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An Epidemic of Aseptic Meningitis due to Coxsackievirus B5 in Nara Prefecture, Japan: An Epidemiological Analysis by PCR-RFLP

Yoshiteru Kitahori*, Yumiko Inoue, Yoshiyuki Maruhashi[1], Osamu Adachi and Shunsuke Imai

Department of Virology, Nara Prefectural Institute for Hygiene and Environment, Nara 630-8131 and [1]Saiseikai-Chuwa Hospital, Nara 633-0054

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The epidemics of aseptic meningitis in the summer season are caused mainly by enteroviruses. A surveillance study in Japan showed that more than 70% of cases of viral meningitis were caused by serotypes 30, 9, 7, and 6 echoviruses and serotype 5 group B coxsackieviruses (CB5) (1). In 2001, we encountered an epidemic of aseptic meningitis, gastroenteritis and herpangina due to CB5 in the central areas of Nara Prefecture. The first case was reported from Kashihara City in June 25 and was a 7-year-old female patient. In the epidemic, a total of 46 CB5 isolates were obtained from 57 aseptic meningitis patients. The epidemic appeared to consist of a local outbreak at Sakurai City area and sporadic outbreaks involving the whole central area of Nara Prefecture. In order to determine whether or not CB5 viruses causing local and sporadic epidemics were genetically different, we conducted a PCR-RFLP and sequencing analysis of the virus isolates.

Cerebrospinal fluids were obtained from patients in Saiseikai-Chuwa Hospital, Saiseikai-Gose Hospital, Mimuro Hospital, and Kokuho-Central Hospital. Virus was isolated by using HEp-2, RD-18S, and MA cells; the virus developed CPE in HEp-2 cells in 2-3 days after inoculation. The isolated viruses were identified by neutralization assay with specific antibodies (Denka-Seiken Co., Tokyo). RT-PCR of the VP1-2C region (about 1500 bp) was carried out according to Caro et al.'s protocol (2). RFLP analysis was conducted on 50 μl of PCR products after *Dde*I (C↓TNA), *Hpa*II (C↓CGG) or *Hae*III (GG↓CC) restriction enzyme (Invitrogen, Carlsbad, Calif., USA) digestions. DNA sequences were determined by using a sequencing kit (Thermo Sequenase Cy5.5 Dye Terminator Cycle Sequencing Kit, Amersham Biosciences, Buckinghamshire, UK). As a standard strain, RNA from Faulkner strain was used.

The geographical distribution of the patients is shown in Figure 1. On one hand, the disease affected 27 patients in Sakurai City in a short, local outbreak (From July 17 to August 5), while 19 patients occurred in wide areas involving Kashihara City, Yamatotakada City, and Gose City over a long period (from June 25 to November 19), on the other. The age of the patients ranged from 3 months to 10 years and the highest incidence was found in the 5-year-old children's group. The male: female ratio was about 2: 1. All of the patients had a fever lasting for 5.9 days in average. Thirty-four of 46 patients (74%) had nausea or vomiting, and 27 (59%) had

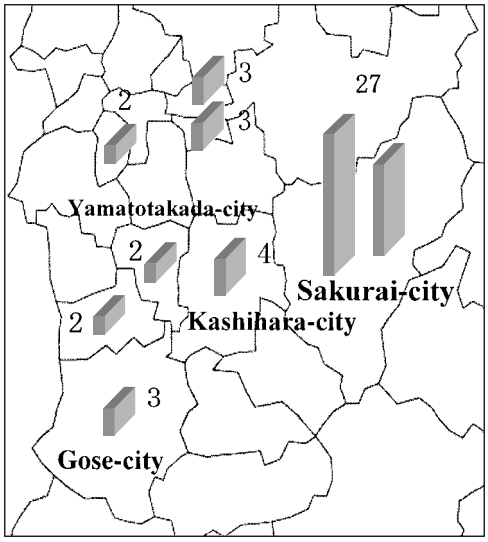

Fig. 1. Map of the distribution of meningitis patients in the central of Nara Prefecture.

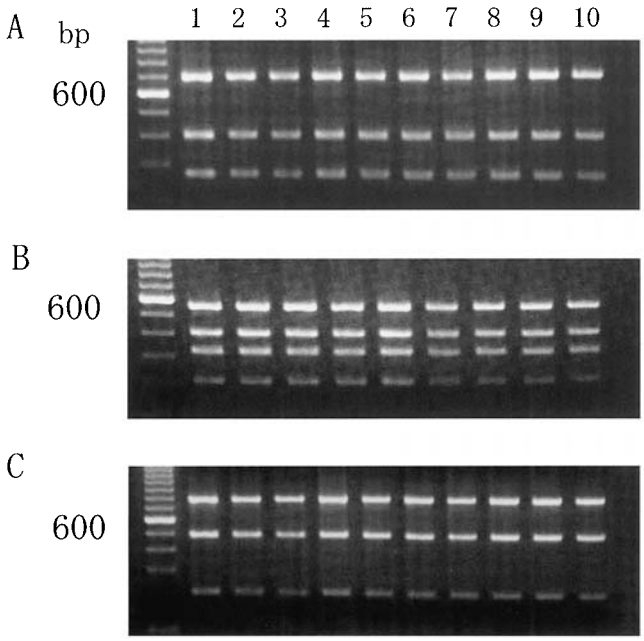

Fig. 2. Results of PCR-RFLP analysis. Specimen Nos. 1-5: separated CB5 from local epidemic. Nos. 6-10: separated CB5 from sporadic epidemic. (A) *Dde*I digesion. (B) *Hae*III digestion. (C) *Hpa*II digestion.

*Corresponding author: Mailing address: Department of Virology, Nara Prefectural Institute for Hygiene and Environment, Ohmori-cho 57-6, Nara 630-8131, Japan. Tel: +81-742-20-2887, Fax: +81-742-27-0634, E-mail: y.kitahori@ihe.pref.nara.jp

(A)

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Faulkner: ACG GAG GGC AGT GCC CCA CCT CGG ATG TCG ATA CCC TTC ATT AGC ATA GGG AAT
   #8100:   A   A   G       A       C   A       A       A                   T

GCA TAT AGC ATG TTT TAT GAT GGA TGG GCT AGA TTT GAC AAA CAA GGG ACA TAT GGC ATT
      C               C                                           A           T   A

AAC ACA CTT AAC AAC ATG GGG ACG CTA TAC ATG AGA CAC GTG AAC GAC GGA AGC CCT GGC
        T G   T                   G                       T   T   C       C

CCC ATT GTG AGC ACA GTT CGT ATC TAC TTT AAA CCG AAA CAC GTG AAG ACG TGG GTC CCA
                  T   G   C   T   T           C   G   T   T       A       T

CGA CCA CCC AGA TTG TGC CAG TAC CAG AAA GCT GGG AAC GTG AAC TTT GAG CCA TCT GGA
              G           A       A       A   T   T       T           T A

GTG ACT GAG GGC AGA ACA GAC ATA ACG ACC ATG CAA ACC ACT  VP1
      C C   A T           G       A   T       G       A

GGT GCC TTT GGA CAG CAA TCC GGT GCC GTG TAT GTC GGT AAT TAT AGA GTG GTG AAT AGA
  C   T       G       G   A         TGT       T   C       C               C

CAC TTG GCC ACC
  T   T   A
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(B)

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TEGSAPPRMSIPFISIGNAYSMFYDGWARFDKQGTYGINT
.....

LNNMGTLYMRHVNDGSPGPIVSTVRIYFKPKHVKTWVPRP
.....

PRLCQYQKAGNVNFEPSGVTEGRTDITTMQTT
.........Q......T...QS..G.......  VP1

GAFGQQSGAVYVGNYRVVNRHLAT
.........C.............
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Fig. 3A, B. Nucleotide (A) and amino acid sequences (B) of separated CB5.

headache. Neck stiffness was observed in 30% (14/46). Thirty patients (65%) had lymphocytosis exceeding 50 cells/μl at diagnosis, and the highest was 1,178 cells/μl. A predominance of neutrophils (123-578 cells/μl) was seen in six cases (13%). The reason for this predominance of neutrophils in the early stage of enterovirus meningitis is not well known (3). *Dde*I, *Hae*III, and *Hpa*II enzyme digestions produced the same PCR-RFLP profile for all the samples irrespective of the outbreak patterns (Fig. 2), suggesting that all cases were caused by a virus of the same lineage. Nucleotide (Fig. 3A) and amino acid (Fig. 3B) sequences of the region covering most of VP1 and a part of 2A (nucleotides 2960-3367) were the same among the isolates and slightly different from the Faulkner strain sequence (GenBank accession No. AF114383). The isolated CB5 (specimen No. 8100) had the same number of nucleotides as the Falkner strain in the PCR amplified region. There were 66 nucleotide differences; 58 of them were silent mutations. The deduced amino acid sequences were well conserved among the isolates and the Faulkner strain (96.3%, 131/136). In conclusion, PCR-RFLP analysis can provide very useful information especially to epidemiological investigations.

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