6, sTNFR1, and CSF IL-6. Four of the seven infants in whom two of serum IL-6, sTNFR1, and CSF IL-6 levels were elevated, had neurologic

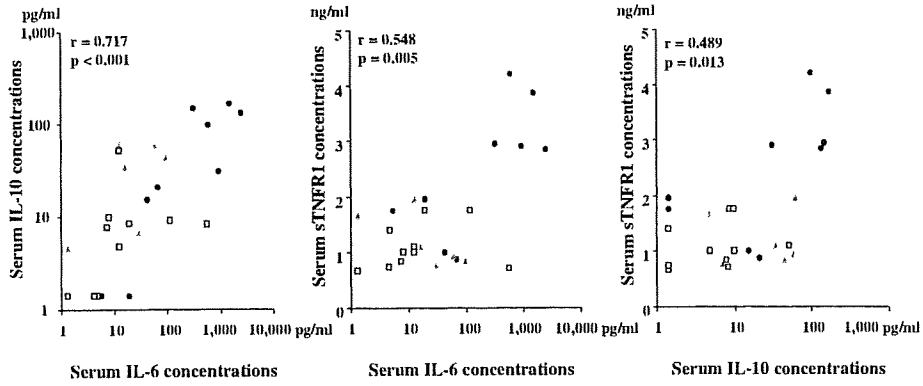

Fig. 2. The relationship among serum IL-6, IL-10, and sTNFR1 concentrations in infants with acute encephalopathy and febrile seizures associated with HHV-6 infection. Circles, triangles, and squares indicate acute encephalopathy with neurological sequelae, acute encephalopathy without neurological sequelae, and febrile seizures, respectively. *r*, Pearson's coefficient.

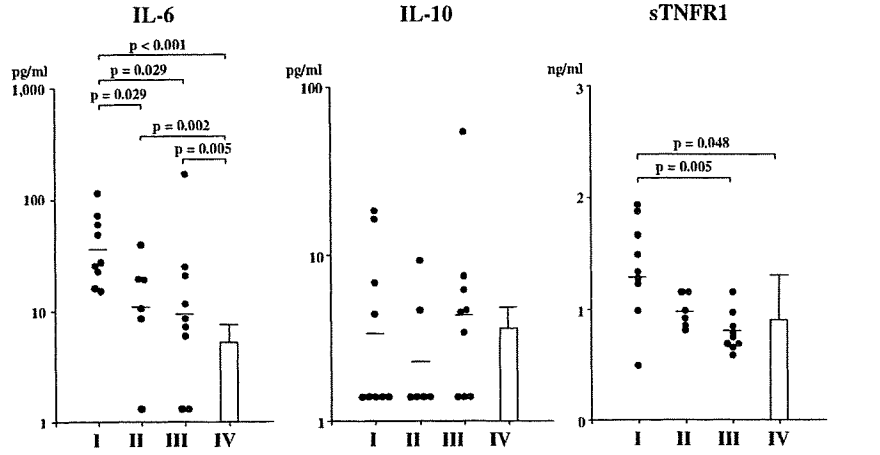

Fig. 3. CSF concentrations of IL-6, IL-10, and sTNFR1 in infants with acute encephalopathy and febrile seizures associated with HHV-6 infection, and controls. (I) Acute encephalopathy with neurological sequelae; (II) acute encephalopathy without neurological sequelae; (III) febrile seizures; (IV) controls. Horizontal lines show geometric means. Data of controls are presented as means + 1 SD. The shaded area indicates under the detection limits.

sequelae, but none of the three infants in whom one or none of those were elevated, exhibited such sequelae (Table 2).

In infants with acute encephalopathy, serum IL-6 and sTNFR1 levels were correlated with the durations of consciousness disturbance (IL-6, $p = 0.025$ and $r = 0.575$; sTNFR1, $p = 0.003$ and $r = 0.716$), but not seizure durations, as shown in Tables 1 and 2. There were no correlations between CSF cytokine levels and the duration of seizures or consciousness disturbance in infants with acute encephalopathy.

Fig. 4 shows serum and CSF concentrations of IFN-γ, IL-6, IL-10, and sTNFR1 in infants with acute encephalopathy. The serum IFN-γ, IL-6, IL-10, and sTNFR1 levels were significantly higher than those in the CSF ($p = 0.037$, $p = 0.037$, $p < 0.001$, and $p = 0.021$, respectively).

3.4. HHV-6 DNA in the CSF of infants with acute encephalopathy

Six of the 15 infants were positive for HHV-6 DNA by PCR in the CSF (4 in Group 1 and 2 in Group 2), as shown in Table 2. There were no significant differences in serum or CSF cytokine levels between infants who were positive ($n = 6$) compared to those who were negative ($n = 9$) for HHV-6 DNA in the CSF.

4. Discussion

The pathogenesis of HHV-6 infection-associated neurologic complications remains unclear. In adult immunocompromised patients, HHV-6 encephalitis is followed by transplantation [16–19]. Our present study revealed that the serum IL-6, IL-10, sTNFR1, CSF

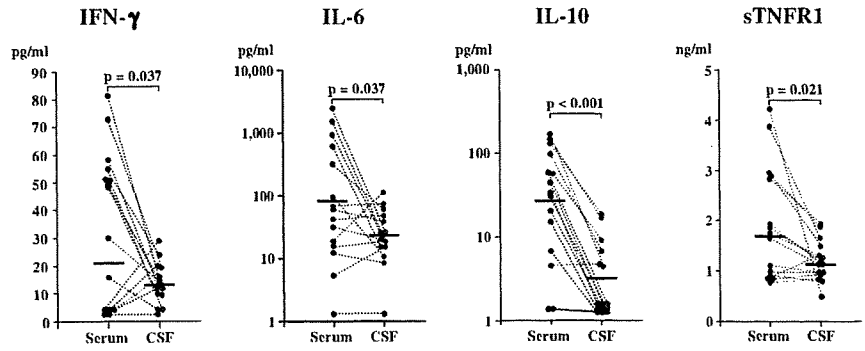

Fig. 4. Serum and CSF concentrations of IFN-γ, IL-6, IL-10, and sTNFR1 in infants with acute encephalopathy associated with HHV-6 infection. Dotted lines represent samples from the same infants on the same day. Horizontal lines show geometric means. The shaded area indicates under the detection limits.

IL-6, and sTNFR1 levels in infants with acute encephalopathy who had neurological sequelae were elevated. In particular, their serum IL-6, sTNFR1, and CSF IL-6 levels were significantly higher than those without neurological sequelae and those with febrile seizures, and the serum IFN-γ, IL-6, IL-10 and sTNFR1 levels were significantly higher than those of the CSF in acute encephalopathy. These findings suggest the existence of hypercytokinemia in acute encephalopathy. Previous studies have shown that sTNFR is an inhibitor and natural homeostatic regulator of the action of TNF-α, and that its level, rather than that of TNF-α, reflects the true biological activity of TNF-α [20–22]. TNF-α has a short half-life of 30 min but induces IL-6 [23], and this IL-6 inhibits TNF-α secretion [24]. This explains why TNF-α was not elevated while sTNFR1 was significantly elevated in the serum of affected infants. High levels of IL-6 and TNF-α in the serum may result in neuronal and vascular endothelial cell damage in acute encephalopathy. IL-10 as an anti-inflammatory cytokine decreases the production of IL-6 and TNF-α [25,26]. Therefore, we suggest that IL-10 is induced in response to the production of IL-6 and TNF-α in acute encephalopathy. IL-6 protects against excitotoxic and ischemic damage to neurons [27,28]. Therefore, IL-6 may be induced in the CSF to protect the neurons damaged by acute encephalopathy.

CSF IFN-γ levels were not elevated in all 15 infants with acute encephalopathy. We previously demonstrated that CSF IFN-γ levels were elevated in CNS disorders due to direct viral invasion, such as viral meningitis and herpes simplex encephalitis [29–31], but not in immune-mediated CNS disorders, such as acute disseminated encephalomyelitis, influenza-associated encephalopathy, acute encephalopathy following prolonged febrile seizures, and hemolytic uremic syndrome with encephalopathy [32–35]. IFN-γ, which is produced by NK cells, CD8+ and Th1 type CD4+ T lymphocytes, plays an important role in host defense against viral infection, and inhibits viral replication [36]. Therefore, IFN-γ elevating in the CSF may exert an inhibitory effect against viruses invading the CNS. Although HHV-6 DNA was positive in the CSF in 6 of the 15 infants with acute encephalopathy, their CSF IFN-γ levels were not elevated. With respect to CSF IFN-γ levels, we suggest that the main pathogenesis of acute encephalopathy associated with HHV-6 infection is not caused by the direct invasion of HHV-6 into the CNS. Our speculation supports several reports of acute encephalopathy following HHV-6 infection [6–11,37].

In infants with acute encephalopathy, the serum IL-6, sTNFR1, and CSF IL-6 levels in infants with were significantly higher than those without neurological sequelae, and serum IL-6 and sTNFR1 levels were correlated with durations of consciousness disturbance. These cytokine levels seemed to be related to the severity and prognosis in infants with acute encephalopathy. In cases whereby infants with acute encephalopathy associated with HHV-6 infection show elevated levels of serum IL-6, sTNFR1, and CSF IL-6, it is likely that they are at a high risk of developing neurological sequelae. However, a part of infants with acute encephalopathy associated with HHV-6 infection who developed neurological sequelae did not have elevated levels of cytokines in the serum or CSF. Therefore, serum or CSF cytokines' levels could not predict the outcome of all infants with acute encephalopathy associated with HHV-6 infection.

In conclusion, our results suggest that cytokines, such as IL-6, IL-10, and TNF-α, mediate the pathogenesis of acute encephalopathy associated with HHV-6 infection, and that the levels of serum IL-6, sTNFR1, and CSF IL-6 are related to the neurologic outcome.

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References

[1] Yamanishi K, Okuno T, Shiraki K, Takahashi M, Kondo T, Asano Y, et al. Identification of human herpesvirus-6 as a causal agent for exanthem subitum. Lancet 1988;1:1065–7.

[2] Asano Y, Yoshikawa T, Suga S, Yazaki T, Hata T, Nagai T, et al. Viremia and neutralizing antibody response in infants with exanthem subitum. J Pediatr 1989;114:535–9.

[3] Yoshikawa T, Asano Y. Central nervous system complications in human herpesverus-6 infection. Brain Dev 2000;22:307–14.

[4] Suga S, Yoshikawa T, Asano Y, Kozawa T, Nakashima T, Kobayashi I, et al. Clinical and virological analyses of 21 infants with exanthem subitum (roseola infantum) and central nervous system complications. Ann Neurol 1993;33:597–603.

[5] Kamei A, Ichinohe S, Onuma R, Hiraga S. Acute disseminated demyelination due to primary human herpesvirus-6 infection. Eur J Pediatr 1997;156:709–12.

[6] Oki J, Yoshida H, Tokumitsu A, Takahashi S, Miyamoto A, Yoda M, et al. Serial neuroimages of acute necrotizing encephalopathy associated with human herpesvirus 6 infection. Brain Dev 1995;17:356–9.

[7] Ohsaka M, Houkin K, Takigami M, Koyanagi I. Acute necrotizing encephalopathy associated with human herpesvirus-6 infection. Pediatr Neurol 2006;34:160–3.

[8] Enoki H, Takeda S, Matsubayashi R, Matsubayashi T. Steroid therapy in an infant with human herpesvirus 6 encephalopathy. Brain Dev 2006;28:597–9.

[9] Kubo T, Sato K, Kobayashi D, Motegi A, Kobayashi O, Takeshita S, et al. A case of HHV-6 associated acute necrotizing encephalopathy with increase of $CD56^{bright}$ NK cells. Scand J Infect Dis 2006;38:1122–5.

[10] Yoshinari S, Hamano S, Minamitani M, Tanaka M, Eto Y. Human herpesvirus 6 encephalopathy predominantly affecting the frontal lobes. Pediatr Neurol 2007;36:13–6.

[11] Nagasawa T, Kimura I, Abe Y, Oka A. HHV-6 encephalopathy with cluster of convulsions during eruptive stage. Pediatr Neurol 2007;36:61–3.

[12] Chen R, Lowe L, Wilson JD, Crowther E, Tzeggai K, Bishop JE, et al. Simultaneous quantification of six human cytokines in a single sample using microparticle-based flow cytometric technology. Clin Chem 1999;45:1693–4.

[13] Cook EB, Stahl JL, Lowe L, Chen R, Morgan E, Wilson J, et al. Simultaneous measurement of six cytokines in a single sample of human tears using microparticle-based flow cytometry: allergics vs. non-allergics. J Immunol Methods 2001;254:109–18.

[14] Metelitsa LS, Naidenko OV, Kant A, Wu HW, Loza MJ, Perussia B, et al. Human NKT cells mediate antitumor cytotoxicity directly by recognizing target cell CD1d with bound ligand or indirectly by producing IL-2 to activate NK cells. J Immunol 2001;167:3114–22.

[15] Kawada J, Kimura H, Hara S, Ito Y, Kawashima H, Okuno T, et al. Absence of associations between influenza-associated encephalopathy and human herpesvirus 6 or human herpesvirus 7. Pediatr Infect Dis J 2003;22:115–9.

[16] Drobyski WR, Knox KK, Majewski D, Carrigan DR. Brief report: fatal encephalitis due to variant B human herpesvirus-6 infection in a bone marrow-transplant recipient. N Engl J Med 1994;330:1356–60.

[17] Zerr DM, Corey L, Kim HW, Huang ML, Nguy L, Boeckh M. Clinical outcomes of human herpesvirus 6 reactivation after hematopoietic stem cell transplantation. Clin Infect Dis 2005;40:932–40.

[18] Yamane A, Mori T, Suzuki S, Mihara A, Yamazaki R, Aisa Y, et al. Risk factors for developing human herpesvirus 6 (HHV-6) reactivation after allogeneic hematopoietic stem cell transplantation and its association with central nervous system disorders. Biol Blood Marrow Transplant 2007;13:100–6.

[19] Vu T, Carrum G, Hutton G, Heslop HE, Brenner MK, Kamble R. Human herpesvirus-6 encephalitis following allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant 2007;39:705–9.

[20] Duncombe AS, Brenner MK. Is circulating tumor necrosis factor bioactive? N Engl J Med 1988;319:1227.

[21] Seckinger P, Issaz S, Dayer JM. A human inhibitor of tumor necrosis factor α. J Exp Med 1988;167:1511–6.

[22] Engelmann H, Novick D, Wallach D. Two tumor necrosis factor-binding proteins purified from human urine: evidence for immunological cross-reactivity with cell surface tumor necrosis factor receptors. J Biol Chem 1990;265:1531–6.

[23] Tracey KJ, Vlassara H, Cerami A. Cachectin/tumour necrosis factor. Lancet 1989;1:1122–6.

[24] Yasukawa H, Ohishi M, Mori H, Murakami M, Chinen T, Aki D, et al. IL-6 induces an anti-inflammatory response in the absence of SOCS3 in macrophages. Nat Immunol 2003;4:551–6.

[25] Howard M, Muchamuel T, Andrade S, Menon S. Interleukin 10 protects mice from lethal endotoxemia. J Exp Med 1993;177:1205–8.

[26] Paris MM, Hickey SM, Trujillo M, Ahmed A, Olsen K, McCracken Jr GH. The effect of interleukin-10 on meningeal inflammation in experimental bacterial meningitis. J Infect Dis 1997;176:1239–46.

[27] Ali C, Nicole O, Docagne F, Lesne S, MacKenzie ET, Nouvelot A, et al. Ischemia-induced interleukin-6 as a potential endogenous neuroprotective cytokine against NMDA receptor-mediated excitotoxicity in the brain. J Cereb Blood Flow Metab 2000;20:956–66.

[28] Yamashita T, Sawamoto K, Suzuki S, Suzuki N, Adachi K, Kawase T, et al. Blockade of interleukin-6 signaling aggravates ischemic cerebral damage in mice: possible involvement of Stat3 activation in the protection of neurons. J Neurochem 2005;94:459–68.

[29] Matsubara T, Matsuoka T, Katayama K, Yoshitomi T, Nishikawa M, Ichiyama T, et al. Mononuclear cells and cytokines in the cerebrospinal fluid of echovirus 30 meningitis patients. Scand J Infect Dis 2000;32:471–4.

[30] Asaoka K, Shoji H, Nishizaka S, Ayabe M, Abe T, Ohori N, et al. Non-herpetic acute limbic encephalitis: cerebrospinal fluid cytokines and magnetic resonance imaging findings. Intern Med 2004;43:42–8.

[31] Ichiyama T, Maeba S, Suenaga N, Saito K, Matsubara T, Furukawa S. Analysis of cytokine levels in cerebrospinal fluid in mumps meningitis: comparison with echovirus type 30 meningitis. Cytokine 2005;30:243–7.

[32] Ichiyama T, Shoji H, Kato M, Sawaishi Y, Ozawa H, Matsubara T, et al. Cerebrospinal fluid levels of cytokines and soluble tumor necrosis factor receptor in acute disseminated encephalomyelitis. Eur J Pediatr 2002;161:133–7.

[33] Ichiyama T, Morishima T, Isumi H, Matsufuji H, Matubara T, Furukawa S. Analysis of cytokine levels and NF-κB activation in peripheral blood mononuclear cells in influenza virus-associated encephalopathy. Cytokine 2004;27:31–7.

[34] Ichiyama T, Suenaga N, Kajimoto M, Tohyama J, Isumi H, Kubota M, et al. Serum and CSF levels of cytokines in acute

encephalopathy following prolonged febrile seizures. Brain Dev 2008;30:47–52.
[35] Shiraishi M, Ichiyama T, Matsushige T, Iwaki T, Iyoda K, Fukuda K, et al. Soluble tumor necrosis factor receptor 1 and tissue inhibitor of metalloproteinase-1 in hemolytic uremic syndrome with encephalopathy. J Neuroimmunol 2008;196:147–52.
[36] Samuel CE. Antiviral actions of interferon. Interferon-regulated cellular proteins and their surprisingly selective antiviral activities. Virology 1991;183:1–11.
[37] Akasaka M, Sasaki M, Ehara S, Kamei A, Chida S. Transient decrease in cerebral white matter diffusivity on MR imaging in human herpes virus-6 encephalopathy. Brain Dev 2005;27:30–3.

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ORIGINAL COMMUNICATION

Serum matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 levels in non-herpetic acute limbic encephalitis

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Abstract The pathogenesis of non-herpetic acute limbic encephalitis (NHALE) has been not clear. Matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase 1 (TIMP-1) play important roles in the function of the blood–brain barrier. We measured the serum concentrations of MMP-9 and TIMP-1 by using enzyme-linked immunosorbent assay (ELISA) in 23 patients with NHALE in the acute and convalescent stages. Serum MMP-9 concentrations and ratios of serum MMP-9/TIMP-1 were significantly higher (1) in patients with NHALE in acute and convalescent stages than in control patients (all $P < 0.001$); (2) in patients with NHALE at the acute stage compared with those at the convalescent stage ($P = 0.004$, and $P = 0.014$, respectively). In contrast, serum TIMP-1 concentrations were significantly lower in patients with NHALE in the acute and convalescent stages than in control patients (both $P < 0.001$) but did not differ in patients with NHALE in the acute and convalescent stages. Our preliminary study suggests that the prolonged imbalance of MMP-9 and TIMP-1 is associated with the pathogenesis of NHALE.

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Keywords Blood–brain barrier · Glutamate receptor ε2 · Matrix metalloproteinase-9 · Non-herpetic acute limbic encephalitis · Tissue inhibitor of metalloproteinase-1

Introduction

In Japan, non-herpetic acute limbic encephalitis (NHALE) has been identified as a new subgroup of limbic encephalitis [1, 17, 26]. The clinical picture of NHALE is similar to that of herpes simplex encephalitis (HSE). However, NHALE is not caused by herpes simplex virus (HSV) infection or a paraneoplastic disease process. Many patients with NHALE have a better neurological prognosis than those with HSE [1, 23]. Autopsies on patients with NHALE have demonstrated neuronal loss and severe gliosis with inflammatory cell infiltrations in the hippocampus and amygdala [20, 23]. However, the pathogenesis of NHALE is still unclear.

Matrix metalloproteinases (MMPs) are a family of enzymes that mediate the degradation of extracellular matrix proteins [3]. MMPs play important roles in normal and pathological processes, including embryogenesis; wound healing; inflammation; and the development of arthritis, cardiovascular diseases, pulmonary diseases, and cancer [2]. MMP-9, a member of this family which is capable of degrading collagen IV, is a major component of the basement membrane of the cerebral endothelium and promotes the migration of cells through tissues and across the blood–brain barrier (BBB) [19]. The activity of MMPs is controlled by specific tissue inhibitors of metalloproteinases (TIMPs) [24]. TIMP-1 has high affinity for MMP-9 [18]. We have reported imbalances in the ratio of MMP-9 to TIMP-1 in neurological diseases such as acute disseminated encephalomyelitis, subacute sclerosing

panencephalitis, influenza-associated encephalopathy, acute encephalopathy following prolonged febrile seizures, haemolytic uremic syndrome with encephalopathy, and perinatal asphyxia [5, 7, 9, 12, 25, 28, 29].

To investigate the roles of MMP-9 and TIMP-1 in the pathogenesis of NHALE, we determined the serum concentrations of MMP-9 and TIMP-1 in patients with NHALE in the acute and convalescent stages. Moreover, the presence of autoantibodies against the *N*-methyl-D-aspartate glutamate receptor (GluR) ε2 subunit was determined in the serum and cerebrospinal fluid (CSF) of affected patients, and the relationships between MMP-9 and TIMP-1 levels and the presence of these autoantibodies were analyzed.

Patients and methods

Informed consent was obtained from the families of the patients and controls enrolled in this study. The protocol was approved by the institutional review board of the National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders.

Non-herpetic acute limbic encephalitis

Serum samples were obtained from 23 patients with NHALE (6 men and 17 women, ages 15–79 years; median age 32 years) who were admitted to 20 collaborating research hospitals from April 2002 to August 2007 (Table 1). The criteria for diagnosis of NHALE were:

1 acute or subacute onset of neurological disorder with limbic-associated symptoms such as amnesia, delirium, panic, anxiety, and excitation;
2 absence of HSV DNA in CSF on nested polymerase chain reaction (PCR) assay and absence of HSV antibodies in CSF on enzyme-linked immunosorbent assay (ELISA);
3 visible lesions in the temporal lobe, especially in the hippocampus and amygdala, on magnetic resonance imaging (MRI) (Fig. 1);
4 absence of malignancy and paraneoplastic disorders;
5 absence of bacteria or fungi on CSF culture; and
6 exclusion of all other neurological, vascular, metabolic, endocrine, toxic, and drug-induced disorders.

Table 1 Clinical data of patients with NHALE and control subjects

	NHALE $N = 23$	Control subjects $N = 41$
Age (median, range)	32 years, 15–79 years	39 years, 15–78 years
Gender (male:female)	6:17	5:36

Serum samples obtained during the acute and convalescent stages were stored at −70°C. Convalescent-stage samples were obtained 21–247 days (median 94 days) after sample collection at the acute stage. These samples were collected during the convalescent stage, several weeks after the neurological status started to improve, or during the chronic stage, when the patient was still morbid. Immunological therapy was administered as follows: 17 of 23 patients were treated with corticosteroids, one patient was treated with intravenous immunoglobulin (IVIG), and three patients were treated with corticosteroids and IVIG.

Controls

The control subjects were 41 healthy adult volunteers (5 men and 36 women, aged 15–78 years; median, 39 years), as shown in Table 1. There were no significant differences in age or sex between the patients with NHALE and the controls as determined using the Mann–Whitney *U* test or the chi-squared test.

Determination of serum MMP-9 and TIMP-1 concentrations

The serum concentrations of MMP-9 and TIMP-1 were determined using sandwich-type ELISA kits (Amersham, Buckinghamshire, UK). A monoclonal coating antibody was adsorbed on to polystyrene microwells to bind MMP-9 or TIMP-1 in the samples or in the standard. A horseradish peroxidase-conjugated monoclonal antibody with neutralizing activity toward MMP-9 or TIMP-1 was added to bind to MMP-9 or TIMP-1 captured by the first antibody. A substrate solution, reactive with horseradish peroxidase, was then added to the wells to produce a colour reaction proportional to the amount of MMP-9 or TIMP-1, and the absorbance was measured. The detection limits were 2.5 ng/mL for MMP-9 and 2.4 ng/mL for TIMP-1. The assay for MMP-9 recognized both the pro and active forms of MMP-9.

Detection of autoantibodies against GluRε2 in serum and CSF

Serum and CSF levels of IgG and IgM autoantibodies against GluRε2 were measured, as described previously, in 22 of 23 patients with NHALE in the acute and convalescent stages [30].

Statistical analysis

The differences between the patients and controls were analysed using the Mann–Whitney *U* test. The differences in the results obtained during the acute and the convalescent stages were analysed using the Wilcoxon matched-

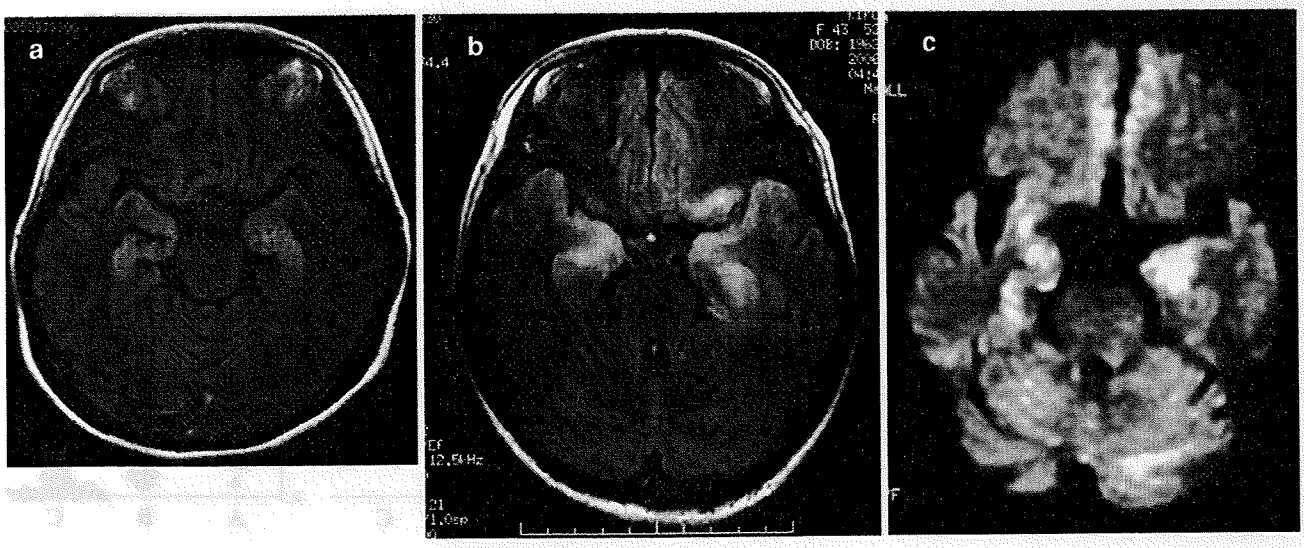

Fig. 1 **a** Fast fluid-attenuated inversion-recovery (FLAIR) MRI of a 42-year-old female patient revealing a high-signal-intensity lesion in both temporal lobes. **b** FLAIR MRI of a 43-year-old female patient showing a high-signal-intensity lesion in both temporal lobes. **c** Diffusion-weighted MRI of a 45-year-old female patient showing bilateral high-signal-intensity lesions in the temporal and frontal lobes

pairs rank-sum test. P values less than 0.05 were considered significant. Analyses and calculations were performed using SPSS-12.0 (SPSS, Chicago, IL, USA).

Results

Serum MMP-9 and TIMP-1 concentrations

The serum MMP-9 concentrations in the control subjects were 6.3–231.8 ng/mL (median 27.5 ng/mL); the TIMP-1 concentrations were 69.3–206.6 ng/mL (median 120.4 ng/mL); and the MMP-9/TIMP-1 ratios were 0.05–2.15 (median 0.22) (Fig. 2). Serum MMP-9 concentrations and the serum MMP-9/TIMP-1 ratio were significantly higher in patients with NHALE in the acute and convalescent stages than in the control subjects ($P < 0.001$). Further, these values were significantly higher in patients with NHALE in the acute stage than in patients in the convalescent stage ($P = 0.004$ and $P = 0.014$, respectively). In contrast, serum TIMP-1 concentrations were significantly lower in patients with NHALE in the acute and convalescent stages than in the control subjects ($P < 0.001$); further, these concentrations did not differ between patients with NHALE in the acute and convalescent stages.

Serum MMP-9 concentrations were not significantly correlated with serum TIMP-1 concentrations in patients with NHALE in the acute and convalescent stages (data not shown). Further, serum MMP-9 and TIMP-1 concentrations in the acute stage were not correlated with the corresponding concentrations in the convalescent stage in patients with NHALE (data not shown).

Autoantibodies against GluRε2

Autoantibodies against GluRε2 in the serum and/or CSF were detected in 18 of 22 patients with NHALE (82%) in the acute stage and in 17 of 22 patients (77%) in the convalescent stage. In the acute stage, IgG and IgM autoantibodies against GluRε2 were detected in the serum of 10 and 14 of 22 patients with NHALE, respectively, and in the CSF of 7 and 5 patients, respectively (Table 2). In the convalescent stage of NHALE, IgG and IgM autoantibodies against GluRε2 were present in the serum of 9 of 21 patients, and in the CSF of 2 of 15 patients, respectively (Table 2). There were no significant differences in serum levels of MMP-9 and TIMP-1 or in the MMP-9/TIMP-1 ratios between patients with and without autoantibodies in the serum or CSF in the acute or convalescent stage (data not shown).

Discussion

The lesions observed in NHALE were primarily located in both the temporal lobes, particularly in the hippocampus and amygdala, and these lesions were similar to those observed in HSE. However, neither HSV DNA nor anti-HSV antibodies were detected in the CSF of patients with NHALE. In previously conducted autopsies on patients with NHALE, HSV-1 and HSV-2 were not detected in the brain [20, 23]. Therefore, NHALE is regarded as a novel type of encephalitis, especially in Japan [1, 17, 23, 26]. Several autoantibodies, including those against the *N*-methyl-D-aspartate GluR and voltage-gated potassium channels, were detected

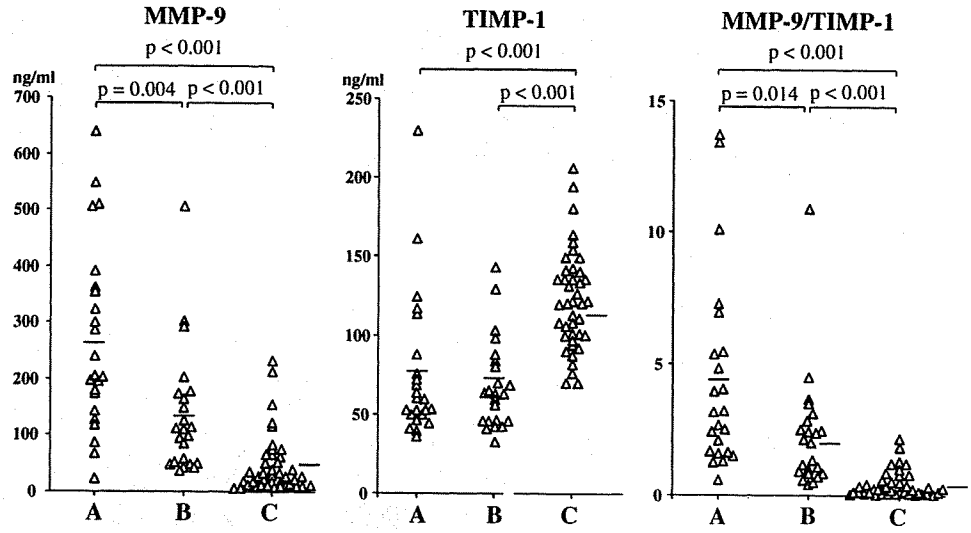

Fig. 2 Serum concentrations of MMP-9 and TIMP-1 and the MMP-9/TIMP-1 ratio in patients with NHALE ($n = 23$) and in controls ($n = 41$). **a** Patients with NHALE in the acute stage, **b** patients with NHALE in the convalescent stage, and **c** controls. The *horizontal lines* indicate median values

Table 2 The presence of autoantibodies against glutamate receptor ε2 subunit in patients with NHALE

	Serum		CSF	
	IgG	IgM	IgG	IgM
Acute stage	10/22 (45%)	14/22 (64%)	7/22 (32%)	5/22 (23%)
Convalescent stage	9/21 (43%)	9/21 (43%)	2/15 (13%)	2/15 (13%)

in patients with NHALE [4, 15, 16, 23, 31]. In addition, some cases of limbic encephalitis associated with autoimmune disease, including Hashimoto's disease, Sjögren's syndrome, and systemic lupus erythematosus, have been reported [14, 22, 27]. Moreover, we recently demonstrated that the CSF concentrations of interferon-γ (IFN-γ) were not elevated in patients with NHALE [11]. We previously demonstrated that CSF IFN-γ levels were elevated in central nervous system (CNS) disorders caused by direct viral invasion, for example viral meningitis and HSE [1, 6, 21], but not in immune-mediated CNS disorders, for example acute disseminated encephalomyelitis, influenza-associated encephalopathy, acute encephalopathy following prolonged febrile seizures, and haemolytic uremic syndrome with encephalopathy [8, 10, 13, 25]. Taking these findings into consideration, because NHALE was not associated with elevated CSF IFN-γ levels in this study, it can be concluded that NHALE is not caused by direct viral infection. The results of the above-mentioned studies suggest that NHALE is an immune-mediated form of encephalitis.

We have previously investigated serum MMP-9 and TIMP-1 levels in other neurological diseases [5, 7, 9, 12, 25, 28, 29], and our findings suggest that imbalances in the MMP-9/TIMP-1 ratio could affect the pathogenesis of each neurological disorder and/or be related to the outcome. In this study, serum MMP-9 levels and MMP-9/TIMP-1 ratios were high and serum TIMP-1 levels were low in patients with NHALE in both the acute and convalescent stages. We believe that high MMP-9 levels and low TIMP-1 levels tend to injure the BBB. Prolonged injury to the BBB may promote invasion of the CNS by leukocytes from the peripheral blood, production of autoantibodies against the CNS, and/or continued inflammation in the CNS. It is likely that MMP-9 and TIMP-1 may cause secondary modifications in the pathogenesis and clinical course of NHALE. Moreover, we have demonstrated that most patients with NHALE exhibit autoantibodies against GluRε2 in the serum and/or CSF during the illness. The autoantibodies may play roles in the pathogenesis of NHALE. However, it is still unclear whether these autoantibodies affect MMP-9 and TIMP-1 production and BBB function. A further large-scale study on the relationship between the presence of autoantibodies and serum MMP-9 and TIMP-1 levels is required to clarify this point.

In conclusion, we have shown that serum MMP-9 levels and MMP-9/TIMP-1 ratios were high and serum TIMP-1 levels were low in patients with NHALE at the acute and convalescent stages. Therefore, the imbalance of MMP-9 and TIMP-1 may affect the inflammatory process and clinical course of NHALE.

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References

1. Asaoka K, Shoji H, Nishizaka S, Ayabe M, Abe T, Ohori N, Ichiyama T, Eizuru Y (2004) Non-herpetic acute limbic

encephalitis: cerebrospinal fluid cytokines and magnetic resonance imaging findings. Intern Med 43:42–48
2. Chakraborti S, Mandal M, Das S, Mandal A, Chakraborti T (2003) Regulation of matrix metalloproteinases: an overview. Mol Cell Biochem 253:269–285
3. Chandler S, Miller KM, Clements JM, Lury J, Corkill D, Anthony DC, Adams SE, Gearing AJ (1997) Matrix metalloproteinases, tumor necrosis factor and multiple sclerosis: an overview. J Neuroimmunol 72:155–161
4. Fauser S, Talazko J, Wagner K, Ziyeh S, Jarius S, Vincent A, Schulze-Bonhage A (2005) FDG-PET and MRI in potassium channel antibody-associated non-paraneoplastic limbic encephalitis: correlation with clinical course and neuropsychology. Acta Neurol Scand 111:338–343
5. Ichiyama T, Kajimoto M, Suenaga N, Maeba S, Matsubara T, Furukawa S (2006) Serum levels of matrix metalloproteinase-9 and its tissue inhibitor (TIMP-1) in acute disseminated encephalomyelitis. J Neuroimmunol 172:182–186
6. Ichiyama T, Maeba S, Suenaga N, Saito K, Matsubara T, Furukawa S (2005) Analysis of cytokine levels in cerebrospinal fluid in mumps meningitis: comparison with echovirus type 30 meningitis. Cytokine 30:243–247
7. Ichiyama T, Matsushige T, Siba P, Suarkia D, Takasu T, Miki K, Furukawa S (2008) Cerebrospinal fluid levels of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in subacute sclerosing panencephalitis. J Infect 56:376–380
8. Ichiyama T, Morishima T, Isumi H, Matsufuji H, Matubara T, Furukawa S (2004) Analysis of cytokine levels and NF-κB activation in peripheral blood mononuclear cells in influenza virus-associated encephalopathy. Cytokine 27:31–37
9. Ichiyama T, Morishima T, Kajimoto M, Matsushige T, Matsubara T, Furukawa S (2007) Serum levels of matrix metalloproteinase-9 and tissue inhibitors of metalloproteinases 1 in influenza-associated encephalopathy. Pediatr Infect Dis J 26:542–544
10. Ichiyama T, Shoji H, Kato M, Sawaishi Y, Ozawa H, Matsubara T, Furukawa S (2002) Cerebrospinal fluid levels of cytokines and soluble tumor necrosis factor receptor in acute disseminated encephalomyelitis. Eur J Pediatr 161:133–137
11. Ichiyama T, Shoji H, Takahashi Y, Matsushige T, Kajimoto M, Inuzuka T, Furukawa S (2008) Cerebrospinal fluid levels of cytokines in non-herpetic acute limbic encephalitis: comparison with herpes simplex encephalitis. Cytokine 44:149–153
12. Ichiyama T, Siba P, Suarkia D, Takasu T, Miki K, Kira R, Kusuhara K, Hara T, Toyama J, Furukawa S (2007) Serum levels of matrix metalloproteinase-9 and tissue inhibitors of metalloproteinases 1 in subacute sclerosing panencephalitis. J Neurol Sci 252:45–48
13. Ichiyama T, Suenaga N, Kajimoto M, Tohyama J, Isumi H, Kubota M, Mori M, Furukawa S (2008) Serum and CSF levels of cytokines in acute encephalopathy following prolonged febrile seizures. Brain Dev 30:47–52
14. Ide T, Iizuka T, Suzuki N (2003) Limbic encephalitis associated with autoimmune diseases (in Japanese). Shinkei Naika 59:31–37
15. Iranzo A, Graus F, Clover L, Morera J, Bruna J, Vilar C, Martínez-Rodriguez JE, Vincent A, Santamaría J (2006) Rapid eye movement sleep behavior disorder and potassium channel antibody-associated limbic encephalitis. Ann Neurol 59:178–181
16. Kimura A, Sakurai T, Suzuki Y, Hayashi Y, Hozumi I, Watanabe O, Arimura K, Takahashi Y, Inuzuka T (2007) Autoantibodies against glutamate receptor ε_2-subunit detected in a subgroup of patients with reversible autoimmune limbic encephalitis. Eur Neurol 58:152–158
17. Kusuhara T, Shoji H, Kaji M, Ayabe M, Hino H (1994) Non-herpetic acute limbic encephalitis (in Japanese). Rinsho Shinkeigaku 34: 1083–1088
18. Lacraz S, Nicod LP, Chicheportiche R, Welgus HG, Dayer JM (1995) IL-10 inhibits metalloproteinase and stimulates TIMP-1 production in human mononuclear phagocytes. J Clin Invest 96:2304–2310
19. Lukes A, Mun-Bryce S, Lukes M, Rosenberg GA (1999) Extracellular matrix degradation by metalloproteinases and central nervous system diseases. Mol Neurobiol 19:267–284
20. Maki T, Kokubo Y, Nishida S, Suzuki H, Kuzuhara S (2008) An autopsy case with non-herpetic acute limbic encephalitis (NHALE). Neuropathology 28:521–525
21. Matsubara T, Matsuoka T, Katayama K, Yoshitomi T, Nishikawa M, Ichiyama T, Furukawa S (2000) Mononuclear cells and cytokines in the cerebrospinal fluid of echovirus 30 meningitis patients. Scand J Infect Dis 32:471–474
22. Mocellin R, Walterfang M, Velakoulis D (2007) Hashimoto's encephalopathy: epidemiology, pathogenesis and management. CNS Drugs 21:799–811
23. Mochizuki Y, Mizutani T, Isozaki E, Ohtake T, Takahashi Y (2006) Acute limbic encephalitis: a new entity? Neurosci Lett 394:5–8
24. Murphy G, Knäuper V (1997) Relating matrix metalloproteinase structure to function: why the "hemopexin" domain? Matrix Biol 15:511–518
25. Shiraishi M, Ichiyama T, Matsushige T, Iwaki T, Iyoda K, Fukuda K, Makata H, Matsubara T, Furukawa S (2008) Soluble tumor necrosis factor receptor 1 and tissue inhibitor of metalloproteinase-1 in hemolytic uremic syndrome with encephalopathy. J Neuroimmunol 196:147–152
26. Shoji H, Asaoka K, Ayabe M, Ichiyama T, Sakai K (2004) Non-herpetic acute limbic encephalitis: a new subgroup of limbic encephalitis? Intern Med 43:348
27. Stübgen JP (1998) Nervous system lupus mimics limbic encephalitis. Lupus 7:557–560
28. Suenaga N, Ichiyama T, Kubota M, Isumi H, Tohyama J, Furukawa S (2008) Roles of matrix metalloproteinase-9 and tissue inhibitors of metalloproteinases 1 in acute encephalopathy following prolonged febrile seizures. J Neurol Sci 266:126–130
29. Sunagawa S, Ichiyama T, Honda R, Fukunaga S, Maeba S, Furukawa S (2009) Matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in perinatal asphyxia. Brain Dev (in press)
30. Takahashi Y, Mori H, Mishina M, Watanabe M, Kondo N, Shimomura J, Kubota Y, Matsuda K, Fukushima K, Shiroma N, Akasaka N, Nishida H, Imamura A, Watanabe H, Sugiyama N, Ikezawa M, Fujiwara T (2005) Autoantibodies and cell-mediated autoimmunity to NMDA-type GluRε2 in patients with Rasmussen's encephalitis and chronic progressive epilepsia partialis continua. Epilepsia 46(suppl 5):152–158
31. Vincent A, Buckley C, Schott JM, Baker I, Dewar BK, Detert N, Clover L, Parkinson A, Bien CG, Omer S, Lang B, Rossor MN, Palace J (2004) Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis. Brain 127:701–712

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Original Article

Characteristics of asthma attack with long-term management for bronchial asthma

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Abstract ***Background***: There have been no reports on the evaluation of the usefulness of long-term asthma management based on the Japanese Pediatric Guideline for the Treatment and Management of Bronchial Asthma 2005 (JPGL 2005).
Methods: The purpose of the present study was to retrospectively investigate the records of 350 patients admitted to Yamaguchi University Hospital who had asthma attacks from January 2006 to June 2008. There were 149 patients who were treated for more than 3 months in accordance with the guideline (long-term management group) and 201 who were not (non-long-term management group). The patients were divided into three age groups: 100 infants, 159 toddlers, and 91 schoolchildren.
Results: The onset age of asthma in the long-term management group was earlier than that in the non-long-term management group in toddlers and schoolchildren. The white blood cell counts and C-reactive protein levels were higher in the non-long-term management group in schoolchildren, suggesting the complication of some infections. The severity of asthma in the long-term management group was greater than that in the non-long-term management group among all three age groups. There were no significant differences, however, in the severity of asthma attack at admission between the long-term and non-long-term management groups in the three age groups.
Conclusion: Patients who had severe asthma tended to be treated with long-term management, which suggests that long-term asthma management according to JPGL 2005 may reduce the severity of asthma attack at that admission, because the severity of asthma in patients undergoing long-term management correlates with the severity of asthma attack.

Key words asthma attack, Japanese Pediatric Guideline for the Treatment and Management of Bronchial Asthma, long-term management.

Asthma is a leading cause of chronic illness in childhood. There is no universally accepted definition of asthma; it may be regarded as a diffuse, obstructive lung disease with (i) hyperreactivity of the airways to a variety of stimuli; and (ii) a high degree of reversibility of the obstructive process, which may occur either spontaneously or as a result of treatment.[1] Also known as reactive airway disease, the asthma complex likely includes wheezy bronchitis, viral-associated wheezing, and atopic-related asthma. There are some differences between children and adults in the mechanism of an asthma attack, and the pathophysiology of asthma attacks in children is not fully understood.

There have been a number of guidelines for asthma treatment published throughout the world.[2–5] Furthermore, it has been proposed that childhood asthma guidelines must be developed with regard to the background of the individual countries.[6] Recently, the third version of the Japanese Pediatric Guideline for the Treatment and Management of Bronchial Asthma 2005 (JPGL 2005) was published by the Japanese Society of Pediatric Allergy and Clinical Immunology in November 2005.[7,8] The JPGL 2005 include a classification system of asthma severity, and recommendations for long-term management organized by age.

In the present study, to evaluate the usefulness of long-term asthma management according to the guideline, we retrospectively investigated the clinical details of patients with asthma attacks during the past 2.5 years.

Methods

Patients

This study involved asthma attack patients who were admitted to Yamaguchi University Hospital from January 2006 to June 2008 and diagnosed according to criteria given in the *Nelson Textbook of Pediatrics*.[1] Cases of re-admission, such as second admission of the same patient during the present study, were excluded. We

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Table 1 Characteristics of asthma attack vs duration of treatment for bronchial asthma in infants (<2 years old)

	Long-term management (group A)	Non-long-term management (group B)	*P*
n	31	69	–
Age (years)	1.4 ± 0.3	1.2 ± 0.5	0.105
Age at asthma onset (years)	0.76 ± 0.24	0.77 ± 0.32	0.508
Duration from attack onset to admission (days)	3.1 ± 1.6	4.4 ± 2.9	0.030
Duration of hospitalization (days)	7.8 ± 1.5	8.4 ± 3.5	0.628
Consolidation in chest X ray	18/31 (58%)	40/69 (58%)	1.000
SpO_2 (%)	92.9 ± 6.3	94.8 ± 3.9	0.087
WBC (/mm^3)	11,258 ± 3,809	12,599 ± 4,433	0.176
CRP (mg/dL)	1.55 ± 1.94	1.33 ± 1.78	0.325
pH	7.39 ± 0.04	7.39 ± 0.05	0.950
pCO_2 (mmHg)	40.8 ± 8.0	38.5 ± 7.7	0.299
pO_2 (mmHg)	48.2 ± 11.4	61.0 ± 28.5	0.150
HCO_3 (mEq/L)	23.7 ± 2.7	22.3 ± 2.2	0.183

CRP, C-reactive protein; SpO_2, percutaneous oxygen saturation; WBC, white blood cell.

also determined the severities of bronchial asthma and asthma attacks according to JPGL 2005.[7,8] We evaluated the severity of asthma using a clinical score as follows: intermittent, 1; mild persistent, 2; moderate persistent, 3; severe persistent, 4; and severity of the asthma attack at admission as follows, mild attack, 1; moderate attack, 2; severe attack, 3; respiratory failure, 4. We studied 350 patients, including 100 infants (<2 years old; mean, 1.7 years; male, $n = 63$; female, $n = 37$), 159 toddlers (2–5 years; mean, 3.2 years old; male, $n = 66$; female, $n = 93$), and 91 schoolchildren (6–15 years old; mean, 9.1 years; male, $n = 55$; female, $n = 36$).

The laboratory data, including white blood cell (WBC) counts, C-reactive protein (CRP), pH, pCO_2, pO_2, HCO_3 levels, and chest X-ray were obtained at the time of admission.

Study design

We divided the bronchial asthma patients into two groups: long-term management (oral medication, inhalation etc.) for ≥3 months (group A); and non-long-term management (group B). We compared the severity of bronchial asthma and asthma attack at admission, and the data including WBC, CRP, pH, pCO_2, pO_2, HCO_3, consolidation in chest X-ray and percutaneous oxygen saturation (SpO_2) between the two groups for each age group.

Statistical analysis

Differences in the results were analyzed using Mann–Whitney *U*-test, Wilcoxon matched paired test, and χ^2 test, and $P < 0.05$ was significant. Analyses and calculations were performed using SPSS version 12.0 (SPSS, Chicago, IL, USA).

Results

Tables 1,2 present the clinical profiles of the patients in the infant group. The duration from attack onset to admission for group A was significantly shorter than that for group B ($P = 0.030$). There were no significant differences in the age, age at asthma onset, duration of hospitalization, percentage of consolidation in chest X-ray, or the blood examination findings. The severity of bronchial asthma in group A was significantly more severe than that of group B ($P < 0.001$), as shown in Table 2.

In Tables 2,3 we list the clinical features in the toddler group. In group A the age at asthma onset was significantly lower compared with group B ($P < 0.001$). There were no significant differences in the age, age at onset, dates for hospitalization, percentage of consolidation in chest X-ray, or blood examination findings. In group A the severity of bronchial asthma was significantly more severe compared with group B ($P < 0.001$; Table 2).

Tables 2,4 list the results for schoolchildren. In group A the age at asthma onset was significantly lower compared with group B ($P < 0.001$). We also found that there were lower percentages of SpO_2, higher WBC counts and CRP levels in group B compared with group A ($P = 0.001$, $P = 0.03$ and $P = 0.005$,

Table 2 Severity of asthma vs duration of bronchial asthma treatment

	Group A	Group B
Infants (<2 years)		
n	31	69
Intermittent	0	39
Mild persistent	5	21
Moderate persistent	15	7
Severe persistent	11	2
Average of severity score	3.2 ± 0.7	1.6 ± 0.8 ($P < 0.001$)
Toddlers (2–5 years)		
n	73	86
Intermittent	1	43
Mild persistent	17	23
Moderate persistent	35	20
Severe persistent	20	0
Average of severity score	3.0 ± 0.8	1.7 ± 0.8 ($P < 0.001$)
Schoolchildren (6–15 years)		
n	45	46
Intermittent	0	25
Mild persistent	6	7
Moderate persistent	26	10
Severe persistent	13	4
Average of severity score	3.2 ± 0.6	1.8 ± 1.1 ($P < 0.001$)

Score of severity: 1, intermittent; 2, mild persistent; 3, moderate persistent; 4, severe persistent.

Group A, long-term management ≥3 months; group B, management <3 months.

Table 3 Characteristics of asthma attack vs duration of treatment for bronchial asthma in toddlers (2–5 years)

	Long-term management (group A)	Non-long-term management (group B)	*P*
n	73	86	–
Age (years)	3.6 ± 1.0	3.6 ± 1.1	0.768
Age at asthma onset (years)	1.3 ± 1.0	2.3 ± 1.3	<0.001
Duration from attack onset to admission (days)	3.2 ± 2.2	3.3 ± 3.6	0.363
Duration of hospitalization (days)	8.9 ± 6.8	7.7 ± 5.3	0.252
Consolidation in chest X ray	38/73 (52%)	43/86 (58%)	0.874
SpO_2 (%)	94.5 ± 2.7	93.8 ± 4.1	0.504
WBC (/mm^3)	13,268 ± 5,716	13,649 ± 4,871	0.432
CRP (mg/dL)	1.11 ± 1.63	1.15 ± 1.50	0.232
pH	7.37 ± 0.06	7.38 ± 0.04	0.885
pCO_2 (mmHg)	41.9 ± 6.7	40.6 ± 5.0	0.527
pO_2 (mmHg)	45.5 ± 12.7	48.8 ± 12.7	0.198
HCO_3 (mEq/L)	23.3 ± 1.8	23.2 ± 1.7	0.809

CRP, C-reactive protein; SpO_2, percutaneous oxygen saturation; WBC, white blood cell.

respectively). In group A the severity of bronchial asthma was significantly more severe compared with group B ($P < 0.001$; Table 2).

We then focused on the severity of asthma attack at admission in the two groups. In Table 5 we compared the severity of attack at admission in infants, toddlers and schoolchildren, respectively. There were no significant differences between groups A and B among the three age groups.

Discussion

The JPGL 2005 now emphasizes the importance of asthma control, a stepwise approach to asthma management, and the importance of early diagnosis and intervention. In the present study we focused on the clinical characteristics of the asthma attack in patients with or without long-term asthma management for >3 months. Regarding the duration of long-term management for bronchial asthma, we also investigated patients with or without long-term management for ≥1 month, and the results were similar to the present ones (data not shown). But we selected the ≥3 month long-term asthma management because of the reliability of the long-term asthma management. In the present study many patients were treated in clinics around Yamaguchi University Hospital, especially those in group B, and we recognize that the JPGL 2005 is not yet well known among the clinicians in this area.

In infants the duration from the onset of asthma attack to admission in group A was shorter than that in group B. The present result indicates that the parents of group A patients had all received education in relation to bronchial asthma, with the result that they would consult a doctor earlier than those in group B during the asthma attack. In both toddlers and schoolchildren the age of asthma onset in group A was lower than that in group B. It is generally thought that the age at asthma onset correlates with the severity. It has been reported that the age at asthma onset in toddlers or schoolchildren correlates with the severity of bronchial asthma.[9] The present results also indicate that age at asthma onset correlates with the severity of asthma.

In schoolchildren we found a significant difference in WBC counts and CRP levels during asthma attacks at admission. In group B these levels were higher than that in group A. It has been reported that some kinds of bacterial infection, such as *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*, and so

Table 4 Characteristics of asthma attack vs duration of treatment for bronchial asthma in schoolchildren (6–15 years)

	Long-term management (group A)	Non-long-term management (group B)	*P*
n	45	46	–
Age (years)	10.0 ± 2.2	9.1 ± 2.5	0.054
Age at asthma onset (years)	2.7 ± 2.1	5.0 ± 2.8	<0.001
Duration from attack onset to admission (days)	3.9 ± 3.6	3.4 ± 4.5	0.251
Duration of hospitalization (days)	6.7 ± 5.7	6.6 ± 3.1	0.424
Consolidation in chest X ray	9/45 (20%)	14/46 (30%)	0.334
SpO_2 (%)	95.8 ± 2.3	93.6 ± 3.6	0.001
WBC (/mm^3)	9,461 ± 3,416	11,054 ± 3,395	0.03
CRP (mg/dL)	0.38 ± 0.50	1.04 ± 1.35	0.005
pH	7.38 ± 0.04	7.40 ± 0.04	0.149
pCO_2 (mmHg)	43.5 ± 4.7	40.1 ± 4.7	0.112
pO_2 (mmHg)	49.0 ± 28.5	50.6 ± 23.3	0.349
HCO_3 (mEq/L)	25.3 ± 1.9	24.5 ± 1.8	0.28

CRP, C-reactive protein; SpO_2, percutaneous oxygen saturation; WBC, white blood cell.

Table 5 Severity of asthma attack on admission vs duration of bronchial asthma treatment

	Group A	Group B
Infants (<2 years)		
n	31	69
Mild attack	2	11
Moderate attack	25	54
Severe attack	4	4
Respiratory failure	0	0
Average of severity score	2.1 ± 0.4	1.9 ± 0.5 ($P = 0.094$)
Toddlers (2–5 years)		
n	73	86
Mild attack	7	8
Moderate attack	56	58
Severe attack	10	19
Respiratory failure	0	1
Average of severity score	2.0 ± 0.5	2.2 ± 0.6 ($P = 0.203$)
Schoolchildren (6–15 years)		
n	45	46
Mild attack	1	5
Moderate attack	41	31
Severe attack	3	10
Respiratory failure	0	0
Average of severity score	2.0 ± 0.3	2.1 ± 0.6 ($P = 0.502$)

Score of asthma attack severity: 1, mild attack; 2, moderate attack; 3, severe attack; 4, respiratory failure.

Group A, long-term management ≥3 months; group B, management <3 months.

on, worsen asthma attack in childhood.[10,11] The present results suggest that there may be a risk of asthma attack complicated by bacterial infections in group B compared with group A. Although the SpO_2 during the asthma attack at admission was lower in the group B schoolchildren, there were no significant differences in the severity of asthma attack between the two groups, suggesting that the lower SpO_2 may be a transient change.

The severity of asthma in group A was higher than that of group B in all three age groups. This means that patients with severe asthma tended to be treated with long-term asthma management. In the present study we were not able to consider whether the stepped up long-term management corresponded to the severity of bronchial asthma, because there were many patients who were managed in clinics in Yamaguchi. We were not able to obtain information regarding whether or not pediatricians in clinics treated the patients with the stepped up long-term management. JPGL 2005 recommends stepped up or stepped down long-term management corresponding to the severity of asthma, so further studies are needed to determine whether the stepped up long-term management corresponds to the severity of asthma.

Next we compared the severity of asthma attack at admission between the two treatment groups for each age group. Although we expected that the score of asthma attack severity in group A might be higher than that of group B, we could not find any differences between the two groups. The results suggest that long-term management according to JPGL 2005 may reduce the severity of the asthma attack, because the severity of asthma in patients with long-term management correlated with the severity of asthma attack. Stepped up management was initiated according to JPGL 2005 in some cases, because the levels of asthma severity were worsened by asthma attack during hospitalization. Taking the these results into consideration, we propose that prospective studies are needed to compare the severity levels of asthma attack between long-term management and non-management groups in the respective severity of asthma, monitoring the changes of asthma severity caused by treatment in long-term management group.

In conclusion, the present study indicates that patients who had severe asthma tended to be treated with long-term management, and suggests that the long-term management for asthma according to JPGL 2005 may reduce the severity of asthma attack at that admission.

References

1 Behrman RE, Kliegman RM, Jenson HB. Asthma. In: Sly RM (ed). *Nelson Textbook of Pediatrics*, 16th edn. WB Saunders, Philadelphia, PA, 2000; 664–80.

2 Myers TR. Guidelines for asthma management: A review and complication of 5 current guidelines. *Respir. Care* 2008; **53**: 767–9.

3 Global Initiative for Asthma. *Global Strategy for Asthma Management and Prevention*. NHLBI/WHO Workshop Report. GINA 2002, Report No. 02-3659. National Institute of Health, National Heart, Lung, and Blood Institute, Bethesda. 2002.

4 Global Initiative for Asthma. *Global Strategy for Asthma Management and Prevention. GINA 2006*. Medical Communications Resources, Gig Harbor, WA, USA, 2006.

5 Szefler SJ. Advances in pediatric asthma in 2007. *J. Allergy Clin. Immunol.* 2008; **121**: 614–19.

6 Guideline Committee on the Treatment and Management in Childhood Asthma, Japanese Society of Allergy and Clinical Immunology. *Japanese Pediatric Guidelines for the Treatment and Management of Asthma 2000*, 1st edn. Kyowa Kikaku, Tokyo, 2000.

7 Morikawa A, Nishima S. New Japanese pediatric guidelines for the treatment and management of bronchial asthma. *Pediatr. Int.* 2007; **49**: 1023–31.

8 Japanese Society of Allergy and Clinical Immunology. *Japanese Pediatric Guidelines for the Treatment and Management of Asthma 2005*, 1st edn. Kyowa Kikaku, Tokyo, 2005.

9 Martyn M, Weaver AL, Jacobson RM, Juhn YJ. Characterization of the duration from onset of asthma symptoms to asthma disease. *Ann. Allergy Asthma Immunol.* 2008; **100**: 589–95.

10 Annagür A, Kendirli SG, Yilmaz M, Altintas DU, Inal A. Is there any relationship between asthma and asthma attack in children and atypical bacterial infections; *Chlamydia pneumoniae*, *Mycoplasma pneumoniae* and *Helicobacter pylori*. *J. Trop. Pediatr.* 2007; **53**: 313–18.

11 Esposito S, Blasi F, Bellini F, Allegra L, Principi N; Mowgli Study Group. *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* infections in children with pneumonia. Mowgli Study Group. *Eur. Respir. J.* 2001; **17**: 241–5.

<Original Article>

Exacerbation of Myasthenia Gravis in the Treatment for Graves' disease

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Key words: Myasthenia gravis, Graves' disease, Anti-microsomal antibody, Hashimoto's disease, Methimazole

ABSTRACT

We studied the exacerbation of myasthenia gravis(MG) during treatment for Graves' disease(GD). Comparing 6 of 13 patients with both MG and GD who experienced exacerbation of MG to 7 whose symptoms were unchanged after antithyroid therapy, we studied risk factors for exacerbation related to clinicopathologic findings and treatment. Results suggested that presence of the generalized form of MG, high values of anti-AchR antibody and anti-microsomal antibody, severe hyperthyroidism, and rapid normalization of FT4 were correlated with exacerbation of MG.

INTRODUCTION

The prevalence of hyperthyroidism in myasthenia gravis (MG) has been reported as between 3 and 7 % [1]. In 1954, Maclean reported a "see-saw relationship" between MG and Graves' disease (GD), characterized by MG being exacerbated by treatment of GD and vice versa. However, despite several reports of MG symptoms fluctuating during the treatment of GD, the nature of the relationship between the two diseases remains obscure. Moreover, there is uncertainty as to the form of MG affected and the types of treatment for GD can aggravate MG symptoms. This study examined the risk factors for exacerbation of MG during the treatment of GD.

MATERIALS and METHODS

Of 76 patients admitted to our hospital for the treatment of MG during the last ten years, we examined 13 patients who were diagnosed with MG in addition to GD (6 males, 7 females, mean age 34.9± 14 years). The diagnosis of MG was based on clinical features, neurological examination, and Edrophonium stimulation test, repetitive stimulation test or detection of serum antiacetylcholine receptor (anti-AchR) antibodies. The severity of MG was based on Myasthenia Gravis foundation of America (MGFA) clinical classification [2]. Anti-AchR antibody were all measured by RIA method. The diagnosis of GD was based on clinical symptoms, undetectable TSH, increased serum FT4, and positive anti-TSH

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receptor antibody. All patients were treated with oral methimazole (MMI) and the first dosage of MMI was 15-30 mg/ day. Patients were classified into three groups according to whether MG symptoms were improved, unchanged, or exacerbated during MMI therapy. We defined that improved group were 1 grade up, exacerbated group were 1 grade down on MGFA clinical classification. We then examined correlation between demographic factors (age and gender), clinico- pathologic factors (ocular vs generalized form of MG, and values of AchR antibody, antimicrosomal antibody, anti-thyroglobulin antibody, thymus histology) and therapeutic factors (FT4 before therapy, dose of MMI, rate of FT4 normalization after treatment) and effect of GD treatment on MG symptoms.

In terms of the maintenance treatment regimens for MG before the state of GD therapy, eight patients were receiving oral pyridostigmine, two a combination of plasmapheresis and oral pyridostigmine, and two a combination of oral prednisolone and pyridostigmine. All cases were treated according to the guideline of Japanese Society of Neurological Therapeutis. Differences between groups were stastically analyzed using Mann-Whitney Test and paired t-test.

RESULTS

Changes in MG symptoms after MMI administration were as follows: no patients experienced improvement in symptoms, 7 patients reported symptoms to be unchanged, and 6 patients experienced an exacerbation of their disease (3 of these developed a myasthenic crisis).

Comparison of patient factors between the two groups was shown in table 1. Age, gender and histology of thymus tissue were not associated with exacerbation of MG symptoms. Patients whose symptoms were exacerbated by treatment of thyroid disease all suffered from the generalized form of MG, whereas in patients whose symptoms were unchanged, 1 had the generalized form and 6 the ocular form. All patients whose symptoms remained unchanged were negative for anti-AchR antibody, whereas all patients whose symptoms were exacerbated had positive to this antibody. Furthermore, of patients having reported a exacerbation of symptoms during treatment of GD, 66.7 % were positive for anti-microsomal antibody compared to 14.3 % of patients who reported no change in symptoms.

The effect of type of antithyroid treatment on MG symptoms was shown in table 2. In terms of temporal relationship between appearance of the two diseases, 8 patients presented with simultaneous symptoms of both diseases, development of MG symptoms preceded those of GD in 3 cases, while appearance of GD symptoms preceded those of MG in 2 cases.

The average FT4 level before antithyroid treatment in patients having shown exacerbation of symptoms (mean ± SD: 5.8 ± 2.57 μg/dl) was significantly higher than that in the group who experienced no change in symptoms (3.1 ± 0.80 μg/dl)($p < 0.05$). In patients whose symptoms exacerbated, the rate of FT4 reduction at 4 weeks after starting MMI therapy was 50.1 %, compared with a rate of 28.7 % in patients whose symptoms were unchanged. The normalization of thyroid function in patients who experienced a exacerbation of MG occurred significantly earlier than that in patients who did not ($p < 0.05$).

Table 1

	Unchanged group	Exacerbated group	Myasthenic crisis
Patient(Number)	7 male 4: female 3	6 2:4	(3) (2:1)
Age(yrs)	37.1±14.9	32.3±10.6	(38.7±8.3)
Type	generalized 1 ocular 6	generalized 6*	(generalized3)
Tymus tissue	hyperplasia 5 thymoma 1	hyperplasia 3 fat tissue 1	(hyperplasia 2)
Anti-AchR abtibody Positive	0/7	6/6*	(3/3)
Anti-microsomal antibody positive	1/7	4/6*	(2/3)

*P < 0.05

Table 2

Patients	FT4 before therapy (μg/dl)	Initial dosage of MMI (mg/day)	Timing of exacerbation during GD therapy (days)	Combined MG therapy	first appearance of symptom
(exacerbated group)					
1. 19 yo F	4.2	30	15	pyridostigmine 60 mg/day	simultaneity
2. 46 yo F	7	30	14	pyridostigmine 180 mg/day	simultaneity
3. 20 yo M	10.7	15	28	pyridostigmine 240 mg/day	MG
4. 37 yo F	2.6	20	30	pyridostigmine 60 mg/day	GD
5. 43 yo F	5.0	30	28	prednisolone 20 mg/day plasmapheresis	MG
6. 27 yo M	5.5	15	30	pyridostigmine 180 mg/day	simultaneity
(unchanged group)					
7. 53yo M	2.5	20		pyridostigmine 60 mg/day	simultaneity
8. 31 yo M	2.2	15		pyridostigmine 60 mg/day	MG
9. 49 yo F	3.6	15		pyridostigmine 120 mg/day	simultaneity
10. 11 yo M	3.2	15		pyridostigmine 60 mg/day	simultaneity
11. 40 yo M	4.0	15		pyridostigmine 60 mg/day	GD
12. 23 yo F	2.5	15		prednisolone 20 mg/day	simultaneity
13. 53 yo F	3.2	20		pyridostigmine 60 mg/day	simultanity

In all patients, values of anti-AchR increased after MMI therapy (before treatment 5.5 ± 4.87 nMol/l, after treatment 18.8 ± 8.97 nMol/l).

DISCUSSION

The existence of a see-saw relationship between MG and GD remains controversial. The rates of exacerbation of MG associated with treatment for GD have been reported to be between 0 and 67 % [3, 4]. In this study, the rate of exacerbation of MG was observed to be 46 % during treatment for GD. The variation in reported exacerbation rates could be due to differences in patients and therapeutic factors.

In terms of disease pathology, both the generalized form of MG and a high anti-AchR antibody values were identified as factors correlated with exacerbation of MG. Although anti-AchR antibody values do not always reflect the degree of clinical severity of MG, presence of a high anti-AchR antibody should alert clinicians to the possibility of exacerbation of MG during anti-thyroid treatment. In all patients who exacerbated, anti-AchR antibody values increased during administration of MMI. This phenomenon might indicate that MMI affected MG through an immunological mechanism. Hirata et al showed a MG with GD that anti-insulin antibody appeared and hypoglycemia was induced [4]. Many cases were reported with appearance of some autoantibodies by administration of MMI. A theory would be supported by numerous reports on development of autoantibodies after the administration of MMI [5, 6].

In this study, a high proportion (66.7 %) of patients who developed exacerbations of MG during anti-thyroid therapy were anti-microsomal antibody positive. These cases might be complicated by Hashimoto's diseae in addition to GD. This finding suggested that the complication of Hashimoto's disease might be a factor influencing exacerbation of MG symptoms. Generally, a few % of cases of MG are complicated by Hashimoto's disease, and in patient in whom the two conditions coexist, the treatment of one has not been reported to adversely affect the other, and in most cases of MG accompanied by Hashimoto's disease, it was reported that MG existed as the ocular form of the disease and symptoms were mild [7]. In contrast, other authors have a higher frequency of Hashimoto's disease to accompany generalized MG to compared to ocular MG [8]. It is reported that MG complicated by Hashimoto's disease is not well-controlled by anticholinesterase drugs [9]. As the patients in our study suffered from the generalized form of MG and were anti-AchR antibody positive, the role of Hashimoto's disease as an independent risk factor requires further investigation.

Severe degree of hyperthyroidism before therapy and rapid improvement of thyroid function are exacerbation factors of MG. In this study, serum level of FT4 before GD therapy and FT4 reduction rate in 4 weeks after treatment in exacerbated group were significantly higher than those in unchanged group. A case report also suggested that rapid improvement of thyroid function induced exacerbation for MG [10]. In MG with severe hyperthyroidism, it seems prudent to administer low dose oral antithyroid drugs in order to gradually achieve a euthyroid state.

The effects of different forms of GD treatment on MG symptoms remain unclear. In Japan, use of oral anti-thyroid drugs is more common than radioactive iodine or partial thyroidectomy. All patients in this study underwent initial treatment with MMI, whereas the cases which developed exacerbation reported by Maclean et al, were treated by radioisotope or propylthiouracil. Gaelen et al reported that radioisotope treatment of GD exacerbated MG in both hyper-and hypo-thyroid states [11]. Iwata et al reported a patient who was treated with partial thyroidectomy and did not experience exacerbation of MG [12]. In these studies, characteristics of patients and disease were not identified. It is necessary to consider that different forms of treatment of GD have different effects on the course of MG. Cholinesterase inhibitors used in the maintenance treatment of MG did not prevent exacerbation induced by antithyroid treatment in this study. Similarly, the two patients having received plasmapheresis treatment for MG also experienced exacerbations during treatment for GD.

The results suggested high anti-AchR values, severe hyperthyroidism, rapid correction to the euthyroid state and possibly the complication of Hashimoto's disease are risk factors for exacerbation of MG during anti-thyroidtherapy. Prophylactic immunosuppresant or corticosteroid therapy prior to or during antithyroid therapy might reduce the risk of MG exacerbation in those patients exhibiting risk factors for exacerbation. Further studies are therefore needed to clarify the necessity of GD therapy and concomitant immunosuppressive therapy to prevent exacerbation of MG.

REFERENCES

1. Goulon M, Estournet B, Tullieez M. Myasthenia gravis and associated disease. Intern J Neurol 1980;14:61-72.
2. Task Force of the Medical Scientific Advisory Board of the Myasthenia Gravis Foundation of America;JaretzkiⅢA, Barohn RJ, Ernstoff RM et al. Myasthenia gravis;Recommendations for clinical research standards. Neurology 2000;55:16-23.
3. Ohno M, Hamada N, Yamakawa J, Noh J, Morii H, Ito K. Myasthenia gravis with Graves' disease in Japan. Jpn J Med 1987;26;2-6.
4. Maclean B, Wilson JAC. See-saw relationship between hyperthyroidism and myasthenia gravis. Lancet ; 1:950-1.
5. Hirata Y, Tominaga M, Ito J, Noguchi A. Spontaneous hypoglycemia with insulin autoimmunity in Graves' disease. Ann Intern Med 1974;81;214-8.
6. Sanke T, Kondo M, Moriyama Y, Nanjo K, Iwao K, Miyaura K. Glucagon binding autoantibodies in a patient with hyperthyroidism treated with methimazole. J Clin Endocrinol Metab 1983;57; 1140-1.
7. Marino H, Roberta R, Pinchera A, Manetti L, Chiovato L, Robbi B, et al. Mild clinical expression of myasthenia gravis associated with autoimmune thyroid disease. J Clin Endoclinol Metab 1997;82:438-41.
8. Thoracius S, Aali JA, Riise T, Matre R, Johnsen H. Associated disorder in myasthenia gravis: Autoimmune disease and their relation to thymectomy. Acta Neurol Scand 1989;80:107-11.
9. Takamori M, Cutmann L, Crosby TW, Martin JD. Myasthenic syndrome in hypothyroidism. Arch Neurol 1968;18:107-12.
10. Takanami I, Imamuma T, Yamamoto Y, Kodaira S. The rapid transformation of hyperthyroidism to hypothyroidism complicated by myasthenia gravis. J thorac Cardiovas Surg 1995;852:110-1.
11. Gaelen LH, Levitan S. Myasthenia gravis and thyroid function. Arch Neurol 1968;18:107-12.
12. Iwata S, Osamu M, Usuku M, Usuku K, Okada A, Igata A. A case of myasthenia gravis with Basedows' disease, treated by subtotal thyroidectomy. Clin Neurol 1987;27:1055-8.

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