Central Nervous System Involvement in Patients with Scrub Typhus

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Scrub typhus, which is caused by Orientia tsutsugamushi, is a systemic illness that causes generalized vasculitis. The central nervous system (CNS) is the most crucial target in other rickettsial diseases; however, there have been several reports of encephalitis or meningitis without direct evidence of rickettsial invasion of the CNS in cases of scrub typhus. To investigate CNS involvement in cases of scrub typhus, we analyzed the CSF profiles (cell count and levels of protein and glucose) and amplified rickettsial DNA in CSF specimens by means of nested polymerase chain reaction (PCR) for 25 patients with the infection. Mild pleocytosis was present in 48% of the patients: CSF white blood cell counts ranged from 0 to 110/mm³ (mean ± SD count, 16.3 ± 27.0/mm³), and the mean (±SD) lymphocyte proportion was 51.9% ± 23.9%. The CSF protein level was increased (>50 mg/dl) in seven patients. Nester PCR amplified six products from the 25 CSF specimens: four of the products were Boryong genotypes, and two were Karp genotypes. The results of this study suggest that O. tsutsugamushi does invade the CSF and that scrub typhus should be considered one of the causes of mononuclear meningitis in areas of endemicity.

We conducted a prospective study to investigate CNS involvement in cases of scrub typhus. CSF samples were analyzed and assayed for O. tsutsugamushi DNA by nested PCR.

Material and Methods

Clinical data. Twenty-five cases of scrub typhus were diagnosed serologically [20] from 1 October 1994 to 31 December 1995 at our hospital, which is located in the west-central part of Korea. All patients were seen by an infectious diseases specialist on the day of admission and daily during hospitalization. Blood specimens for serological tests were drawn on the first day and the seventh day. CSF specimens were obtained on the day of admission, before doxycycline therapy was begun for all patients except patient 13 (table 1).

CSF samples from these patients were analyzed for the cell profile and levels of glucose and protein. The CSF samples were amplified by PCR with use of the sequence of genes encoding the 56-kD protein of O. tsutsugamushi to detect the presence of the organism’s DNA. We used one CSF sample from a patient with sagittal sinus thrombophlebitis as a negative control.

CSF sample preparation. For PCR amplification, each 500-µL sample of CSF was centrifuged at 2,000g for 5 minutes, then the pellets and supernatants were stored at −20°C. To isolate DNA, the pellets were mixed with lysis buffer containing 0.1% SDS (wt/v), 10 mM Tris-HCl (pH, 8.0), and 25 mM EDTA (pH, 8.0) and digested with proteinase K (50 µg/mL) at 56°C for 3 hours. The DNA in the lysate was purified by extraction two times with equal volumes of phenol and chloroform and precipitated with 2 volumes of 95% ethanol. The precipitate was resuspended in 50 µL of water [21–23].

PCR amplification of CSF. The nested PCR was performed as described by Furuya et al., with minor modifications [21].
The following oligonucleotide primers were synthesized. Primer R56f and R56r were used for the first assay: R56f 5'-ATTGCT TAGTG CAATG TCTGC-3' and R56r 5'-CTTCT TGGGC GTGAT GTTGA-3'. For the second assay, R56c and the genotype-specific reverse primers Bor, KP, KT, or GM were used, which correspond to the nucleotide sequence of genes that encode the 56-kD protein of each genotype (Boryong, Karp, Kato, and Gilliam): R56c 5'-CAGCC TACTA TGAGT CCTAT-3', Bor 5'-CACCG GATTG ACCAT CATAT-3', KP 5'-ACAAT ATCGG ATTTA TAACC-3', KT 5'-GGAAT ATTTA ATAGG ACTGG-3', and GM 5'-AGGGA TCCCT GCTGC TTGCT GCG-3'.

Nested PCR was performed with the nested PCR primer pairs. The PCR amplification mixture (total volume, 100 μL) contained 10 mM Tris-HCl (pH, 7.4), 1.5 mM MgCl2, 50 mM KCl, 0.1% octoxynol, 200 μM each deoxynucleoside triphosphate (dNTP), 0.5 μM primers R56f and R56r, 2.5 U of Taq polymerase (Amplitaq, Perkin-Elmer-Cetus, Norwalk, CT), and 10 μL of template DNA. The mixture was denatured at 95°C for 1 minute and annealed at 60°C for 1.5 minutes; then the chain was extended at 72°C for 2 minutes in a thermal cycler (Perkin-Elmer-Cetus). This cycle was repeated 30 times.

Amplified products were separated from any residual primers and dNTPs with a Centricon-100 microconcentrator (Amicon, Beverly, MA). For the second amplification, 10 μL of the purified first PCR product was amplified as described above, except that primer pairs of R56c and Bor, R56c and KP, R56c and KT, and R56c and GM were substituted. An aliquot of the amplified PCR product (10 μL) was electrophoresed in a 2% agarose gel containing 0.75 μL of ethidium bromide.

Results

Clinical findings. The durations of fever ranged from 2 days to 20 days (mean duration ± SD, 6.9 ± 3.5 days). All 25 patients had headaches, but only patient 21 had signs of altered mental status. This patient was drowsy but had no signs of nuchal rigidity. An MRI did not show any abnormalities. Twelve (48%) of 25 patients had mild pleocytosis (WBC count, >5/μm3) (table 1). The WBC counts for these patients ranged from 0/μm3 to 110/μm3 (mean count ± SD, 16.3 ± 27.0/μm3); the proportion of lymphocytes ranged from 11% to 83% (mean proportion ± SD, 51.9% ± 23.9%).

The level of protein in CSF was elevated (>50 mg/dL) in seven patients, but this elevation did not correspond to the increased number of WBCs in the CSF. As we expected, CSF glucose levels were not decreased (table 1). All of these patients recovered completely, without any sequelae.

PCR amplification of rickettsial DNA in CSF. We amplified rickettsial DNA in the CSF samples from all of these patients. A 214-bp DNA fragment was amplified by nested PCR with the primers R56c and Bor from the CSF samples of patients 3, 22, 23, and 25 (figure 1). A 220-bp DNA fragment was amplified by nested PCR with the primers R56c and KP from the CSF of patients 13 and 21 (figure 1). Gilliam genotype— and Kato genotype—specific amplification yielded negative results (data not shown). Six of 25 samples were found to contain DNA of O. tsutsugamushi. Four of the rickettsiae were found to be the Boryong genotype (the most common genotype in the west-central part of South Korea [24]), and two were the Karp genotype.

Discussion

Scrub typhus is a febrile disease that is endemic in Asian-Pacific areas, including the Korean Peninsula. It is a clinically important disease because of its high incidence in areas of endemicity and because it is associated with many serious complications [1, 3, 4, 6–11]. Of the clinical manifestations, myocarditis and encephalitis are the most life-threatening complications [1, 3, 6, 9, 11, 17–19]. There have been sporadic reports of CNS involvement [6, 9, 11] that described patients with altered mental status, stupor, nuchal rigidity, or coma. In some cases, these patients had mild-to-moderate pleocytosis (in general, 10–100 cells/mm3, which were predominantly mononuclear) [6].

Pathological studies of autopsy specimens have clearly shown CNS pathology in patients with fatal cases of scrub typhus; findings include mononuclear cell infiltration of the leptomeninges and the presence of typhus nodules (clusters of microglial cells) and hemorrhages in the brain substance [17–19]. These results suggest that O. tsutsugamushi infects the CNS. However, there have not been any previous reports in which O. tsutsugamushi was found in CSF or CNS tissues.

We have clearly demonstrated the presence of rickettsiae in CSF samples from patients with scrub typhus by using nested PCR. Since there were no fatal cases during this endemic outbreak, we were not able to obtain brain tissue specimens. In addition, our results did not allow differentiation between meningitis and encephalitis. Studies of CSF revealed mild-to-moderate pleocytosis (mainly mononuclear) in 48% of the patients, normal glucose levels, and a mild increase in the protein levels in 30% of the patients. These findings are similar to those of viral meningitis. Erythrocytes were seen in the CSF in several cases (table 1), which could be explained by the presence of generalized vasculitis, the pathological mechanism of this disease [1].

Involvement of the CNS that results in residual neurological sequelae has been well documented in cases of Rocky Mountain spotted fever (RMSF) [12, 13]. However, neurological sequelae in patients with RMSF manifest only as mild electroencephalographic changes in an otherwise clinically normal patient. On the other hand, many patients treated sufficiently early in the course of RMSF have no neurological sequelae [12]. Our patients recovered completely, without obvious neurological sequelae.

We believe the good recoveries of our patients, without sequelae, can be explained in several ways. First, our patients sought medical attention early (all patients except patient 23...
Table 1. CSF findings for 25 patients with scrub typhus.

| Patient no. | Duration of fever (d) | RBC count/mm³ | WBC count/mm³ | Lymphocytes (%) | Polymorphonuclear leukocytes (%) | Protein (mg/dL) | Glucose (mg/dL) | Nested PCR result^

1.  7  50  110  42  44  54  76
2.  7  100  0  ND  ND  Boryong
3.  8  50  40  56  30  43  56.5
4.  10  0  7  80  20  48  53.4
5.  6  5  4  ND  50  82
6.  10  0  50  35  40  44  ND
7.  9  0  4  ND  40  57
8.  7  0  4  ND  42  52
9.  8  0  15  ND  51  61.6
10.  5  10  0  ND  40  ND
11.  6  5  8  ND  25  56
12.  6  6  11  83  17  63  61
13.  2  15  8  75  25  71  47.3
14.  7  80  1  ND  76
15.  8  0  2  38  63.2
16.  5  0  4  110  65
17.  5  2  3  43  59.3
18.  5  0  3  49  80.2
19.  7  0  70  11  67  47  67
20.  6  3  3  10  58.8
21.  4  0  5  65  74.9
22.  10  120  7  40  60  47  63.2
23.  20  0  0  28  84 Boryong
24.  1  620  49  45  50  48  67.3
25.  6  10  0  34  85 Boryong

^NOTE. ND = not done.

*Orientia tsutsugamushi* genotype.

1 Differential counts were not performed for patients with WBC counts of <5/mm³.

came to the hospital after ≤10 days of febrile illness), and we started doxycycline therapy on the day of admission. Second, because we did not perform electroencephalography for any of these patients, we could have missed minor neurological abnormalities. Third, comparative study of the brain pathology associated with scrub typhus and other rickettsial diseases showed that scrub typhus more commonly causes meningitis but that the parenchymal lesions are milder, and there is no involvement of the white matter [17].

During the recovery stage of scrub typhus, healing of the nodules was observed, but the patients had no focal areas of demyelination, both of which findings have been considered characteristic of RMSF [18]. Thus, the residual neurological effects of scrub typhus that involves the CNS require further study.

Patients with scrub typhus usually have typical manifestations, but some patients have atypical findings such as the absence of rash and eschar formation. In the absence of typical manifestations, scrub typhus could be misdiagnosed as aseptic meningitis when fever, myalgia, and headache are present and the CSF findings indicate aseptic meningitis. In Southeast Asia—especially Thailand, where scrub typhus and malaria are prevalent [25, 26]—the differential diagnosis of altered mental status with fever should include cerebral scrub typhus as well as cerebral malaria. Therefore, we suggest that scrub fever be considered a cause of febrile illness and altered mental status in areas where *O. tsutsugamushi* is endemic.

Serological tests (indirect immunofluorescence assay or ELISA) are generally performed to diagnose scrub typhus [20]. However, PCR has been reported to be more sensitive and specific [21–23]. *O. tsutsugamushi* expresses many surface proteins; the 56-kD protein is known to be strain-specific and most abundant [20, 21, 27]. A PCR assay of human blood samples that is based on the sequence of genes encoding the 56-kD protein has proven to be useful for the early diagnosis of scrub typhus [21–23]. In addition, the nested PCR assay with use of genotype-specific sequences of genes encoding the 56-kD protein allows differentiation of the genotypes of *O. tsutsugamushi* [21].

We used Furuya’s protocol with minor modifications to amplify the rickettsial DNA in the CSF samples from our patients with scrub typhus [21]. The results showed that six of 25 specimens contained rickettsial DNA. Although three patients (patients 1, 6, and 19) had pleocytosis (WBC counts, >15/mm³), the DNA of *O. tsutsugamushi* was not detected.
with use of nested PCR in their CSF samples. On the other hand, two patients (patients 23 and 25) who had CSF WBC counts of <5/mm³ had DNA amplified in their samples.

The CSF specimens from patients 1–20 were obtained in 1994 and stored at −20°C until use, while the specimens from patients 21–25 had been freshly collected in 1995. Only two of the 20 specimens collected in 1994 were positive for DNA by nested PCR, whereas four of five specimens obtained in 1995 contained amplified products. The low yield of amplified product from the 1994 specimens might be due to inappropriate storage of the specimens. By using nested PCR, we also tried to identify which rickettsial genotypes invade the CNS more frequently. Only the Karp and Boryong genotypes have been detected in the west-central area of South Korea [24], and both of these genotypes were identified in the CSF from our patients. These results suggest that the Karp and Boryong genotypes (and possibly other genotypes) invade the CNS in patients with scrub typhus.

There are two possibilities with respect to CNS invasion by *O. tsutsugamushi*. One is that pleocytosis occurs transiently in the early period of the infection and is followed by spontaneous clearing, and the other is that only a few patients have CNS invasion by the organism and pleocytosis. We also speculate that since *O. tsutsugamushi* is an obligatory intracellular organism, it must either enter the CSF in a monocyte or grow through the endothelium (i.e., it enters via the luminal cell membrane, replicates in the capillary endothelial cytoplasm, and is released via the basal cell membrane into the perivascular space). Further studies are needed to confirm the exact pathophysiology of CNS infection in patients with scrub typhus.

We studied the correlation between CNS invasion and the severity of scrub typhus by comparing the clinical manifestations and laboratory findings for two groups of patients: those with CSF WBC counts of ≥5/mm³ and those with normal CSF WBC counts (i.e., <5/mm³). We evaluated both groups in terms of age; gender; duration of fever; the presence of rash, lymphadenopathy, or headache; and changes in mental status. We reviewed the results of the following laboratory studies: complete blood count; urinalysis; tests for liver function and levels of creatine phosphokinase, lactic dehydrogenase, myoglobin, fibrinogen degradation products, fibrinogen, blood urea nitrogen, and creatinine; and coagulation tests. There were no significant differences between the two groups with respect to clinical findings or the results of laboratory tests (data not shown).

Our study is the first prospective study to determine the mechanism of CNS involvement in patients with scrub typhus and to detect *O. tsutsugamushi* in the CSF of such patients. Our results suggest that *O. tsutsugamushi* does invade the CSF, and, therefore, scrub typhus should be considered a cause of mononuclear meningitis in areas where *O. tsutsugamushi* is endemic.

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References

