

Meningococcal Disease in the Republic of Korea Army: Incidence and Serogroups Determined by PCR

This study was performed to determine the incidence and serogroups of meningococcal disease in the Korean Army. From August 2000 to July 2001, we identified prospective cases in the Korean Army. Meningococcal disease was confirmed by isolation of *Neisseria meningitidis* or detection of its antigen by latex agglutination from cerebrospinal fluid (CSF) or blood. Polymerase chain reactions (PCRs) were performed in the *crgA* gene to identify *N. meningitidis* regardless of its serogroup, and then in *orf-2* (serogroup A) and *siaD* (serogroups B, C, Y, and W135) respectively for serogroup prediction. During the study period, twelve patients (four meningitis and eight septicaemia) were identified. The annual incidence was 2.2 per 100,000 (95% confidence interval, 1.3-3.8) among 550,000 private soldiers. Latex agglutinations were positive to A/C/Y/W135 polyvalent latex, but not to B latex in all patients. PCRs of *crgA* gene were positive in ten patients, whose samples (2 isolates from CSF, 2 CSFs, and 6 sera) were stored. In PCRs for serogroup prediction, one isolate was serogroup A, and one isolate and two sera were serogroup C. The need for meningococcal vaccination would be considered in the Korean Army through the cost-benefit analysis based on the result of this study.

Key Words : *Neisseria meningitidis*, Serogroup A; *Neisseria meningitidis*, Serogroup B; *Neisseria meningitidis*, Serogroup C; *Neisseria meningitidis*, Serogroup W-135; *Neisseria meningitidis*, Serogroup Y; Incidence; Military Personnel; Korea

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INTRODUCTION

Neisseria meningitidis is a leading cause of bacterial meningitis and septicemia among children and young adults (1). The annual incidence of meningococcal disease in the U.S.A. was 0.8-1.0 per 100,000 population during 1992-1996 (1). In European countries, the overall incidence of meningococcal disease was 1.1 per 100,000 population during 1993-1996 (2). Northern European countries tend to have a higher rate of incidence (2). The annual incidence in Norway, for example, was 2.4-4.6 per 100,000 population during 1992-1995 (3). The majority of cases are sporadic and due to serogroups B and C in the U.S.A. and Europe (4). In China and the African 'meningitis belt', the annual incidence due to serogroup A is more than 500 per 100,000 population during epidemic periods (5). In Korea, however, data of the incidence and serogroups of meningococcal disease are not yet available.

Military personnel are considered to have a higher risk for meningococcal disease than the civilian population. The increased risk is probably related to crowded living conditions among the people from various geographic areas, as well as to the specific age group (6). In the U.S. Army, the rate of hospitalizations resulting from meningococcal disease was

25.2 per 100,000 population during 1964-1970, and as a result vaccination campaigns were begun in 1971. During 1990-1998, the overall rate of hospitalizations from meningococcal disease among the enlisted, active-duty service members of the U.S. Army was 0.5 per 100,000 population (6).

In the Republic of Korea Army, the need for meningococcal vaccination was not considered because data of the incidence and serogroups were not available. Thus, this study was performed to determine the incidence and serogroups of meningococcal disease in the Korean Army.

MATERIALS AND METHODS

We identified the prospective cases of meningococcal meningitis or septicemia that occurred in Armed Forces Capital Hospital (AFCH) from August 2000 to July 2001. AFCH is the final referral medical center in the Korean Army. The computerized database of the Armed Forces Medical Command (AFMC) was examined retrospectively to identify additional cases from other Korean military hospitals during the same periods. In the Korean Army, individual cases of communicable disease including meningococcal disease are com-

pulsory notifiable to the Department of Preventive Medicine, AFMC. We defined an outbreak as 3 or more cases of meningococcal disease occurring within a 3-month period among the residents of a camp. The incidence was defined as cases per 100,000 population per year among 550,000 private soldiers.

The definition of meningococcal disease was based on clinically compatible symptoms or purpura fulminans with the identification of *N. meningitidis* or its antigen from cerebrospinal fluid (CSF) or blood. Identification of *N. meningitidis* was done using the Vitek system (bioMerieux-Vitek, U.S.A.). The detection of meningococcal antigen was done by latex agglutination test (Murex, Wellcome Diagnostics Limited, Dartford, U.K.) from CSF or serum. The test was performed according to the protocol of the manufacturer. This commercial kit consists of polyvalent latexes for serogroups A/C/Y/W135, serogroup B/*Escherichia coli* K1, or the positive control. The positive result is defined as a clear agglutination of single test latex accompanied by negative reactions with all other test latex reagents.

The meningococcal isolates, CSF, or serum from patients of AFCH were stored at -70°C . These samples were taken at the time of the hospital admission. Serogrouping by PCR was performed on available samples by using the methods described previously (7). To identify *N. meningitidis*, regardless of its serogroup, a PCR screen was performed in the *argA* gene. This gene is involved in the regulation of adhesion of *N. meningitidis* to target cells (7). For serogroup prediction, PCRs were performed with oligonucleotides in the *orf-2* gene (serogroup A) and in the *siaD* gene (serogroups B, C, Y, and W135), respectively. The sizes of the expected amplicons from these PCRs were 230 bp (*argA*), 400 bp (serogroup A), 450 bp (serogroup B), 250 bp (serogroup C), and 120 bp (serogroups Y and W135) (7).

RESULTS

During the study period of one year, a total of twelve patients with meningococcal disease have diagnosed in AFCH, including ten patients who transferred from other military hospitals in Korea. A review of the database of the AFMC showed that there were no patients with meningococcal disease in other hospitals, who were not transferred to AFCH. The annual incidence was 2.2 per 100,000 population (95% confidence interval, 1.3-3.8). Five of the twelve patients occurred in February 2001, but there was no outbreak during the study period (Table 1).

All twelve patients were male. The median age of patients was 21 yr old (ranged, 19-22 yr). The median interval between the enlistment and the first symptom was 80 days (ranged, 17-553 days). Of the twelve patients, four were diagnosed as meningitis, six as septicemia only, and two as septicemia with meningitis. Eight patients with septicemia were admitted to AFCH within 24 hr after the first symptom, and

Table 1. Outcomes of patients with meningococcal disease

Case	Date	Diagnosis	Organ dysfunctions	Outcomes*
1	Aug. 2000	septicemia	DIC	alive
2	Sep. 2000	septicemia	coma, shock, DIC	death (15 hr after purpura)
3	Oct. 2000	septicemia	DIC	skin necrosis
4	Jan. 2001	meningitis		alive
5	Feb. 2001	both	drowsy, shock	alive
6	Feb. 2001	both	drowsy, ARF	right deltoid myopathy
7	Feb. 2001	septicemia		alive
8	Feb. 2001	meningitis	drowsy	alive
9	Feb. 2001	meningitis	drowsy	frontal lobe dysfunction
10	Mar. 2001	septicemia	coma, shock, DIC, ARF	death (8 hr after purpura)
11	May 2001	septicemia	DIC	skin grafts on both lower limbs
12	Jun. 2001	meningitis	drowsy	alive

Both: septicemia with meningitis; DIC: disseminated intravascular coagulopathy; ARF: acute renal failure. *Alive, (alive with) complication, or death.

four patients with meningitis were admitted within 1-4 days.

Two patients with septicemia accompanied by multiorgan dysfunctions died at eighth and fifteenth hours, respectively, after the purpura fulminans (Table 1). These two patients had been treated for meningococcal meningitis during six and fifteen months previously, respectively, in AFCH. Complications developed in four patients. Skin necrosis with secondary infection occurred in two patients (Cases 3 and 11). In Case 11, reconstructive skin surgery was done on nearly the whole area of both lower extremities. In Case 6, right deltoid muscle weakness developed and resolved 3 months later. As sequelae of frontal lobe dysfunction, partial loss of memory and cognition power remained in Case 9.

Latex agglutinations were positive to serogroups A/C/Y/W135 polyvalent latex, but not to serogroup B/E. *coli* K1 latex in all patients. Two of the twelve patients were excluded due to sample loss (Cases 4 and 5). In ten patients whose samples were stored (2 isolates from CSF, 2 CSFs, and 6 sera), PCRs of the *argA* gene were positive at 230 bp (Fig. 1A). In PCRs for serogroup prediction, one isolate (Case 12) was serogroup A (*orf-2*, 400 bp), and one isolate (Case 9) and two sera (Cases 7 and 10) were serogroup C (*siaD*, 250 bp) (Fig. 1B). In other patients (2 CSFs and 4 sera), serogroups could not be determined by PCR. Consequently, we could not determine a serogroup by PCR in six patients, however, there were no cases of serogroup B.

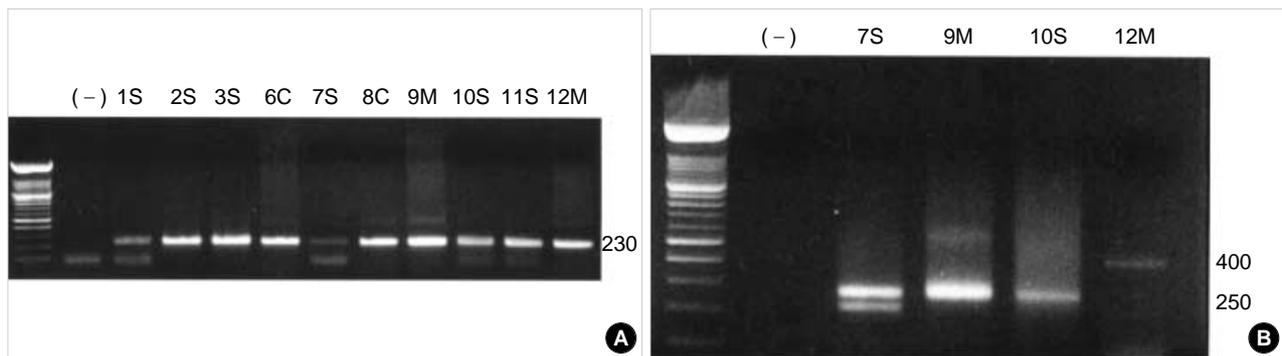


Fig. 1. PCR amplification of the *crgA* gene. The sizes of *crgA* amplicons are 230 bp (A). Serogroup specific PCR of the *orf-2* and *siaDs*. The size of *orf-2* (serogroup A) amplicon is 400 bp. The size of *siaD* amplicon for serogroup C is 250 bp (B). The lane name is case number+specimen: S= serum, C= cerebrospinal fluid, and M= microorganism. Lane (-) is the negative control. Size markers (100 bp ladder) are indicated in base pairs at the right.

DISCUSSION

In Korea, data for the incidence and serogroups of meningococcal disease were not available in military, as well as in civilian population. This study is the first report in Korea despite the data during introduced only a year ago. In the U.S. Army, rates of meningococcal disease have decreased since the early 1970s. However, this was not the only effect of vaccination campaigns. The rates in U.S. Army personnel declined before the 1971 vaccination campaigns, suggesting that the smaller recruit populations at training installations and the natural periodicity of outbreaks may have contributed to the decline in the disease (6). However, routine vaccination of high-risk populations may be a reasonable public health strategy because meningococcal disease may be fatal and fulminant. Routine vaccination for controlling meningococcal disease would be considered in the Korean Army.

A specific group that has several characteristics found common among the military personnel is college students, especially dormitory residents. Recently, the reports of meningococcal disease on college campuses have increased in the U.S.A. and the United Kingdom (6, 8). The U.S.A. surveillance data from 1998 to 1999 school year suggested that the overall rate of meningococcal disease among the college students with dormitory resident was higher than the rate among the same age-group people between persons aged 18 to 23 yr who were not enrolled in college: 2.2 vs. 1.5 per 100,000 (6). The annual incidence in the Korean Army was 2.2 per 100,000 population in this study. This is the same as the incidence of dormitory residents of U.S.A. colleges.

In this study, there was no serogroup B disease in latex agglutination test. A serogroup B meningococcal vaccine is not available. The immunogenicity and clinical efficacy of serogroup A and C vaccines have been well established in young adults (9, 10). The bivalent (A/C) or quadrivalent (A/C/Y/W135) meningococcal vaccine may be effective in the Korean Army. Recently in the U.S.A., there have been

conflicting opinions as to whether college freshmen should be targeted for routine vaccination (6, 11, 12). Vaccination of all freshmen or the specific freshmen who live in dormitories is unlikely to be cost-effective (6). In a military recruit camp, however, the cost of vaccination may be reduced because of the method of mass administration.

Serogroups of six patients could not be determined by PCR in this study. This was because the sensitivity of nonculture based PCR from CSF or serum was low, not because there were serogroups other than A, B, C, Y, or W135 in this study. Many of our samples were of 'category 2' of the original method referenced: culture was negative but antigen detection for *N. meningitidis* was positive (7). As a result of the reference, the sensitivity of CSF of category 2 was 92% and the sensitivity of serum was 71%. In this method, CSF and serum were heated at 100°C for 3 min and centrifuged, then PCR was done. If we performed the procedures of purification of nucleic acids from samples, the sensitivity might be increased (13).

The two patients who died in this study had two episodes of meningococcal disease within six or fifteen months, respectively. Because the first episodes of the two patients occurred before this study period, we could not confirm whether the second episodes were relapses or reinfections. Also, we could not document whether these patients had chronic meningococcemia with complement deficiencies, because they died with septic shock and multiorgan dysfunctions within a few hours after the admission. In a case report of 112 meningococcal infections at the U.S. Army training center, two patients had experienced two or three episodes within four or eight month periods (14). Although a specific antibody against *N. meningitidis* is generally protective, this immunity is not absolute (15, 16). Feldman believed that at least four infections are possible because the acquired immunity is group-specific (17).

In summary, a total of twelve patients of meningococcal disease occurred in the Korean Army from August 2000 to

July 2001. The annual incidence was 2.2 cases per 100,000 population (95% confidence interval, 1.3-3.8). We identified one serogroup A and three serogroup C diseases by PCR among ten patients whose samples were stored. However, there was no serogroup B disease found by latex agglutination tests. The need for meningococcal vaccination would be considered in the Korean Army through the cost-benefit analysis based on this study's results.

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