Journal of Medical Virology 74:355–360 (2004)

Variable Patterns of Varicella-Zoster Virus Reactivation in Ramsay Hunt Syndrome

Hiroshi Aizawa,[1] Fumio Ohtani,[1] Yasushi Furuta,[1]* Hirofumi Sawa,[2] and Satoshi Fukuda[1]

[1]*Department of Otolaryngology-Head & Neck Surgery, Hokkaido University Graduate School of Medicine, Sapporo, Japan*

[2]*Laboratory of Molecular & Cellular Pathology, CREST, JST, Hokkaido University Graduate School of Medicine, Sapporo, Japan*

The mechanism by which reactivation of varicella-zoster virus (VZV) causes facial paralysis in Ramsay Hunt syndrome remains unclear. The relationship between VZV load and the onset of facial paralysis was analyzed in 42 patients with Ramsay Hunt syndrome. The patients were divided into three groups according to the times of appearance of zoster and of facial paralysis; group I (zoster preceding, n = 13), group II (simultaneous, n = 22), group III (paralysis preceding, n = 7). A real-time quantitative PCR assay was used to measure VZV DNA copy number in saliva, and paired sera were assayed for anti-VZV IgG and IgM antibodies. In group I, the VZV DNA-positive rate was low and virus load decreased gradually after the initial hospital visit around the time of onset of paralysis. The level of anti-VZV antibodies had in most cases already increased at that time. In group III, viral load tended to increase after the onset of paralysis and peaked around the time of appearance of zoster. The level of anti-VZV antibodies was low at the onset of paralysis but showed a significant increase when paired sera were tested. In group II, virus load and changes in level of anti-VZV antibodies either resembled group I or group III behavior. These results indicate that facial paralysis in Ramsay Hunt syndrome can occur at various times between the early and the regression phase of VZV reactivation, suggesting that there are variable patterns of development of facial nerve dysfunction caused by VZV reactivation and the progression of neuritis. ***J. Med. Virol. 74:355–360, 2004.*** © 2004 Wiley-Liss, Inc.

KEY WORDS: facial paralysis; real-time quantitative PCR; anti-VZV antibody

INTRODUCTION

Varicella-zoster virus (VZV) reactivation causes facial paralysis in Ramsay Hunt syndrome. In this disease, VZV reactivation occurs mainly in the geniculate or, occasionally, trigeminal or cervical ganglia, and subsequent inflammation of the facial nerve in the temporal bone is thought to cause facial paralysis as well as zoster lesions in the auricle or the oropharyngeal epithelium [Hunt, 1907; Furuta et al., 1992]. However, the mechanism by which VZV causes nerve dysfunction remains to be elucidated. VZV may injure directly the facial nerve since VZV DNA has been detected in facial nerve endoneurial fluid obtained during decompression surgery [Murakami et al., 1996]. On the other hand, facial paralysis can occur a few weeks after the onset of zoster, when vesicles have almost disappeared, suggesting by analogy with the Guillain-Barré syndrome that it involves an immune-mediated post-infection process [Jacobs et al., 1998]. In addition, some patients with Ramsay Hunt syndrome exhibit zoster several days after the onset of facial paralysis. Therefore, some variability exists in the pathogenesis of Ramsay Hunt syndrome, and analysis of the relationship between the patterns of VZV reactivation and the onset of facial paralysis might provide a clue to the mechanism by which VZV causes facial nerve damage.

VZV DNA has been detected in the saliva, tears, cerebrospinal fluid, and peripheral blood mononuclear cells, as well as vesicles, of patients with Ramsay Hunt syndrome [Murakami et al., 1998; Furuta et al., 2001; Hiroshige et al., 2002]. However, only a few studies have analyzed the load of VZV in patients with Ramsay Hunt syndrome [Furuta et al., 2001; Hiroshige et al., 2002]. The previous study showed that salivary VZV load reflects the kinetics of virus reactivation [Furuta et al.,

Grant sponsor: Ministry of Education, Culture, Sports, Science and Technology (Grant-in-Aid for Scientific Research).

*Correspondence to: Dr. Yasushi Furuta, Department of Otolaryngology-Head & Neck Surgery, Hokkaido University Graduate School of Medicine, Kita 15, Nishi 7, Kita-ku, Sapporo 060-8638, Japan. E-mail: yfuruta@med.hokudai.ac.jp

Accepted 14 June 2004

DOI 10.1002/jmv.20181

Published online in Wiley InterScience (www.interscience.wiley.com)

2001]. To determine the role of VZV reactivation in Ramsay Hunt syndrome, this study examined the antibody response to VZV as well as the VZV load in saliva, and analyzed the relationship between the time of VZV reactivation and the onset of facial paralysis.

PATIENTS AND METHODS

Patients

In total, 42 patients with Ramsay Hunt syndrome were examined. The patients visited the Hokkaido University Hospital or its associated hospitals within 7 days of the onset of facial paralysis. Ramsay Hunt syndrome was diagnosed when there was acute peripheral facial paralysis coupled with typical zoster lesions in the skin or the oropharyngeal epithelium. Patients who did not exhibit zoster (zoster sine herpete) were excluded in this study. Informed consent was obtained from all patients.

Quantitative PCR

One to 2 ml of saliva was collected from patients at every visit and stored at −80°C. The samples were centrifuged at 2,200*g* for 5 min and DNA was extracted from 50 μl of the supernatant with a DNA extraction kit (SepaGene, Sanko Junyaku Co., Tokyo, Japan). Detection and quantitation of VZV DNA were carried out by real-time TaqMan PCR method according to the protocol described previously [Furuta et al., 2001]. Patterns of VZV load in saliva were judged as "decrease" if VZV copy number gradually decreased after the initial visit; while they were judged as "increase" if an increase in the viral copy number after the initial visit was observed.

Serological Assays

Paired sera were taken from patients at their initial visit and 2–3 weeks later (convalescent phase). Anti-VZV antibodies were measured using enzyme-linked immunosorbent assay (ELISA) kits (Enzygnost Anti-VZV/IgG and IgM, Dade Behring Marburg GmbH, Marburg, Germany) and an automatic ELISA processor (Processor III, Dade Behring Marburg GmbH). The antibody ELISA values were quantified in international units (mIU/ml) by calculation using the α-method. In each assay, anti-VZV reference serum was run in duplicate for measurement correction to avoid inter-assay variations. Significant changes (greater than 2-fold) in IgG antibody values were considered to indicate a recent VZV infection (in accordance with the manufacturer's instructions). Anti-VZV IgG antibodies were considered to be high if the ELISA value was 50×10^2 mIU/ml (the mean + 3 SD of the healthy controls) or more. Anti-VZV IgM antibodies were judged as negative when absorbance value (ΔA) was less than 0.1.

Treatment

The severity of facial paralysis was evaluated according to the House and Brackmann grading scale during every hospital visit [House and Brackmann, 1985]. Patients with facial paralysis grade IV–VI were usually given 40–60 mg prednisolone for 4 days, and the dosage was then tapered off. Steroids were not administered to patients with grade II or III facial paralysis. In 37 patients, acyclovir (4,000 mg in tablet form or 750 mg per day by infusion) or valaciclovir (3,000 mg in tablets) was administered for 5–7 days. Five patients did not receive anti-viral therapy.

RESULTS

Appearance of Zoster and Facial Paralysis

As shown in Figure 1, the time of appearance of zoster varied from 27 days before, to 13 days after, the onset of facial paralysis. The patients were divided into three groups according to time of appearance of zoster relative to facial paralysis. Thirteen patients exhibited facial paralysis 3 or more days after zoster appeared and were categorized as group I (zoster preceding group). Zoster developed 3 days or more after the onset of facial paralysis in seven patients (group III, paralysis preceding group). The remaining 22 patients were placed in group II (simultaneous group).

Confirmation of VZV Reactivation

VZV reactivation was indicated serologically in 39 of the 42 patients (93%) by either anti-VZV IgM detection (n = 34) or a significant change in anti-VZV IgG response (n = 18). In the remaining three patients, two had extremely high anti-VZV IgG antibodies (more than 100×10^2 mIU/ml) and one was positive for VZV DNA by PCR. Thus, VZV reactivation was confirmed in all patients using either serological assays or PCR. VZV DNA was detected on at least one occasion in the saliva of 25 of the 42 patients (60%) (Fig. 1).

VZV DNA Positivity and Load in Group I

VZV DNA was detected in 4 of the 13 patients (31%) in group I. In each of these four VZV DNA-positive

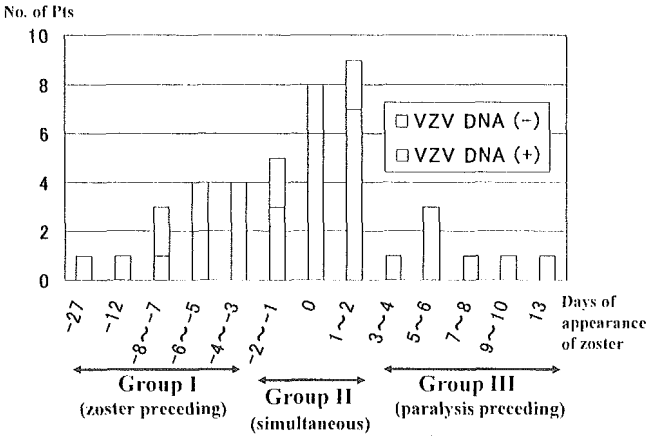

Fig. 1. The distribution of times of appearance of zoster relative to the onset of facial paralysis. Day 0 is the day of appearance of facial paralysis.

TABLE I. Patterns of Salivary VZV DNA Positivity and Load

Group	No. of VZV DNA-positive patients at the onset of facial paralysis	Patterns of VZV load in saliva		
		Gradually decrease	Increase after the initial visit	Not detected
I (n = 13)	4 (31%)	4	0	9
II (n = 22)	16 (73%)	9	7	6
III (n = 7)	5 (71%)	1	4	2
Total (n = 42)	25 (60%)	14	11	17

patients, the copy number in saliva gradually decreased after the initial visit at around the onset of facial paralysis (Table I). Paired sera were obtained from 11 of the 13 group I patients. As shown in Table II, 10 of these (91%) had high anti-VZV IgG at the initial visit and anti-VZV IgM antibody was also elevated in 8 of them (73%). However, none showed a significant increase (more than 2-fold) in their anti-VZV IgG antibody in the paired sera and only two (18%) showed seroconversion of the anti-VZV IgM antibodies. A representative case is shown in Figure 2A.

VZV DNA Positivity and Load in Group II

VZV DNA was detected in 16 of the 22 patients (73%) in group II. VZV copy number gradually decreased after the initial visit in nine of these patients, and increased in the seven other patients (Table I). Paired sera were obtained from 21 of the 22 patients. Eleven of these (52%) showed a high anti-VZV IgG value at the initial visit, and anti-VZV IgM antibodies were elevated in nine of them (43%). Anti-VZV IgG antibody increased in 11 patients (52%) at later times, and 9 (43%) showed seroconversion of the anti-VZV IgM antibodies (Table II). A representative case is shown in Figure 2B.

VZV DNA Positivity and Load in Group III

VZV DNA was detected in five of the seven patients (71%) in group III. In four of these DNA-positive patients, copy number increased after the initial visit that took place around the onset of facial paralysis and peaked near the time of appearance of zoster, while VZV copy number gradually decreased in the remaining DNA-positive patient (Table I). None of the patients in group III had a high anti-VZV IgG or IgM value at the initial visit. However, anti-VZV IgG antibody increased subsequently in all the patients in this group, and five of them (71%) showed seroconversion of the anti-VZV IgM antibodies (Table II). A representative case is shown in Figure 2C.

Influence of Antiviral Therapy on VZV Load

Antiviral agents were given to 37 of the 42 patients; the VZV load was "decreased" in 14 patients, "increased" in 9, and "not detected" in 14. Of the remaining five patients who did not receive antiviral therapy, the viral load was "increased" in two patients, and "not detected" in three. No significant difference in the patterns of salivary VZV load was observed between the two treatment groups. In addition, the patterns of systemic antibody response did not differ significantly between the two groups.

DISCUSSION

The present study analyzed the antibody response to VZV together with the VZV load in saliva in patients with Ramsay Hunt syndrome using an ELISA and a real-time quantitative PCR method. Although the variability in the timing or presence of neurological complications in VZV reactivation has been well known clinically [Echevarría et al., 1997], the findings clearly show that the viral load and host immune responses are related to the presence of zoster, and are independent of facial paralysis. In group I (zoster preceding group), VZV DNA-positive rate was relatively low and the viral load in the VZV DNA-positive patients gradually decreased after the initial visit at around the onset of facial paralysis, suggesting that viral reactivation was in the regression phase at the onset of facial paralysis. In

TABLE II. Antibody Responses to VZV

Group	No. of patients with high IgG or positive IgM antibodies at the onset of facial paralysis		No. of patients with fluctuations of antibody titers	
	High IgG antibody	Positive IgM antibody	IgG, increase more than 2-fold	IgM, seroconversion
I (n = 11[a])	10 (91%)	8 (73%)	0 (0%)	2 (18%)
II (n = 21[a])	11 (52%)	9 (43%)	11 (52%)	9 (43%)
III (n = 7)	0 (0%)	0 (0%)	7 (100%)	5 (71%)
Total (n = 39)	21	16	18	16

[a]Paired sera were not obtained from two patients in group I and from 1 patient in group II. Anti-VZV IgG antibodies were considered to be high if the ELISA value was 50×10^2 mIU/ml or more.

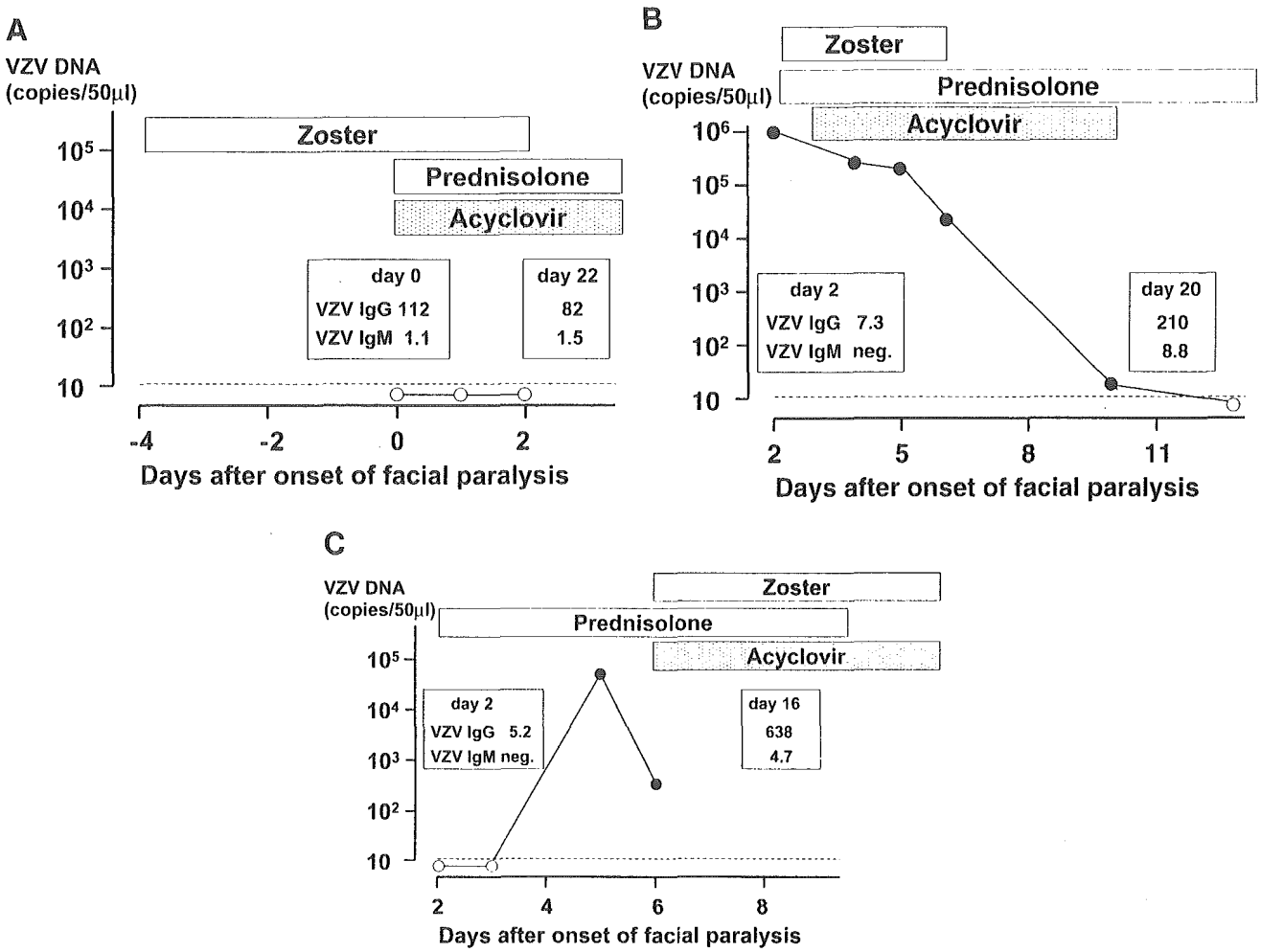

Fig. 2. Varicella-zoster virus (VZV) DNA load in patients with Ramsay Hunt syndrome. ●: DNA copies are expressed as the copy number/50 μl saliva (logarithmic scales). ○: Dotted lines indicate the minimum detection level of VZV DNA; samples below the line are negative for VZV DNA. Antibody ELISA values were expressed as $\times 10^2$ mIU/ml. **A**: A patient in group I who exhibited facial paralysis 4 days after zoster appeared. VZV DNA was not detected on day 0–day 2. This patient showed a high anti-VZV IgG antibody value at the initial visit (day 0) and the level of anti-VZV IgM antibody was also elevated. Anti-VZV IgG antibody had slightly decreased on day 22. **B**: A patient in group II in whom zoster and facial palsy appeared simultaneously. VZV DNA gradually decreased and became undetectable on day 13. The convalescent serum (day 20) showed a significant increase in anti-VZV IgG antibody, and the patient had seroconversion of anti-VZV IgM antibody. **C**: A patient in group III in whom zoster developed 6 days after the onset of facial paralysis. VZV DNA peaked 1 day before the appearance of zoster. Anti-VZV IgG antibody was elevated on day 16 and the patient had seroconversion of the anti-VZV IgM antibody.

addition, in most cases serum levels of anti-VZV IgG and IgM antibodies had already increased at the onset of facial paralysis. These results support the view that in these patients facial paralysis occurred in the regression phase of VZV reactivation. Neurological complications of varicella seem to be caused by immune-mediated post-infectious mechanisms unrelated to direct tissue damage by the viral replication [Echevarría et al., 1997]. In some patients categorized as group I, facial paralysis might be caused by such post-infectious process.

In group III (paralysis preceding group), the amount of VZV DNA tended to increase after the initial visit and peaked at around the time of appearance of zoster. Serum levels of anti-VZV IgG and IgM antibodies were low at the onset of facial paralysis but increased in the paired sera. These observations suggest that facial paralysis occurred in the early phase of VZV reactivation probably due to the direct effect of the viral replication. The subsequent viral replication at the skin or the oropharyngeal epithelium would be responsible for the systemic antibody response, which would begin simultaneously with the appearance of zoster. Because group II (simultaneous group) patients are intermediate between group I and III, it is reasonable that copy number of VZV DNA and antibody response to VZV resembled the pattern in group I in some cases and in group III in others. These findings clearly indicate that facial paralysis occurs at various points in time, from the early to the regression phase of VZV reactivation.

The relationship between the course of VZV reactivation and the onset of facial paralysis revealed by these findings is illustrated in Figure 3. In patients who exhibit facial paralysis several days after the appearance of zoster (group I), viral replication may already have decreased at the onset of paralysis. Therefore, VZV DNA is less frequently detected in such patients. In

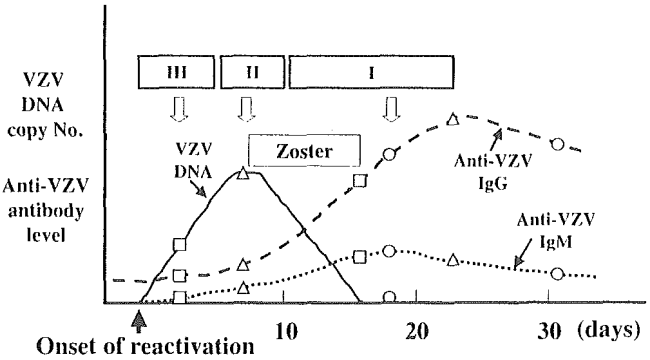

Fig. 3. A schema of the relationship between VZV load, antibody response, appearance of zoster, and the onset of facial paralysis in Ramsay Hunt syndrome. Open arrows indicate examples of the timing of the onset of facial paralysis in each group. ○: An example of VZV load and antibody response in group I (zoster preceding). In this case, VZV DNA was not detectable and the level of anti-VZV IgG and IgM antibodies had already increased at the onset of facial paralysis. No significant increase in antibody was observed on testing paired sera. △: An example in group II (simultaneous). VZV copy number gradually decreased after the onset of facial paralysis and increases in anti-VZV IgG and IgM antibodies were detected. □: An example of group III (paralysis preceding). VZV copy number increased after the onset of paralysis and increases in antibody were detected.

contrast, in patients with Ramsay Hunt syndrome who exhibit paralysis followed by zoster (group III), VZV copy number in the saliva increases after the onset of facial paralysis and peaks near the appearance of zoster. Therefore, we should be cautious in interpreting PCR data in Ramsay Hunt syndrome because the VZV DNA-positive rate differs depending on the reactivation pattern, the day of sample collection or DNA copy number. In addition, because patients tend to visit hospitals at the onset of facial paralysis, and this occurs at different times during VZV reactivation, some patients show no significant increase in anti-VZV IgG antibody value and sometimes even a decrease in this value; their behavior thus differs from the general pattern of viral infection.

Antiviral therapy has resulted in suppression of VZV load in cases of varicella and acute retinal necrosis [Kimura et al., 2000; Asano et al., 2004]. In addition, acyclovir has been reported to reduce the humoral immune responses in zoster [Mitchell et al., 1986]. In the present study, however, it seems to be difficult to evaluate the influence of the treatment on either the immune response or salivary VZV load because most patients received steroids and antiviral agents.

VZV reactivation occurs mainly in the geniculate or, occasionally, in the trigeminal or cervical ganglia, and the subsequent neuritis of the facial nerve in the bony fallopian canal may be the cause of Ramsay Hunt syndrome. There are several indications that inflammation and edema can cause mechanical compression of the facial nerve within the fallopian canal and that this leads to facial paralysis. First, evidence of inflammation within the facial nerves has been found in the temporal bones obtained during post mortems of patients with Ramsay Hunt syndrome [Schknecht, 1993]. Second, increased vascular permeability of the facial nerve, caused by inflammation and edema, has been detected by magnetic resonance imaging of paralyzed facial nerves using gadolinium-enhancement [Korzec et al., 1991]. Third, inflammation and edema of paralyzed facial nerves in the fallopian canal have been demonstrated during decompression surgery in patients with Ramsay Hunt syndrome [Honda et al., 2002]. The unique anatomical features of the facial nerve may play a role in the development of facial paralysis [Adour et al., 1975], since this is the most frequently paralyzed of all the cranial nerves. The present findings suggest that neuritis can occur in the early phase of VZV reactivation. However, the time of onset of facial paralysis may differ in different patients. The following factors are thought to be involved in the variation. First, differences in the causes of neuritis, such as direct damage of the nerve by VZV or an immune-mediated post-infection process, may be related to the differences in degree and progression of inflammation and edema of the facial nerve. Second, the anatomical variation in the facial nerve and the fallopian canal may relate to the progression of mechanical compression and subsequent ischemia in the nerve. These factors may contribute to the variety of patterns in the development of facial paralysis in Ramsay Hunt syndrome.

In conclusion, the present study has shown that facial paralysis in Ramsay Hunt syndrome can occur at different times from the early phase to the regression phase of VZV reactivation. These results suggest that there are variable patterns of development of facial nerve dysfunction induced by VZV reactivation and the progression of neuritis.

ACKNOWLEDGMENTS

We thank Ms. Sanae Yamaguchi, Ms. Erina Katoh, and Ms. Saori Akaishi for excellent technical assistance.

REFERENCES

Adour KK, Bell DN, Hilsinger RL, Jr. 1975. Herpes simplex virus in idiopathic facial paralysis (Bell palsy). JAMA 233:527–530.

Asano S, Yoshikawa T, Kimura H, Enomoto Y, Ohashi M, Terasaki H, Nishiyama Y. 2004. Monitoring herpesvirus DNA in three cases of acute retinal necrosis by real-time PCR. J Clin Virol 29:206–209.

Echevarría JM, Casas I, Martínez-Martín P. 1997. Infections of the nervous system caused by varicella-zoster virus: A review. Intervirology 40:72–84.

Furuta Y, Takasu T, Sato KC, Fukuda S, Inuyama Y, Hondo R, Nagashima K. 1992. Detection of varicella-zoster virus DNA in human geniculate ganglia by polymerase chain reaction. J Infect Dis 166:1157–1159.

Furuta Y, Ohtani F, Sawa H, Fukuda S, Inuyama Y. 2001. Quantitation of varicella-zoster virus DNA in patients with Ramsay Hunt syndrome and zoster sine herpete. J Clin Microbiol 39:2856–2859.

Hiroshige K, Ikeda M, Hondo R. 2002. Detection of varicella zoster virus DNA in tear fluid and saliva of patients with Ramsay Hunt syndrome. Otol Neurotol 23:602–607.

Honda N, Yanagihara N, Hato N, Kisaki H, Murakami S, Gyo K. 2002. Swelling of the intratemporal facial nerve in Ramsay Hunt syndrome. Acta Otolaryngol 122:348–352.

House JW, Brackmann DE. 1985. Facial nerve grading system. Otolaryngol Head Neck Surg 93:146–147.

Hunt JR. 1907. On herpetic inflammations of the geniculate ganglion; a new syndrome and its complication. J Nerv Ment Dis 34:73–96.

Jacobs BC, Rothbarth PH, van der Meché FGA, Herbrink P, Schmitz PIM, de Klerk MA, van Doorn PA. 1998. The spectrum of antecedent

infections in Guillain-Barré syndrome: A case-control study. Neurology 51:1110–1115.

Kimura H, Kido S, Ozaki T, Tanaka N, Ito Y, Williams RK, Morishima T. 2000. Comparison of quantitations of viral load in varicella and zoster. J Clin Microbiol 38:2447–2449.

Korzec K, Sobol SM, Kubal W, Mester SJ, Winzelberg G, May M. 1991. Gadolinium-enhanced magnetic resonance imaging of the facial nerve in herpes zoster oticus and Bell's palsy: Clinical implications. Am J Otol 12:163–168.

Mitchell CD, Gehrz RC, Balfour HH, Jr. 1986. Varicella-zoster-specific immune responses in acute herpes zoster during a placebo-controlled trial of oral acyclovir therapy. Diagn Microbiol Infect Dis 5:113–126.

Murakami S, Mizobuchi M, Nakashiro Y, Doi T, Hato N, Yanagihara N. 1996. Bell palsy and herpes simplex virus: Identification of viral DNA in endoneurial fluid and muscle. Ann Intern Med 124:27–30.

Murakami S, Nakashiro Y, Mizobuchi M, Hato N, Honda N, Gyo K. 1998. Varicella-zoster virus distribution in Ramsay Hunt syndrome revealed by polymerase chain reaction. Acta Otolaryngol 118:145–149.

Schknecht HF, editor. 1993. Pathology of the ear, 2nd edn. Philadelphia: Lea & Febiger. pp 236–238.

JOURNAL OF CLINICAL MICROBIOLOGY, May 2005, p. 2474–2476 Vol. 43, No. 5
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.5.2474–2476.2005

Comparative Molecular Analysis of *Haemophilus influenzae* Isolates from Young Children with Acute Lower Respiratory Tract Infections and Meningitis in Hanoi, Vietnam

Hiroshi Watanabe,[1]* Chiharu Kaji,[1] Dang Duc Anh,[2] Phan Le Thanh Huong,[2] Nguyen Thi Hien Anh,[2] Vu Thi Huong,[2] Hoang Vu Mai Phuong,[2] Ngo Thi Thi,[3] Pham Thi Suu,[3] Nguyen Thi Thu Nguyet,[3] Olivia Sebastian Rusizoka,[1] Kiwao Watanabe,[1] Tsuyoshi Nagatake,[1] and Kazunori Oishi[1]

Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan,[1] and National Institute of Hygiene and Epidemiology[2] and National Institute of Pediatrics,[3] Hanoi, Vietnam

Received 10 December 2004/Accepted 11 December 2004

Thirty-seven *Haemophilus influenzae* strains from nasopharyngeal swabs (NP) and 44 *H. influenzae* strains from cerebrospinal fluid (CSF) were investigated. Of the 37 *H. influenzae* isolates from NP, the serotypes of 30 isolates were nontypeable, 4 were type b, 2 were type c, and 1 was type a, whereas all of the 44 isolates from CSF were type b. The MICs of 16 antibiotics for the *H. influenzae* isolates from NP and CSF were similar, and no β-lactamase-negative ampicillin-resistant strain was found. Molecular typing by pulsed-field gel electrophoresis (PFGE) showed that the 37 *H. influenzae* strains from NP had 22 PFGE patterns, with none predominating, and the 44 *H. influenzae* strains from CSF had 9 PFGE patterns, with patterns α (22 isolates) and β (12 isolates) predominating. Our results indicate that two predominant types of *H. influenzae* type b strains have the potential to spread among children with meningitis in Hanoi, Vietnam.

Nontypeable *Haemophilus influenzae* (NTHi) can cause a variety of infections, including otitis media, bronchitis, and pneumonia (7), whereas *H. influenzae* type b (Hib) is a common cause of meningitis in children (11). Hib infection rates have been dramatically reduced in countries that have implemented Hib conjugate vaccine programs as part of routine infant immunizations (10). It has also recently been reported that β-lactamase-negative ampicillin (AMP)-resistant (BLNAR) strains have increased in some countries (6, 12), although their global prevalence remains low (4, 5). The aim of our study was to investigate the characteristics of *H. influenzae* among children less than 5 years of age in Vietnam.

Thirty-seven *H. influenzae* strains were isolated from the nasopharyngeal swabs (NP) of 37 children aged 2 to 60 months (mean age, 11 months) who were diagnosed with acute lower respiratory tract infections between 2001 and 2002, and 44 *H. influenzae* strains were isolated from the cerebrospinal fluid (CSF) of 44 children aged 1 to 24 months (mean age, 9 months) who were diagnosed with meningitis between 2002 and 2003, in Hanoi, Vietnam. No patient with an acute lower respiratory tract infection overlapped a patient with meningitis. *H. influenzae* isolates were serotyped by slide agglutination with antisera purchased from Difco Laboratories (Detroit, Mich.), and β-lactamase production was detected by a disk impregnated with nitrocefin (Becton Dickinson, Sparks, Md.). PCR was carried out for *H. influenzae* isolates by using mixed primers (Wakunaga Pharmaceutical Co., Hiroshima, Japan), as described previously (3). MICs were determined by the agar dilution method according to the NCCLS guidelines (8). The susceptibilities of 81 *H. influenzae* isolates to the following 16 antibiotics were tested: penicillin G (Meiji Seika Kaisha, Tokyo, Japan), AMP (Meiji Seika Kaisha), amoxicillin-clavulanic acid (AMC) (GlaxoSmithKline K.K., Tokyo, Japan), cefatrizine (Taiyo Yakuhin Co., Nagoya, Japan), cefuroxime (Sankyo Co., Tokyo, Japan), ceftriaxone (Chugai Pharmaceutical Co., Tokyo, Japan), cefotaxime (Aventis Pharma, Tokyo, Japan), imipenem (Banyu Pharmaceutical Co., Tokyo, Japan), minocycline [Lederle (Japan), Tokyo, Japan], chloramphenicol (Sankyo Co.), clarithromycin (Taisho Pharmaceutical Co., Tokyo, Japan), erythromycin (Dainippon Pharmaceutical Co., Osaka, Japan), gentamicin (Schering-Plough K.K., Osaka, Japan), levofloxacin (Daiichi Pharmaceutical Co., Tokyo, Japan), norfloxacin (Kyorin Pharmaceutical Co., Tokyo, Japan), and sulfamethoxazole-trimethoprim (Shionogi & Co., Osaka, Japan). After digestion with SmaI (Takara Shuzo Co., Shiga, Japan), pulsed-field gel electrophoresis (PFGE) was performed on the 37 *H. influenzae* isolates from the NP and the 44 *H. influenzae* isolates from the CSF, as described previously (16), and the interpretation of PFGE patterns was based on the criteria described by Tenover et al. (13).

Of the 37 *H. influenzae* isolates from NP, the serotypes of 30 isolates were nontypeable, 4 were type b, 2 were type c, and 1 was type a, whereas the 44 isolates from CSF were all type b. Twenty-six strains (70.3%) from NP and 23 strains (52.3%) from CSF were β-lactamase producing, and the remaining strains were β-lactamase negative by the nitrocefin disk assay. PCR analysis to identify the resistance genes indicated that 25 strains from NP and 21 strains from CSF were β-lactamase-producing AMP-resistant isolates which had the TEM-1-type β-lactamase gene; 11 strains from NP and 22 strains from CSF were β-lactamase-negative AMP-susceptible isolates, all of which lacked all resistance genes; and 1 strain each from NP and CSF were β-lactamase-producing AMC-resistant isolates

* Corresponding author. Mailing address: Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan. Phone: 81 (95) 849-7842. Fax: 81 (95) 849-7843. E-mail: h-wata@net.nagasaki-u.ac.jp.

TABLE 1. Distribution of MICs against 16 antibiotics for *H. influenzae* strains isolated from nasopharyngeal swabs and cerebrospinal fluid from children in Vietnam

Antibiotic	MIC (μg/ml) for isolates from:					
	NP (n = 37)			CSF (n = 44)		
	Range	50%	90%	Range	50%	90%
Penicillin G	0.5–128	16	32	≤0.004–128	2	32
Ampicillin	0.25–64	8	32	0.125–32	1	8
Amoxicillin-clavulanic acid	0.25–2	0.5	0.5	0.25–1	0.25	0.25
Cefatrizine	2–32	4	8	2–16	4	16
Cefuroxime	0.5–4	1	4	0.016–4	1	2
Ceftriaxone	≤0.004–0.032	0.008	0.016	≤0.004–0.032	0.008	0.008
Cefotaxime	0.008–0.125	0.032	0.032	≤0.004–0.125	0.032	0.063
Imipenem	0.25–4	2	2	0.25–1	0.25	1
Minocycline	0.5–2	1	2	0.5–2	1	1
Chloramphenicol	0.5–16	4	8	0.5–16	8	16
Clarithromycin	0.25–16	8	16	4–16	8	8
Erythromycin	0.25–4	4	4	0.016–8	2	4
Gentamicin	1–2	1	2	0.016–2	0.5	2
Levofloxacin	0.016–0.063	0.032	0.032	≤0.004–0.032	0.032	0.032
Norfloxacin	0.063–0.125	0.125	0.125	0.063–0.125	0.063	0.125
Sulfamethoxazole-trimethoprim	1–≥128	≥128	≥128	0.032–≥128	128	≥128

which had the TEM-1-type β-lactamase gene and the *ftsI* gene with the same substitution as the low-BLNAR strains. Although all isolates from NP which had the TEM-1-type β-lactamase gene were β-lactamase producing by the nitrocefin disk assay, one isolate from CSF which had the TEM-1-type β-lactamase gene was β-lactamase negative and two isolates from CSF which did not have the TEM-1-type β-lactamase gene were β-lactamase producing by the nitrocefin disk assay. No BLNAR strain was found. Table 1 shows the MIC range, the MICs at which 50% of isolates were inhibited (MIC_{50}), and the MIC_{90} of 16 antibiotics for 37 *H. influenzae* isolates from NP and 44 *H. influenzae* isolates from CSF. Although the MICs of the *H. influenzae* isolates from NP against penicillin G and AMP appear to be higher than those from CSF, the antimicrobial susceptibilities of the *H. influenzae* isolates from NP and CSF were similar. Molecular typing by pulsed-field gel electrphoresis (PFGE) showed that the 37 *H. influenzae* strains from NP had 22 PFGE patterns (A to V), without any predominant pattern (Fig. 1). The PFGE patterns of *H. influenzae* types a, b, and c were different from those of NTHi. Four isolates of type b had two PFGE patterns (I and K), and two isolates of type c had two PFGE patterns (H and Q). Forty-four *H. influenzae* strains from CSF had nine PFGE patterns (α to ι), with patterns α (22 isolates) and β (12 isolates) predominating. The PFGE patterns of 4 *H. influenzae* type b strains from NP were quite different from those of the 44 *H. influenzae* type b strains from CSF (Fig. 2).

Infants and young children tend to acquire *H. influenzae* in the upper respiratory tract because of their low immunity (16), and subsequent colonization can become a risk factor for invasive diseases caused by *H. influenzae* (2, 11). Since it has recently been reported that BLNAR NTHi and Hib have increased in some countries (3, 6, 12), the primary objective of this study was to investigate such resistant strains among children in Vietnam. In fact, no BLNAR strains were found in either NP or CSF, although more than half the isolates were β-lactamase producing and had the TEM-1-type β-lactamase gene. Hib remains the major cause of meningitis after the introduction of Hib vaccine in many advanced nations, because that vaccine is not usually available in Vietnam (14). Therefore, a secondary objective of this study was to examine the transmission route of *H. influenzae*. It has recently been reported that children can acquire *H. influenzae* at day care centers (9, 16) or from their parents at home (15). Our PFGE studies showed that NTHi did not have dominant genetic patterns but that Hib had two dominant genetic patterns. The results provide evidence to show that at least two types of Hib strains are spreading horizontally among children with meningitis in Vietnam. The Hib conjugate vaccine appears to be effective, not only for the prevention of invasive diseases, but also for the reduction of nasopharyngeal carriage in young children (1, 10).

In conclusion, our results demonstrate that BLNAR strains are not prevalent and that two predominant types of Hib

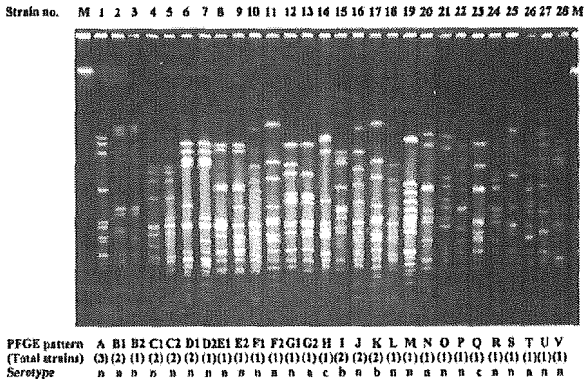

FIG. 1. PFGE patterns of SmaI-digested DNA from 37 *H. influenzae* isolates from NP of 37 children with acute lower respiratory tract infections. Molecular typing by PFGE demonstrated that 37 *H. influenzae* strains from the NP had 22 PFGE patterns (A to V), without any predominant pattern. The PFGE patterns of *H. influenzae* types a, b, and c were different from those of the nontypeable strains.

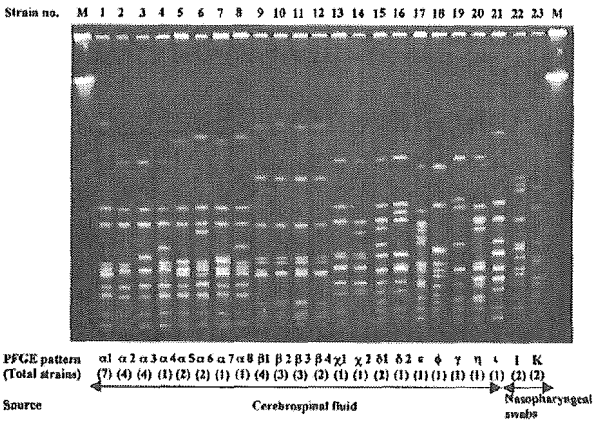

FIG. 2. PFGE patterns of SmaI-digested DNA from 48 Hib isolates from the CSF of 44 children with meningitis and the NP of 4 children with acute lower respiratory tract infections. Molecular typing by PFGE demonstrated that the 44 Hib strains from the CSF had nine PFGE patterns (α to ι), with patterns α (22 isolates) and β (12 isolates) predominating. PFGE patterns of 4 Hib strains from the NP were quite different from those of 44 Hib strains from CSF.

strains have the potential for spreading among children with meningitis in Hanoi, Vietnam. Therefore, the introduction of the Hib conjugate vaccine for young children should be considered in order to prevent invasive diseases caused by Hib.

We thank Akihiro Wada (Department of Bacteriology, Institute of Tropical Medicine, Nagasaki University), Chieko Shimauchi (Miyazaki Prefectural Nursing University), and Matsuhisa Inoue (Kitasato University School of Medicine) for help with completion of the PFGE studies. We also thank Yoko Takashima and Naoko Kitajima (Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University) for help with PCR studies.

This study was supported by the Core University Program, sponsored by the Japan Society for the Promotion of Science (JSPS).

REFERENCES

1. **Barbour, M. L., R. T. Mayon-White, C. Coles, D. W. Crook, and E. R. Moxon.** 1995. The impact of conjugate vaccine on carriage of *Haemophilus influenzae* type b. J. Infect. Dis. **171**:93–98.
2. **Faden, H., L. Duffy, R. Wasielewski, J. Wolf, D. Krystofik, and Y. Tung.** 1997. Relationship between nasopharyngeal colonization and the development of otitis media in children. J. Infect. Dis. **175**:1440–1445.
3. **Hasegawa, K., N. Chiba, R. Kobayashi, S. Y. Murayama, S. Iwata, K. Sunakawa, and K. Ubukata.** 2004. Rapidly increasing prevalence of β-lactamase-nonproducing, ampicillin-resistant *Haemophilus influenzae* type b in patients with meningitis. Antimicrob. Agents Chemother. **48**:1509–1514.
4. **Hoban, D., and D. Felmingham.** 2002. The PROTEKT surveillance study: antimicrobial susceptibility of *Haemophilus influenzae* and *Moraxella catarrhalis* from community-acquired respiratory tract infections. J. Antimicrob. Chemother. **50**:49–59.
5. **Karlowsky, J. A., I. A. Critchley, R. S. Blosser-Middleton, E. A. Karginova, M. E. Jones, C. Thornsberry, and D. F. Sahm.** 2002. Antimicrobial surveillance of *Haemophilus influenzae* in the United States during 2000–2001 leads to detection of clonal dissemination of a beta-lactamase-negative and ampicillin-resistant strain. J. Clin. Microbiol. **40**:1063–1066.
6. **Marco, F., J. Garcia-de-Lomas, C. Garcia-Rey, E. Bouza, L. Aguilar, C. Fernandez-Mazarrasa, and the Spanish Surveillance Group for Respiratory Pathogens.** 2001. Antimicrobial susceptibilities of 1,730 *Haemophilus influenzae* respiratory tract isolates in Spain in 1998–1999. Antimicrob. Agents Chemother. **45**:3226–3228.
7. **Murphy, T. F., and M. A. Apicella.** 1987. Nontypeable *Haemophilus influenzae*: a review of clinical aspects, surface antigens, and the human immune response to infection. Rev. Infect. Dis. **9**:1–15.
8. **National Committee for Clinical Laboratory Standards.** 1998. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7–A4. National Committee for Clinical Laboratory Standards, Wayne, Pa.
9. **Peerbooms, P. G., M. N. Engelen, D. A. Stokman, B. H. van Benthem, M. L. van Weert, S. M. Bruisten, A. van Belkum, and R. A. Coutinho.** 2002. Nasopharyngeal carriage of potential bacterial pathogens related to day care attendance, with special reference to the molecular epidemiology of *Haemophilus influenzae*. J. Clin. Microbiol. **40**:2832–2836.
10. **Peltola, H.** 2000. Worldwide *Haemophilus influenzae* type b disease at the beginning of the 21st century: global analysis of the disease burden 25 years after the use of the polysaccharide vaccine and a decade after the advent of conjugates. Clin. Microbiol. Rev. **13**:302–317.
11. **Saito, M., K. Okada, K. Takemori, and S. Yoshida.** 2000. Clonal spread of an invasive strain of *Haemophilus influenzae* type b among nursery contacts accompanied by a high carriage rate of non-disease-associated strains. J. Med. Microbiol. **49**:845–847.
12. **Suzuki, K., T. Nishimura, and S. Baba.** 2003. Current status of bacterial resistance in the otolaryngology field: results from the Second Nationwide Survey in Japan. J. Infect. Chemother. **9**:46–52.
13. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33**:2233–2239.
14. **Tran, T. T., Q. T. Le, T. N. Tran, N. T. Nguyen, F. K. Pedersen, and M. Schlumberger.** 1998. The etiology of bacterial pneumonia and meningitis in Vietnam. Pediatr. Infect. Dis. J. **17**(Suppl. 9):S192–S194.
15. **Watanabe, H., K. Hoshino, R. Sugita, N. Asoh, K. Watanabe, K. Oishi, and T. Nagatake.** 2004. Possible high rate of transmission of nontypeable *Haemophilus influenzae* including β-lactamase-negative ampicillin-resistant strains between children and their parents. J. Clin. Microbiol. **42**:362–365.
16. **Yano, H., M. Suetake, A. Kuga, K. Irinoda, R. Okamoto, T. Kobayashi, and M. Inoue.** 2000. Pulsed-field gel electrophoresis analysis of nasopharyngeal flora in children attending a day care center. J. Clin. Microbiol. **38**:625–629.

Am. J. Trop. Med. Hyg., 73(2), 2005, pp. 435–440

COMPARISON OF CLINICAL FEATURES AND HEMATOLOGIC ABNORMALITIES BETWEEN DENGUE FEVER AND DENGUE HEMORRHAGIC FEVER AMONG CHILDREN IN THE PHILIPPINES

CELIA C. CARLOS, KAZUNORI OISHI,* MARIA T. D. D. CINCO, CYNTHIA A. MAPUA, SHINGO INOUE, DEU JOHN M. CRUZ, MARY ANN M. PANCHO, CAROL Z. TANIG, RONALD R. MATIAS, KOUICHI MORITA, FILIPINAS F. NATIVIDAD, AKIRA IGARASHI, AND TSUYOSHI NAGATAKE

Department of Pediatrics, and Research and Biotechnology Division, St. Luke's Medical Center, Quezon City, The Philippines; Institute of Biology, University of the Philippines Diliman, Quezon City, The Philippines; Department of Internal Medicine and Virology, Institute of Tropical Medicine Nagasaki University, Nagasaki, Japan

Abstract. To demonstrate the differences of clinical features and hematologic abnormalities between dengue fever (DF) and dengue hemorrhagic fever (DHF), 359 pediatric patients admitted St. Luke's Medical Center in Quezon City, between 1999 and 2001 in Metro Manila, and adjoining provinces the Philippines, with a laboratory-confirmed dengue virus infection were evaluated. One third of the patients had DHF, and most of these patients were without shock. Restlessness, epistaxis, and abdominal pain were more associated with DHF. The platelet count was significantly lower in the DHF group than in the DF group before and after defervescence. In the DHF patients, the hematocrit was significantly increased before defervescence, and decreased the day after due to administration of intravenous fluid. Coagulation abnormalities associated with most DHF patients were thrombocytopenia and an increased fibrinolysis, but not disseminated intravascular coagulation. We present recent data on readily obtained clinical and laboratory data that can be used for early diagnosis and consequently earlier appropriate treatment of dengue virus infections.

INTRODUCTION

Dengue virus, a mosquito-borne human viral pathogen, has recently become a major public health concern particularly in tropical and subtropical countries, predominantly in urban and periurban areas. The geographic distribution of dengue viruses has greatly expanded and the number of cases has dramatically increased during the past three decades.[1] Two and a half billion people in more than one hundred countries are at risk of infection, with an estimated 50 million infections per year.[2] Since the first report of an outbreak of dengue hemorrhagic fever (DHF) in the Philippines in 1956,[3] dengue epidemics have occurred in the country at approximately five-year-intervals.[4,5] Previous reports have also characterized the view that dengue has been hyperendemic and a leading cause of childhood hospitalization during the 1980s in the Philippines.[6,7] Although dengue fever (DF) is a self-limited febrile illness, DHF is characterized by prominent hemorrhagic manifestations with thrombocytopenia, an increased vascular permeability, and is associated with a high mortality rate.[8]

An early clinical diagnosis of DHF is difficult because the World Health Organization (WHO) clinical and laboratory criteria for DHF may be manifested only in the late phase of acute illness.[9] Although previous reports have characterized the clinical features of DF and DHF,[9,10] differences in these features, including hematologic abnormalities between the two conditions, are poorly defined in hospitalized pediatric patients under appropriate treatment according to WHO guidelines. Therefore, this prospective study was undertaken to determine the differences in the clinical features and hematologic abnormalities between DF and DHF among hospitalized pediatric patients in Metro Manila, the Philippines from 1999 to 2001.

* Address correspondence to Dr. Kazunori Oishi, Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan. E-mail: oishi-k@net.nagasaki-u.ac.jp

PATIENTS AND METHODS

Patients and study design. All patients admitted at the St. Luke's Medical Center in Quezon City, the Philippines between January 1999 and December 2001 who satisfied the following criteria were enrolled in the study: 1) age between 2 and 17 years, 2) fever for ≤ 5 days, 3) temperature of at least 37.8°C, and 4) no apparent focus of infection. Informed consent was obtained from the patient's legal guardian.

Medical histories were obtained and physical examinations were conducted by one of the pediatrician investigators (CCC and MTDDC.) on recruitment and on a daily basis until discharge. The clinical symptoms and signs, including nutritional status, were recorded on case record forms. The day of defervescence was defined as day 0.[11] The days before and after defervescence were reported consecutively as follows: −2, −1, 0, +1, +2, etc.

Blood was drawn on the first, third, fourth, and seventh days of the hospital stay. Serial complete blood counts were obtained until the day of discharge. Diagnostic tests for dengue included reverse transcriptase–polymerase chain reaction (RT-PCR) for flaviviruses and determination of IgM antibody to dengue viruses by an enzyme-linked immunosorbent assay (ELISA).[12,13] Because the diagnostic sensitivity was 90–93% for the IgM ELISA and 80–100% for the RT-PCR, the combined diagnostic sensitivity of the RT-PCR and the IgM ELISA will be greater than 90%.[14–16]

All cases with dengue virus infections confirmed by any of the diagnostic tests were categorized as either DF or DHF according to the criteria of the WHO.[17] The diagnostic criteria included a platelet count nadir of less than 100,000/μL, hemorrhagic manifestations, and an increase in hematocrit greater than 20% above the average or the presence of pleural effusion or ascites. Cases of DHF were further graded as I–IV. A chest radiograph (posterioanterior view) was routinely done to detect pleural effusion on the third day of hospitalization. Treatment, including intravenous fluids (IVF) and fresh frozen plasma (FFP) was given to each patient based on the WHO guidelines,[17] and the total

TABLE 1
Disseminated intravascular coagulation (DIC) score*

Items	Test results	Score
Underlying disease	Yes	1
Clinical symptoms		
Hemorrhagic manifestations	Yes	1
Visceral symptoms†	Yes	1
Test results		
Serum FDP level (μg/mL)	≥ 40	3
	≥ 20 to < 40	2
	≥ 10 to < 20	1
Platelet count (× 10^4/μL)	≤ 5	3
	> 5 to ≤ 8	2
	> 8 to ≤ 12	1
Plasma fibrinogen level (mg/dL)	≤ 100	2
	> 100 to ≤ 150	1
Prothrombin time ratio	≥ 1.67	2
(divided by the normal value)	≥ 1.25 to < 1.67	1

* FDP = fibrin degradation product.
† Signs of circulatory insufficiency due to microthrombus caused by DIC.

volume of IVF or FFP administered to each patient was recorded.

Disseminated intravascular coagulation (DIC) score. To assess the presence of DIC in the patients, all patients enrolled between September 2000 and December 2001 were examined for DIC scores.[18] The DIC scoring system used is shown in Table 1. The DIC score included an evaluation of the following parameters: the underlying disease and clinical symptom (hemorrhagic manifestations or visceral signs), and an assessment of platelet count, fibrinogen, prothrombin time (PT) ratio (divided by the normal value), and fibrin degradation product (FDP). Dengue virus infection was the underlying disease referred to the DIC scoring system for purposes of this study. The concentration of fibrinogen was measured with Dadethrombin reagent (Dade Behring, Inc., Newark, DE), and PT was determined with Thromborel S reagent (Dade Behring, Inc.). Citrated blood was used for the determination of the PT ratio and fibrinogen levels. The concentration of FDP was determined by means of a commercially available kit (Eiken Chemical Co., Ltd., Tokyo, Japan). The study protocol was reviewed and approved by the Institutional Ethics Review Board of the St. Luke's Medical Center.

Statistical analysis. All data are expressed as the mean ± SD or as frequencies and proportions. Differences in laboratory data between patients with DF and DHF were analyzed using the Student's *t*-test for continuous variables. Differences in the demographic and clinical data and DIC scores between patients with DF and DHF were tested by the chi-square test or Fisher's exact test for nominal variables, whichever was appropriate. A *P* value less than 0.05 was considered significant. The statistical software SPSS version 12.0 (SPSS, Inc., Cary, NC) was used for data analysis.

RESULTS

Subject characteristics. Of the 503 subjects screened, 359 (71.4%) were confirmed as having a dengue virus infection: 322 (89.7%) by IgM-capture ELISA and 139 (38.7%) by RT-PCR. A total of 102 (28.4%) had positive results for both tests. Of the 359 laboratory-confirmed cases, 239 (66.6%) and 120 (33.4%) were diagnosed as DF and DHF, respectively (Table 2). Forty-two patients (23 with DF and 19 with DHF) were enrolled in 1999, 75 (37 with DF and 38 with DHF) in 2000, and 242 (179 with DF and 63 with DHF) in 2001. The proportion of DHF differed in each year (45.2% in 1999, 50.6% in 2000, and 26.0% in 2001). The distribution of dengue virus serotypes (DEN1, DEN2, DEN3, DEN4) determined by RT-PCR was (6, 1, 1, and 1) in 1999, (7, 5, 0, and 1) in 2000, and (24, 84, 4, and 0) in 2001, respectively. Double-positive reactions in the RT-PCR occurred in 10 cases for serotype DEN 1 + 2, one case each for serotype DEN 1 + 3 and DEN 1 + 4, and three cases for serotype DEN 2 + 3. An outbreak of dengue illness occurred between June and October 2001 (Figure 1). These cases were primarily associated with DEN 2 and DEN 1. The mean age of all subjects was 9.8 years. With respect to severity of disease, 120 patients diagnosed as having DHF were further classified as follows: DHF I (n = 7), DHF II (n = 110), DHF III (n = 2), and DHF IV (n = 1). Although a fatal case with DHF grade IV was observed, most DHF patients were without shock. Of these, 57 (47.5%) were associated with pleural effusion.

The duration of the hospital stay was significantly longer in those with DHF than in those with DF ($P < 0.001$; Table 2). A significant increase in the frequency of abdominal pain was

TABLE 2
Demographic and clinical profile of subjects*

Parameter	DF (n = 239)	DHF (n = 120)	Total (n = 359)	*P*
Mean age (years) (SD)	9.9 (4.2)	9.8 (3.8)	9.8 (4.0)	0.877
Male:female ratio	1.49	1.50	1.49	0.976
Days with fever before admission (SD)	3.4 (1.3)	3.5 (1.4)	3.5 (1.3)	0.670
Duration of hospital stay, days (SD)	4.4 (1.7)	5.6 (1.7)	4.8 (1.8)	< 0.001
Symptoms before admission, no./total no. (%)				
Abdominal pain	76/238 (31.9)	55/119 (46.2)	131/357 (36.7)	0.008
Epistaxis	46/233 (19.7)	23/119 (19.3)	69/352 (19.6)	0.926
Symptoms at time of admission, no./total no. (%)				
Restlessness	0/238 (0.0)	4/119 (3.4)	4/357 (1.1)	0.012†
Epistaxis	26/236 (11.0)	23/117 (19.7)	49/353 (13.9)	0.027
Abdominal pain	69/237 (29.1)	51/119 (42.9)	120/356 (33.7)	0.010
Petechiae	195/239 (81.6)	102/120 (85.0)	297/359 (82.7)	0.420
Gum bleeding	11/232 (4.7)	6/113 (5.3)	17/345 (4.9)	0.819
Hematemesis	1/222 (0.5)	2/108 (1.9)	3/330 (0.9)	0.251†
Breathlessness	0/238 (0.0)	2/119 (1.7)	2/357 (0.6)	0.110†

* DF = dengue fever; DHF = dengue hemorrhagic fever.
† Fisher's exact test.

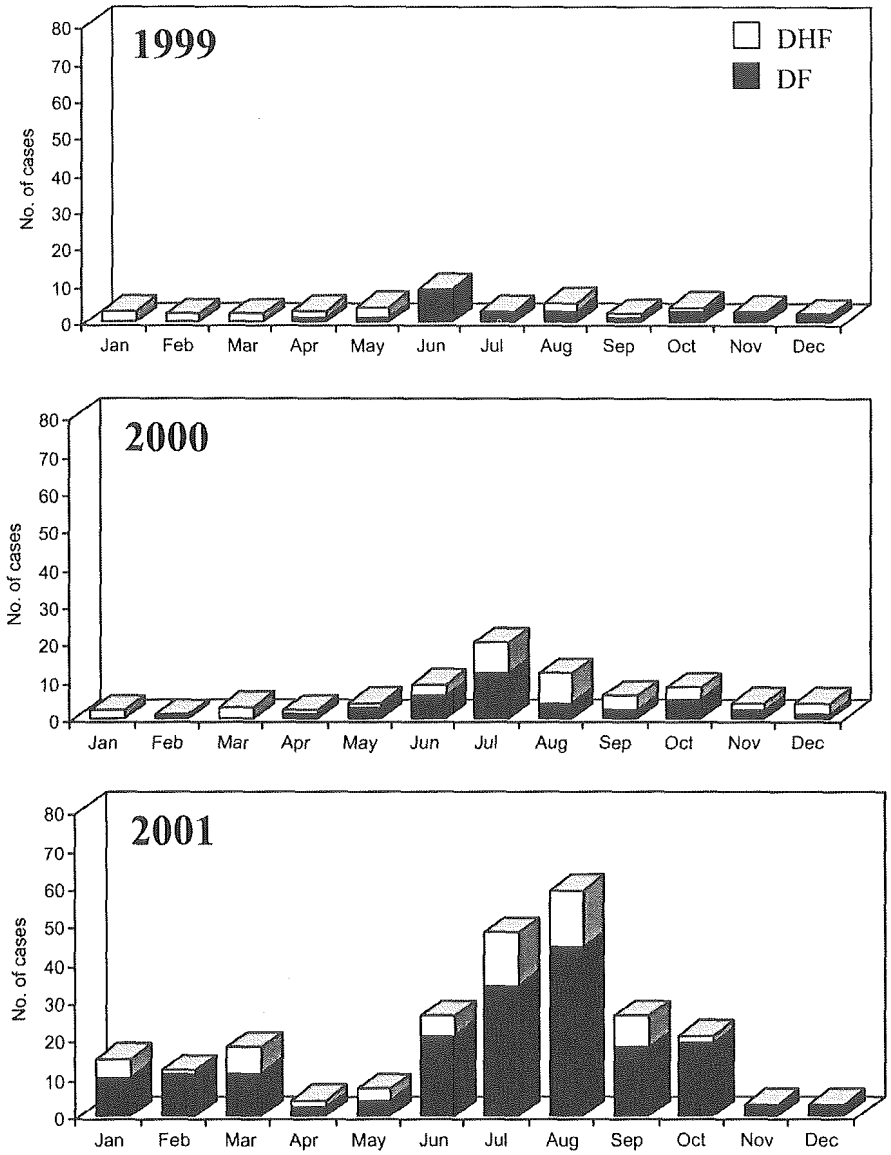

FIGURE 1. Distribution of dengue cases by month and year of enrollment. The number of laboratory confirmed dengue cases is plotted at monthly intervals from January 1999 to December 2001. DF = dengue; DHF = dengue hemorrhagic fever.

found in the DHF group before admission ($P = 0.008$) and at the time of admission ($P = 0.010$), compared with the DF group. The frequency of restlessness ($P = 0.012$) and epistaxis ($P = 0.027$) at the time of admission in the DHF group were also significantly higher than that in the DF group. No significant difference in nutritional status was found between the DF and DHF groups according to the Centers for Disease Control and Prevention classification.[19] The positive and negative predictive values of abdominal pain before admission for the development of DHF were 42.0% and 71.7%. These predictive values of symptoms upon admission were 42.5% and 71.2% for abdominal pain, 100% and 69.0% for restlessness, and 46.9% and 69.1% for epistaxis, respectively.

Laboratory data. Although the peripheral white blood cell (WBC) count of all subjects was generally below normal values prior to defervescence, the peripheral WBC count was significantly higher in the DHF group than in the DF group on days -1, 0, +1, +2, and +3 of defervescence (Figure 2A). The lymphocyte fraction in the peripheral WBC count was also significantly higher in the DHF group than in the DF group from days -1 to +2 (Figure 2B). No difference was found in the absolute monocyte, eosinophil, and basophil counts between the two groups. The laboratory data also confirmed that the platelet count was significantly lower in the DHF group than in the DF group from days -3 to +5 (Figure 2C). The lowest peripheral platelet count was noted at day +1 for both groups. The nadir of platelet count ($\times 10^3/\mu L$) was 113.8 ± 58.3 in the DF group and 58.5 ± 84.1 in the DHF group, respectively. The hematocrit was significantly higher in the DHF group from days -2 to +0 (Figure 2D). The maxi-

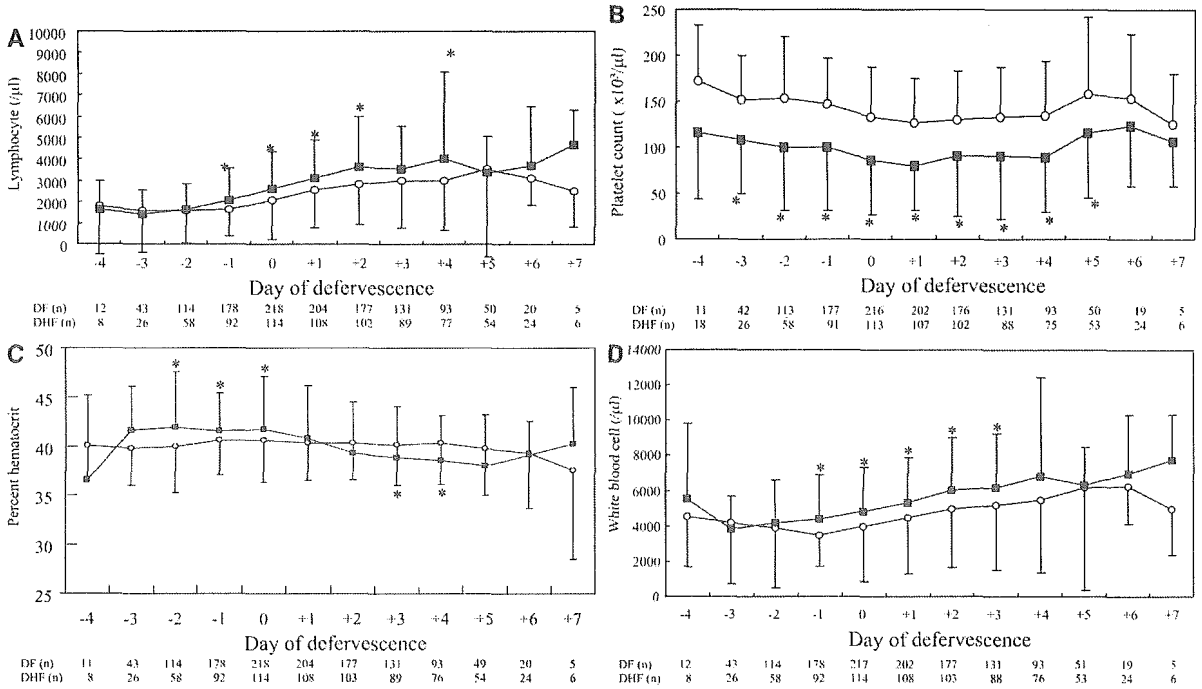

FIGURE 2. Comparison of the total white blood cell count (**A**), lymphocyte count (**B**), platelet count (**C**), and the hematocrit (**D**) in peripheral blood between pediatric patients with dengue fever (DF) and dengue hemorrhagic fever (DHF). The number of cases with DF and DHF are shown below each figure. Open circles show cases with DF and filled squares show cases with DHF. Data represent the mean ± SD $*P < 0.05$ versus patients with DF.

mum increase in the hematocrit was significantly higher in the DHF group (24.3 ± 13.8%) than that in the DF group (11.4 ± 7.7%) ($P < 0.001$). Interestingly, a significant decrease in the hematocrit was also found in the DHF group, compared with the DF group at day +4. This finding may be influenced by an intravenous fluid correction that was previously administered in addition to a shift of fluid to the intravascular space with recovery from the illness in patients with DHF. Therefore, we compared the total volume of IVF or FFP administered to each patient during admission between the DF and the DHF group. The total volume of IVF administered to each patient was significantly higher in the DHF group (n = 117, 3,265 ± 1,560 mL) than that in the DF group (n = 230, 2,687 ± 1,216 mL) during their hospital stay in this study ($P < 0.001$). No significant difference was found in the total volume of FFP used in between the DF group (n = 11, 307 ± 133 mL) and the DHF group (n = 38, 401 ± 471 mL).

DIC score. Among the parameters evaluated for the DIC scores, the frequency distribution of platelet and fibrinogen scores in the DHF group were significantly different than those in the DF group ($P < 0.05$), with higher scores observed for the DHF group (Table 3). Bleeding manifestations were frequently observed in both DF and the DHF patients, and no difference in clinical score was found between the two groups. A few cases of DF and DHF had an increased FDP and PT ratios with no significant differences in these scores between the two groups. Consequently, the frequency of cases with a DIC score ≥ 7 was significantly higher in the DHF group than in the DF group ($P < 0.001$). Of 17 cases with a DIC score of ≥ 7, 13 were DHF and 4 were DF. Of 13 DHF cases, 11 were DHF II and 1 case each of DHF I and IV, respectively. Only one death, a case of DHF grade IV, was associated with a marked increase in the PT ratio. Two cases of DHF were associated with a mild increase in the PT ratio. Only 7 of 17 cases were associated with a mild increase in FDP.

DISCUSSION

The findings herein serve to demonstrate the differences in clinical and laboratory features between DF and DHF during

TABLE 3

Comparison of DIC scores between those with DF and those with DHF*

		DF (n = 163)		DHF (n = 94)		Total (n = 257)		
Parameter	Score	No.	%	No.	%	No.	%	*P*
Platelet score	0	73	44.8	4	4.3	77	30.0	< 0.001
	1	39	23.9	15	16.0	54	21.0	
	2	30	18.4	18	19.1	48	18.7	
	3	21	12.9	57	60.6	78	30.4	
Clinical score	0	23	14.1	12	12.8	35	13.6	0.762
	1	140	85.9	82	87.2	222	86.4	
PT score	0	160	98.2	90	95.7	250	97.3	0.327
	1	3	1.8	3	3.2	6	2.3	
	2	0	0.0	1	1.1	1	0.4	
Fibrinogen score	0	111	68.1	53	56.4	164	63.8	0.027
	1	43	26.4	27	28.7	70	27.2	
	2	9	5.5	14	14.9	23	8.9	
FDP score	0	132	81.0	72	76.6	204	79.4	0.403
	1	31	19.0	22	23.4	53	20.6	
Total score	< 7	159	97.5	81	86.2	240	93.4	< 0.001
	≥ 7	4	2.5	13	13.8	17	6.6	

* DIC = disseminated intravascular coagulation; DF = dengue fever; DHF = dengue hemorrhagic fever; PT = prothrombin time; FDP = fibrin degradation product.

admission under appropriate treatment according to WHO guidelines. Abdominal pain and epistaxis were more commonly associated with DHF patients during the acute phase of the illness in this study, although previous studies reported conflicting data on the frequency of abdominal pain and bleeding manifestations.[5,6] Since abdominal pain and epistaxis were also found in DF, a diagnostic value of these symptoms for the severity of disease is limited. Although DHF required a longer hospital stay, DF also required a hospital stay longer than four days. This finding indicates that DF and DHF impose a considerable burden in the health care system in the Metro Manila, the Philippines. Although the etiology of abdominal pain in dengue illness remains obscure, Setiawan and others reported that most pediatric patients with DHF and epigastric pain also had increased serum levels of amylase or lipases and an enlarged pancreas.[20] Another study reported hemorrhagic gastritis as a most common finding of gastroendoscopy among patients with dengue fever in Taiwan.[21] Since we could not specify any definite reasons for abdominal pain in this study, further studies will be necessary. Although abdominal pain or epistaxis yielded a low positive predictive value for the development of DHF, restlessness was associated with a high positive predictive value. Therefore, this rare symptom could be used as a predictor of DHF.

Our laboratory data confirmed the increasing mean total WBC and lymphocyte counts that approached normal levels around the day of defervescence (Figure 2A and B). These findings are consistent with previous reports, although an examination for atypical lymphocytes was not done.[22–24] The maximum increase in the hematocrit in the DHF group was higher than 20%, and significantly higher than those in the DF group in this study, which supports the WHO definition of the disease.[17] Increased vascular permeability would allow plasma to flow out of the intravascular compartments, leading to hypovolemic shock. The present study also demonstrated that the volume of IVF administered to prevent hypovolemic shock in the DHF group was significantly higher than in the DF group. The increased administration of IVF for preventing dengue shock syndrome subsequently could lead to a significant decrease in the hematocrit in the DHF group, compared with the DF group, after defervescence.

In this study, we attempted to apply the diagnostic criteria of DIC to 257 patients with dengue virus infections.[18] Although thrombocytopenia was more prominent in the DHF group than in the DF group, a few cases of DF and DHF had an increased PT ratio. In addition, only a mild increase in FDP was found in both the DF and DHF group. These data are not in agreement with previous reports,[25,26] and may be explained by the limited number of patients with dengue shock syndrome in this study. The high frequency of low fibrinogen levels in the DHF group is indicative of increased fibrinolysis, which is consistent with previous findings concerning DHF.[25,27,28] Krishnamurti and others also reported an increased activated partial thromboplastin time and decreased fibrinogen levels in patients with DF and DHF.[29] These investigators suggested that platelet activation, rather than consumptive coagulopathy, was likely to cause hemorrhage in dengue without shock.

Although an increased frequency of cases with a DIC score of ≥ 7 was found in the DHF group compared with the DF group, most of these cases were free of consumptive coagulopathy. Serious bleeding manifestations such as melena caused by DIC were found in only one fatal case of DHF grade IV. Collectively, our data suggest that coagulation abnormalities involve a combination of thrombocytopenia and increased fibrinolysis, but not classic DIC in most patients in this study.

In summary, our present data demonstrated a low incidence of dengue shock syndrome among pediatric patients undergoing appropriate treatment in Metro Manila, the Philippines. Our data also show the differences in the frequency of clinical symptoms, such as restlessness, epistaxis, and abdominal pain, between patients with DF and DHF. Administration of increased volumes of IVF during the period of increased vascular permeability, a typical pathophysiologic feature of DHF 2–3 days after defervescence, can prevent dengue shock syndrome. Significantly low platelet counts and increased fibrinolysis in the peripheral blood were found in the DHF group, compared with the DF group. Coagulation abnormalities in most patients involve a combination of thrombocytopenia and increased fibrinolysis, but not classic DIC.

Received May 30, 2004. Accepted for publication April 13, 2005.

Acknowledgments: We are grateful to Dr. Felicitas S. Aguila, the former Chairman, and the medical staff of Department of Pediatrics, St. Luke's Medical Center for enrolling their patients in this study, and the technical staff of Research and Biotechnology Division, St. Luke's Medical Center for the processing of specimens. We also thank Shoko Honda and Mariko Saito for their assistance in the data analysis.

Financial support: This study was supported by a Grant-in-Aid for Scientific Research (B: 14406019) from the Ministry of Education, Science and Culture, Japan and from the St. Luke's Medical Center (Project no. 18-011).

Authors' addresses: Celia C. Carlos and Maria T.D.D. Cinco, Department of Pediatrics, St. Luke's Medical Center, 279 E. Rodriguez Sr. Boulevard, Cathedral Heights, Quezon City 1102, the Philippines, Telephone: 632-809-9763, Fax: 632-809-9763, E-mail: ccarlos@ritm.gov.ph. Kazunori Oishi, Kouichi Morita, Akira Igarashi, and Tsuyoshi Nagatake, Department of Internal Medicine and Virology, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan, Telephone: 81-95-849-7842, Fax: 81-95-849-7843, E-mail: oishi-k@net.nagasaki-u.ac.jp. Cynthia A. Mapua, Deu John M. Cruz, Mary Ann M. Pancho, Carol Z. Tanig, and Filipinas F. Natividad, Research and Biotechnology Division, St. Luke's Medical Center, 279 E. Rodriguez Sr. Boulevard, Cathedral Heights, Quezon City, 1102, the Philippines. Shingo Inoue, Research and Biotechnology Division, St. Luke's Medical Center, 279 E. Rodriguez Sr. Boulevard, Cathedral Heights, Quezon City, 1102, The Philippines and Department of Virology, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan. Ronald R. Matias, Research and Biotechnology Division, St. Luke's Medical Center, 279 E. Rodriguez Sr. Boulevard, Cathedral Heights, Quezon City, 1102, the Philippines and Institute of Biology, University of the Philippines Diliman, Quezon City, the Philippines.

REFERENCES

1. Igarashi A, 1997. Impact of dengue virus infection and its control. *FEMS Immunol Med Microbiol 18:* 291–300.
2. Guzman MG, Kouri G, 2002. Dengue: an update. *Lancet Infect Dis 2:* 33–42.
3. Hammon WM, Rudnick A, Sather GE, 1960. Viruses associated with epidemic hemorrhagic fevers of the Philippines and Thailand. *Science 131:* 1102–1103.
4. Venzon EI, Rudnick A, Marchette NJ, Fabie AE, Dukellis E, 1972. The greater Manila dengue hemorrhagic fever epidemic of 1966. *J Philippine Med Assoc 48:* 297–313.

5. Dizon JJ, 1978. The occurrence of dengue haemorrhagic fever in the Philippines. *Asian J Infect Dis 2:* 15–17.
6. Hayes CG, Corazon RM, Gonzales A, Ranoa CP, 1988. Dengue infections in the Philippines: clinical and virological findings in 517 hospitalized patients. *Am J Trop Med Hyg. 39:* 110–116.
7. Songco RS, Hayes CG, Leus CD, Manaloto COR, 1987. Dengue fever/dengue haemorrhagic fever in Filipino children: clinical experience during the 1983–1984 epidemic. *Southeast Asian J Trop Med Public Health 18:* 284–290.
8. Nimmannitya S, 1987. Clinical spectrum and management of dengue haemorrhagic fever. *Southeast Asian J Trop Med Public Health 18:* 329–397.
9. Kalayanarooj S, Vaughn DW, Nimmannitya S, Gree S, Suntayakorn S, Kunentrasai N, Viramitrachai W, Ratanachu-eke S, Kiatpolpoj S, Innis BL, Rothman AL, Bisalak A, Ennis FA, 1997. Early clinical and laboratory indicator of acute dengue illness. *J Infect Dis 176:* 313–321.
10. Kabra SK, Jain Y, Pandey RM, Madhulika, Sinhai T, Tripathi P, Brooe S, Seth P, Seth V, 1999. Dengue haemorrhagic fever in children in the 1996 Delhi epidemic. *Trans R Soc Trop Med Hyg. 93:* 294–298.
11. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, Rothman AL, Ennis FA, Nisalak A, 1997. Dengue in the early febrile phase: viremia and antibody responses. *J Infect Dis 176:* 322–330.
12. Bundo K, Igarashi A, 1985. Antibody-captured ELISA for detection of immunoglobulin M antibodies in sera from Japanese encephalitis and dengue hemorrhagic fever patients. *J Virol Methods 11:* 15–22.
13. Morita K, Tanaka M, Igarashi A, 1991. Rapid identification of dengue virus serotypes by using polymerase chain reaction. *J Clin Microbiol 29:* 2107–2110.
14. Rigau-Perez JG, Clark GG, Gubler DJ, Reiter P, Sanders EJ, Vorndam AV, 1998. Dengue and dengue haemorrhagic fever. *Lancet. 352:* 971–977.
15. Baalmaseda A, Sandoval E, Perez L, Gutierrez CM, Harris E, 1999. Application of molecular typing techniques in the 1998 dengue epidemic in Nicaragua. *Am J Trop Med Hyg. 61:* 893–897.
16. Raengsakulrach B, Nisalak A, Maneekarn N, Yenchitsomanus P, Limsomwong C, Jairungsri A, Thirawuth V, Green S, Kalayanarooj S, Suntayakorn S, Sittisombut N, Malasit P, Vaughn DW, 2002. Comparisons of four reverse transcription-polymerase chain reaction procedures for the detection of dengue virus in clinical specimens. *J Virol Methods. 105:* 219–231.
17. World Health Organization, 1997. *Dengue Haemorrhagic Fever: Diagnosis, Treatment, Prevention and Control.* Second edition Geneva: World Health Organization.
18. Nishiyama T, Matsukawa T, Hanaoka K, 2000. Is protease inhibitor a choice for the treatment of pre- or mild disseminated intravascular coagulation? *Crit Care Med 28:* 1419.
19. Ogden CL, Kaczmarski RJ, Flegal KM, Mei Z, Guo S, Wei R, Grummer-Strawn LM, Curtin LR, Roche AF, Jhonson CL, 2002. Centers for Disease Control and Prevention 2000 Growth Charts for the United States: improvements to the 1977 National Center for Health Statistics Version. *Pediatrics 109:* 45–60.
20. Setiawan MW, Samsi TK, Wulur H, Sugianto D, Pool TN, 1998. Epigastric pain and sonographic assessment of the pancreas in dengue hemorrhagic fever. *J Clin Ultrasound 26:* 257–259.
21. Wang J, Tseng C, Lee C, Cheng K, 1990. Clinical and upper gastroendoscopic features of patients with dengue virus infection. *J Gastroenterol Hepatol 5:* 664–668.
22. Boonpucknavig S, Lohachitranond C, Nimmanitya S, 1979. The pattern and nature of the lymphocyte population response in dengue hemorrhagic fever. *Am J Trop Med Hyg 28:* 885–889.
23. Suvatte V, Longsaman M, 1979. Diagnostic value of buffy coat preparation in dengue hemorrhagic fever. *Southeast Asian J Trop Med Public Health 10:* 7–12.
24. Thisyakorn U, Nimmannitya S, Ninganond V, Soogarun S, 1984. Atypical lymphocytes in dengue hemorrhagic fever: it's value in diagnosis. *Southeast Asian J Trop Med Public Health 15:* 32–36.
25. Mitrakul C, Poshyachinda M, Futralul P, Sangkawibha N, Abandrik S, 1977. Hemostatic and platelet kinetic studies in dengue hemorrhagic fever. *Am J Trop Med Hyg. 26:* 975–984.
26. Srichaikul T, Nimmannitya S, Artchararit N, Sriasawakul T, Sungpeuk P, 1977. Fibrinogen metabolism and disseminated intravascular coagulation in dengue hemorrhagic fever. *Am J Trop Med Hyg 26:* 525–532.
27. Huang YH, Liu CC, Wang ST, Lei HY, Liu HS, Lin YS, Wu HL, Yeh TM, 2001. Activation of coagulation and fibrinolysis during dengue virus infection. *J Med Virol 63:* 247–251.
28. Wills B, Oraqui EE, Stephens AC, Daramola OA, Dung NM, Loan HT, Chau NV, Chambers M, Stepniewska K, Farrar JJ, Levin M, 2002. Coagulation abnormalities in dengue hemorrhagic fever: serial investigations in 167 Vietnamese children with dengue shock syndrome. *Clin Infect Dis 35:* 277–285.
29. Krishnamurti C, Kalayanarooj S, Cutting MA, Peat RA, Rothwell SW, Reid TJ, Green S, Nisalak A, Endy TP, Vaughn DW, Nimmannitya S, Innis BL, 2001. Mechanisms of hemorrhage in dengue without circulatory collapse. *Am J Trop Med Hyg 65:* 840–847.

Conditions in the DPRK with regard to Safe Motherhood

Danielle Deboutte, MD, MPH
WHO Consultant

Overviews of aids in health sector

Since the beginning of humanitarian aid to the DPRK in 1995, assistance to reproductive health has been varied and sometimes haphazard. Several organisations, including at least one non-resident agency (**Caritas**), have donated medical goods and equipment (e.g. wheelchairs, delivery beds, instruments) for years and continue to do so.

Most of the resident NGOs with a mandate in health that were present in the early days, such as **MSF**, **Capanamur** and **Children's Aid Direct** have since left the country and no longer provide assistance. Except for rehabilitation work, which is easy to spot, the effectiveness of aid that was given in the past is hard to assess.

At the beginning of the crisis, access to the interior of the country was still more limited than now. Agencies were either encouraged, or chose, to concentrate the bulk of their activities in provinces close to the capital Pyongyang. **WFP** food aid is distributed to all the accessible counties (the administrative equivalent of districts). **UNICEF** support for EPI is provided nationwide and includes the areas that are closed for monitoring.

Assistance to the health sector comprises mostly equipment and supplies. Recently, several agencies have become engaged in systematic rehabilitation of a small number of provincial and county hospitals (**WHO** partially rehabilitated 15 county hospitals in 2004), mainly in the northeast of the country.

The range of essential drugs that are generally distributed is limited to oral painkillers, first-line antibiotics, selected vitamins and minerals and ORS. Some agencies now also distribute oxytocin and a limited quantity of IV fluids to target institutions. WHO estimates that 6 dollars /person/ year can cover essential drug needs. Since the

WHO estimates that 6 dollars /person/ year can cover essential drug needs. Since the total annual amount of aid to the health sector in the DPRK is less than 1dollar per person, the selection and quantity of essential drugs provided are necessarily restricted.

Training and Hospital Services

Training of staff at the county level has been minimal. International agencies in the DPRK are instructed to follow a top-down approach. Training starts with specialists from Pyongyang and, to a lesser degree, from provincial capital cities. These specialists are responsible for organizing national training programmes. It is assumed that they will incorporate new knowledge and practices in training programmes as they evolve. The extent to which this has happened and the time it takes for changes to reach the service level has not been investigated. To implement good practice, equipment should be in good working order and essential supplies available at all times. With the possible exception of the Maternity Hospital in Pyongyang, few if any health institutions respond to international standards of health care.

Since 2003, the British NGO **Save the Children Fund (UK)** has started a district based rehabilitation programme, which includes assistance to a local school of midwifery. The agency has no direct influence on the training curriculum, but provides teaching aids and textbooks. **The IFRC** (the International Federation of the Red Cross and Crescent), which has the longest track record of cooperation with the DPR Korea, is allowed to hold training courses for Red Cross Volunteers, doctors and midwives in the provinces they support. The content of these courses is related to the assistance provided, and mainly concerned with hygiene and prescription practices.

Medical Supplies

Lack of IV fluids is compounded by the local production of liquids of dubious quality. Hospitals use small glass distillers and keep the fluid in glass bottles (of the same type as large beer bottles). The mouth of the bottle is covered with thick waxed paper tied around the neck with a piece of string. Re-useable rubber connections and needles tend to be very old and unlikely to be sterile. Local doctors are well aware that using these devices carries an additional risk to the patient. The NGO **Première Urgence** received funding from ECHO in 2002 to build modern IV production units in 5 district hospitals

throughout the country. In 2003, funding was extended to cover 5 more units. There are more than 700 hospitals in the DPRK, spread over 203 districts (154 accessible to international observers). While it might be possible to produce enough fluids for several hospitals in one unit (if electricity supply is stable for at least part of the day), hospitals are not inclined to share supplies. The UN agencies, while initially opposing the project, have failed to propose an alternative solution so far, arguing that building a factory to cover the needs of the country would be too costly (although large and expensive rehabilitation projects are taking place in other sectors, such as water-and sanitation).

UNFPA opened its office in Pyongyang, manned by one expatriate UNV manager (not medically qualified) and local staff in 1998. The organization worked in only 3 (of 10) provinces until 2003. In 2004 they revised their programme of activities, which will be implemented in different geographical areas starting from 2005, with WHO as an implementing partner. The non-availability of an international health professional in UNFPA to assess and monitor the programme in the country has contributed to a heavy influence of, sometimes unreasonable, local demands on the type of inputs provided.

In 2003, an inter-agency working group on safe motherhood was set up, with monthly meetings open to all interested agencies on a voluntary basis. Members of the group, while meeting regularly, continued to develop and execute projects as planned, without apparently achieving a greater level of overall coherence. On the positive side, information exchanged during these meetings helped to avoid duplication in present assistance and future plans.

A minimum initial service package (MISP) has been described for assistance to refugees (and) in complex emergencies.[1] The assumption that clinics and hospitals, in districts receiving additional assistance (for example abdominal ultrasound equipment), are implementing the MISP, or at least the part of services with a direct bearing on maternal well-being needs to be confirmed. This would include the availability of consumables, especially soap, disinfectant and essential medical supplies.

Delivery

[1] Reproductive Health in refugee situations, an inter-agency field manual; 1999, UNHCR

Official reports state that up to 99% of deliveries are assisted by a skilled attendant. There is some disagreement over the proportion of women who deliver in a health institution. International observers are of the opinion that many women deliver at home, especially during winter, when health centres and hospitals are not well heated. Transport problems, together with a lack of standardized criteria for emergency obstetric care, may cause late referral and increased maternal (and child) mortality and morbidity. Doctors at county hospitals are able to perform caesarean sections, but ill equipped to deal with bleeding or infection.

While the rate of induced abortion is stated to be 23/1000 pregnancies, the shortage of contraceptive options and field observations suggest that the true figure might be higher. Health workers and government officials are reluctant to discuss this as an area of concern. Only UNFPA assisted provinces (3) were assured a regular supply of oral contraceptives, IUDs and condoms. Contraceptives may be available at county hospitals, but are rarely found at health centres. Overall, condom use is very low (less than one percent of married men). There are no official data regarding the incidence of sexually transmitted infections.

Conclusion

Assistance to safe motherhood and reproductive health would benefit from a more comprehensive approach. Improvements in hygiene, blood transfusion services (or alternatives to transfusion) and availability of essential drugs have a general effect on the quality of care. In addition, the referral system for obstetric emergencies needs to be strengthened. Rehabilitation of surgical facilities at county hospitals is an important (and costly) aspect. Training of primary health care workers (doctors and midwives) in the use of the partograph as a tool for timely referral would enhance the effectiveness of available facilities for emergency care.

<<This report was prepared following field survey by Dr. Danielle Deboutte during early 2005. >>

2004年8月9日
社会開発と国際保健論（夏学期）レポート
担当教授：喜多悦子

人権から考える国際保健

－北朝鮮から見た人権

イ・ウンジョン

第25条、「生活水準についての権利」すべての者は、衣食住、医療および必要な社会的施設等により、自己および家族の健康および福祉に十分な生活水準を保持する権利並びに失業、疾病、心身障碍、配偶者の死亡、老齢その他の不可抗力による生活不能の場合は、保障を受ける権利を有する。

－世界人権宣言（1948）

I

約115人に一人、あるいは、120人に一人という多数の人たちが、不安、危険、不安定のなかに、自分の住んでいる所から追われ、動いているという状況があるわけです。
国連難民条約によれば、難民は、自国に帰ったら「人種、宗教、国籍若しくは特定の社会集団の構成員であること又は政治的意見を理由」に、政府などから迫害を受ける人のことをいう。しかし、この狭義の難民以外にも、暴力、人権侵害、紛争、環境破壊、貧困などを理由に、住み慣れた土地を離れざるを得ない人たちが世界に何千万人もいる。
難民問題は９０年代に入って、もっと深刻に拡大している。冷戦中にいろいろな形で抑えられていた国内の多くの民族、宗教団体、政治的な利害関係というものの対立が、冷戦のくびきが取れて、非常に強い形で爆発したのだと考えていただいていいのではないか。
そして、21世紀の朝鮮半島でも多くの人々が基本的な権利さえもらえなくて、国の去る決心をしたり、どうしようもなくそのまま死んで行く。個人的には朝鮮半島の政治に関心がある。しかし、いつも政治問題で人権という壁を超えることは難しい。北朝鮮政権を崩すのは難しくないが、そこで住んでいる３千人余りのことを考えると迷わざるを得ないのだ。

II

北朝鮮の場合は他の難民問題とはちょっと違う。まず、脱北者はまだ正式に難民として認めてもらわなかった。だから、法的な難民庇護が与えられない。北朝鮮からの難民はほとんど食糧を求めて国境を越える。食べるものがないということが主な理由である。90年代に入って、ソ連などからの支援がなくなり、そして洪水や日照りなどは北朝鮮の食糧状況をもっと悪化させた。独裁政権から逃げ出すための政治的な理由もあったけど、ほとんど