剤は存在せず，どのような集団にスクリーニングを行うべきかという議論も，一部の対象者には有意義であるとの報告もあるものの，今後の検討が必要である。

約3割程度で再発するとされており，可能であれば経過観察が必要である。実際には，どの性感染症にも当てはまるが，再診率は低い。定期的に受診することも少ない。したがって，患者には再発の可能性を十分に伝える必要がある。また，性的パートナーも同時に罹患している可能性があり，パートナーの検査も奨めるべきである[2)]。

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ORIGINAL ARTICLE

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Incidence of sexually transmitted diseases in Hokkaido, Japan, 1998 to 2001

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Abstract The objective of this study was to provide precise data on the incidence of sexually transmitted diseases (STDs) in Hokkaido. The goal of this prospective surveillance, study was to clarify the STD incidence between 1998 and 2001 in Hokkaido, Japan. The incidence of gonococcal infection in men was found to be 127–199 per 100000 people per year, which was three or four times higher than that for women. Female genital chlamydial infection had an incidence of 300–400 with a female to male ratio of two or three to one. Younger adults had higher incidences of gonococcal and chlamydial infections than older people. In conclusion, the current study of STDs revealed high incidences of gonococcal and chlamydial infections in the Hokkaido area, and there was no decreasing trend in STD incidence during these 4 years.

Key words Sexually transmitted diseases · Surveillance · Hokkaido

Introduction

Chlamydia trachomatis and *Neisseria gonorrhoeae* are commonly prevalent in Japan. While there have been a few reports in Japan of *C. trachomatis* resistant to antimicrobial agents, many studies have indicated an increase of *N. gonorrhoeae* resistant to the conventional agents, especially to quinolone.[1] Thus, information on the incidence of sexually transmitted diseases (STDs) must be delivered to the public to establish effective countermeasures against the diseases. Unfortunately, until now, there have been no sources of data in Japan to determine the current incidences of STDs.

In this context, the Selected Prefectures Survey for STDs started in 1998 in eight prefectures of Japan with the support of Health and Labor Sciences Research Grants (Research on Emerging and Re-emerging Infectious Diseases) from the Ministry of Health, Labor, and Welfare of Japan.[2–5] The results of the studies in all selected prefectures will be reported separately. We are actively engaged in the study and responsible for data collection in Hokkaido which is the northern main island of Japan. We determined in this study the incidence of STDs in Hokkaido from 1998 through 2001.

Patients and methods

Subjects and data collection

Hokkaido has a population of 5700000, and approximately 1800000 people live in Sapporo, the capital. The study consisted of collecting the age and sex of all newly diagnosed symptomatic patients with STDs, including syphilis; chancroid; genital herpes infection; condyloma acuminatum; and gonococcal, chlamydial, and nongonococcal and nonchlamydial infections of the urethra or uterine cervix, in June and November in 1998, 1999, 2000, and 2001. The data were requested from all clinics and hospitals that were engaged in the treatment of patients with STDs. By mail, we asked all these clinics and hospitals in Hokkaido to participate in the study and report these data.

Diagnosis of STDs

The early stage of symptomatic syphilis was diagnosed by skin manifestation and standard serum tests. Chancroid, genital herpes infection, and condyloma acuminatum were

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basically diagnosed through inspection by physicians to identify typical clinical lesions. Symptomatic patients with urethritis or cervicitis were diagnosed as having gonococcal infection when *N. gonorrhoeae* was detected in urethral discharge or the first voided urine in male patients and cervical smears in female patients. The detection methods for this organism depended on the clinic and included Gram staining, culture, polymerase chain reaction (PCR), and ligase chain reaction (LCR). Symptomatic patients with *C. trachomatis* infection were diagnosed as having the infection by enzyme-linked immunoassay, PCR, or LCR methods in specimens similar to those used in gonococcal detection. When neither *C. trachomatis* or *N. gonorrhoeae* was detected in symptomatic patients, they were diagnosed as having nonchlamydial and nongonococcal (NC-NG) infection of the urethra or cervix. If examination to detect *C. trachomatis* was not done and patients showed no typical findings of gonococcal infection, they were diagnosed as having nongonococcal infection with chlamydia not determined (NG-CND) of the urethra or cervix.

Estimation of incidence of STDs

The incidence of STDs was determined as the number of patients per 100000 people per year, based on the results of the two months (June and November) and the total population of Hokkaido in the corresponding year. The final incidence was adjusted by the response rates of institutes in a given year.

Results

During the 4 years of the study, the number of institutes asked to report information about patients with STDs varied from 578 to 711 as a result of the opening of new opened hospitals and the closure of old ones (Table 1). However, response rates were consistently high at around 80%, suggesting that most of the clinics and hospitals actively participated in the study.

When all STDs were taken into consideration, the mean incidences from 1998 through 2001 were 590 male patients and 816 female patients per 100000 people per year (Table 2). Although classic STDs such as syphilis and chancroid showed very low incidences, the rates of gonococcal and chlamydial infections in male patients and chlamydial infection in female patients were high in Hokkaido. In particular, the incidence of chlamydial infection in female patients was

Table 1. Number of institutes asked to participate and response rates in June and November each year

	No. of institutes	Response rate (%)
1998		
June	584	82.2
November	578	80.3
1999		
June	711	78.9
November	697	84.1
2000		
June	683	78.6
November	679	79.2
2001		
June	656	87.8
November	644	87.1
Mean		82.3

Table 2. Incidence (per 100000 people per year) of sexually transmitted diseases (STDs) from 1998 through 2001 in Hokkaido prefecture

STD	1998		1999		2000		2001	
	Male	Female	Male	Female	Male	Female	Male	Female
All STDs	444 (436.0–453.7)	688 (677.5–698.7)	630 (619.6–640.4)	910 (897.7–921.7)	621 (610.2–631.2)	853 (840.9–864.6)	663 (652.2–672.9)	813 (801.7–823.6)
Syphilis	1.4 (0.9–1.9)	1.3 (0.8–1.8)	1.9 (1.3–2.5)	1.5 (1.0–2.0)	2.5 (1.9–3.2)	5 (0.2–0.8)	1.0 (0.6–1.4)	0.7 (0.4–1.0)
Chancroid	0	0	0	0	0	0	0	0.2 (0.1–0.4)
Genital herpes infection	31 (29.1–33.8)	67 (63.4–70.0)	33 (30.6–35.4)	64 (61.2–67.6)	35 (32.4–37.4)	71 (68.0–74.9)	33 (30.2–34.8)	65 (61.6–67.8)
Condyloma acuminatum	20 (17.9–21.6)	26 (24.0–28.1)	26 (23.5–27.7)	33 (31.0–35.6)	28 (25.9–30.4)	35 (32.8–37.6)	23 (21.0–24.8)	29 (26.7–30.8)
Gonococcal infection	127 (121.9–131.3)	30 (27.7–32.1)	190 (184.4–195.8)	51 (48.7–54.4)	162 (156.8–167.6)	53 (50.5–56.4)	199 (193.4–204.9)	62 (59.1–65.2)
Chlamydial infection	100 (96.2–104.6)	273 (266.1–279.5)	146 (141.4–151.5)	378 (370.3–385.8)	156 (150.7–161.3)	341 (333.6–348.6)	178 (172.8–183.5)	353 (345.5–360.0)
NC-NG infection	139 (134.4–144.3)	240 (233.9–246.3)	197 (191.3–203.0)	316 (308.6–322.8)	210 (203.7–215.9)	313 (305.8–320.1)	217 (211.1–222.9)	281 (274.5–287.5)
CND-NG infection	26 (23.7–28.0)	51 (48.4–54.2)	36 (33.3–38.2)	41 (38.4–43.5)	26 (24.3–28.6)	16 (15.9–14.3)	11 (10.1–12.8)	5 (4.1–5.8)

Data are median incidence values with the 95% confidence interval in parentheses
NC-NG, nonchlamydial and nongonococcal infection; CND-NG, nongonococcal infection with chlamydia not determined

two to three times higher than in male patients. This finding was consistent throughout the 4 years. In contrast, gonococcal infection was predominantly found in male patients.

Gonococcal infection (Table 3) and genital chlamydial infection (Table 4) were high in younger people. The peak incidence was found at the ages of 20–24 years for both infections, followed by 15–19 and 25–29. In younger people, there was a much higher incidence of female chlamydial infection than male gonococcal infection.

Discussion

Countermeasures to prevent the spread of STDs have been a major medical and public health issue. Some countries have been very active in the prevention of STDs because prevention is closely linked with a decrease in the incidence of HIV infection.[6,7] Thus, it is crucial as a first step in the prevention of STDs to understand current trends in STD incidence. Unfortunately, we have not had appropriate sources of data in Japan to estimate the incidence of STDs. The major purpose of this study is to provide such data and to estimate the incidences of STDs.[2–5,8]

Hokkaido is located in northern Japan and is the largest prefecture in Japan. It is rich in natural resources and has many tourist attractions and many tourists from not only other cities in Japan but also other Asian countries. Sapporo is the capital of Hokkaido with a population of approximately 2 million. Sapporo's Susukino entertainment district has been thought to be a major source of STDs. In addition, the style of sexual activity has been changing slowly since the late 1990s, with oral sex becoming more common. We have already investigated differences in STD incidence between urban Sapporo and the rural areas of Hokkaido.[8] The levels of chlamydial infection were almost the same in urban and rural areas; however, the incidence of gonococcal infection in male patients was higher in urban areas than in rural areas.

The Centers for Disease Control and Prevention (CDC), USA, reported the overall rates of chlamydial infection in the USA in 1995 to be 290.3 in women and 52.1 in men per 100000 population.[9] In the CDC report, the incidence had declined substantially for all age groups over a seven-year period, although they were persistently highest among young adolescents. In Birmingham, UK, the overall prevalence of chlamydia was reported to be 129 per 100000.[10] Northern Australia had a reported incidence of female chlamydial infection of 250 per 100000.[11] The rate for women was approximately six times higher than that for men. In addition, the report indicated that those aged 15–19 years accounted for 46% of those infected, followed 20–24 years at 33% and 14 years and younger at 4%. Thus, the report cautioned that a higher incidence of the infection was evident in the younger generation. Similar findings were apparent in our study. Moreover, the incidence of chlamydial infection in the study was higher than those reported in other countries. Simple comparison of the incidence levels

Table 3. Incidence (per 100000 people per year) of gonococcal infections according to age category

Age (years)	Gonococcal infection (male)				Gonococcal infection (female)			
	1998	1999	2000	2001	1998	1999	2000	2001
10–14	0	4 (0.9–7.6)	0	8 (3.5–12.3)	0	13 (7.2–19.4)	23 (14.7–31.0)	0
15–19	166 (146.2–186.0)	252 (227.4–275.8)	241 (216.6–264.8)	245 (221.7–267.9)	170 (139.2–190.3)	224 (201.0–247.9)	232 (207.6–256.0)	227 (204.5–249.9)
20–24	513 (478.6–547.4)	715 (674.6–755.1)	579 (541.9–615.5)	613 (576.6–648.6)	165 (145.4–184.5)	309 (282.6–335.6)	263 (238.6–288.3)	312 (285.8–337.1)
25–29	401 (368.3–435.3)	749 (703.5–793.9)	545 (506.6–584.4)	875 (827.9–922.3)	41 (30.5–50.9)	100 (83.9–115.6)	185 (163.5–207.4)	205 (182.6–226.4)
30–34	325 (295.2–355.3)	517 (480.0–554.5)	414 (379.7–447.9)	522 (485.6–558.3)	24 (20.0–37.1)	60 (47.7–72.3)	54 (41.9–65.5)	93 (78.4–108.0)
35–39	172 (150.4–193.6)	219 (195.0–243.3)	252 (226.1–278.8)	295 (267.6–321.6)	21 (13.2–27.9)	36 (26.7–45.9)	21 (13.4–28.2)	49 (38.1–59.6)
40–44	89 (74.8–103.1)	129 (112.2–145.9)	133 (115.8–150.7)	172 (153.4–191.0)	0	10 (5.3–14.4)	14 (8.2–19.0)	21 (15.0–27.9)
45–49	65 (53.1–76.1)	73 (60.8–84.9)	56 (44.9–66.3)	80 (67.5–91.9)	0	6 (2.6–9.1)	9 (4.9–13.2)	5 (2.4–8.5)
50–54	53 (41.6–65.1)	68 (55.2–81.6)	71 (57.0–84.3)	34 (24.8–42.7)	7 (3.2–11.4)	0	4 (0.8–6.7)	13 (8.1–18.7)
55–59	9 (3.8–13.6)	17 (10.3–23.9)	13 (7.2–19.3)	10 (12.9–27.0)	8 (3.4–12.2)	0	0	7 (3.1–11.2)
60–64	13 (7.2–19.3)	9 (3.8–13.5)	22 (14.4–30.3)	40 (30.2–50.4)	4 (0.9–7.4)	8 (3.6–12.7)	0	0
65+	4 (1.9–6.6)	2 (0.4–3.7)	11 (6.9–14.6)	4 (1.7–6.1)	2 (0.3–2.8)	2 (0.3–2.8)	0	0

Table 4. Incidence (per 100000 people per year) of chlamydial infections according to age category

Age (years)	Chlamydial infection (male)				Chlamydial infection (female)			
	1998	1999	2000	2001	1998	1999	2000	2001
10–14	0	4 (0.9–7.6)	0	8 (3.5–12.3)	9 (4.0–14.1)	31 (21.7–40.3)	23 (14.7–31.0)	12 (6.7–18.1)
15–19	185 (163.9–205.9)	377 (347.7–407.1)	290 (263.9–316.8)	303 (277.7–329.1)	1255 (1199.4–1311.2)	1540 (1479.0–1601.6)	1547 (1484.1–1608.9)	1439 (1381.7–1496.1)
20–24	451 (418.4–483.0)	665 (625.8–703.4)	664 (624.6–703.4)	723 (684.0–762.1)	1481 (1422.5–1539.5)	2286 (2213.8–2357.6)	1893 (1826.1–1959.1)	1892 (1828.9–1955.3)
25–29	269 (241.9–296.7)	381 (348.6–413.1)	608 (566.3–649.1)	653 (612.5–694.1)	757 (713.0–801.2)	1125 (1072.1–1178.6)	940 (890.1–988.9)	1253 (1199.0–1307.4)
30–34	277 (249.2–304.6)	293 (264.8–321.2)	285 (256.5–313.0)	381 (350.3–412.5)	326 (297.4–355.4)	380 (349.0–410.9)	405 (372.3–437.2)	432 (400.4–464.2)
35–39	103 (86.5–119.9)	156 (135.6–176.3)	192 (168.6–214.5)	208 (185.5–230.9)	115 (97.8–132.3)	153 (133.4–172.8)	175 (153.3–196.1)	195 (173.7–216.7)
40–44	68 (55.3–79.9)	84 (70.1–97.3)	79 (65.8–92.7)	120 (104.5–136.0)	40 (31.0–49.5)	49 (39.2–59.4)	68 (55.8–79.9)	31 (22.9–38.3)
45–49	32 (24.2–40.4)	51 (40.6–60.7)	43 (33.2–51.9)	71 (59.3–82.3)	24 (17.2–30.6)	29 (21.9–36.6)	18 (12.3–24.0)	22 (15.7–27.9)
50–54	29 (20.1–37.4)	28 (19.7–36.6)	54 (42.1–65.9)	49 (38.0–59.5)	11 (5.9–16.0)	29 (20.7–36.8)	26 (18.2–33.7)	17 (10.8–22.7)
55–59	13 (7.1–19.1)	30 (21.0–38.9)	18 (10.7–24.7)	24 (16.2–31.7)	16 (9.5–21.9)	12 (6.6–16.8)	4 (0.8–7.1)	7 (3.1–11.2)
60–64	4 (0.9–7.9)	4 (0.9–7.8)	9 (3.9–14.0)	12 (6.5–17.6)	12 (6.7–18.1)	12 (6.6–17.7)	8 (3.7–13.1)	8 (3.3–11.8)
65+	2 (0.4–3.8)	2 (0.4–3.7)	2 (0.4–3.9)	4 (1.7–6.1)	2 (0.3–2.8)	3 (1.4–4.8)	3 (1.4–5.0)	0

sometimes is not appropriate because of different backgrounds of study design. However, the results of our study suggest that we need to provide immediately effective countermeasures to prevent chlamydial infection in the younger generation.

In our study, the incidence of male gonococcal infection was 127–199 per 100000 people per year and the incidence for women was 30–62. In other reports, the overall incidence of male gonococcal infection was 98.4 per 100000 men and the estimated incidence of female gonococcal infection was 370 per 100000.[11] In the USA, the incidence of gonococcal infection declined 71.3% between 1981 and 1996.[12] However, there were some regions still having high rates of infection, such as 547.4 per 100000 in Kansas City, MO, 669.7 in Detroit, MI, 939.8 in Baltimore, MD, and 942.5 in Newark, NJ. Interestingly, there were some states, such as Montana (4.4 per 100000) and North Dakota[9,12] with low infection rates. In Hokkaido, there was no declining trend of the disease during the 4 years of the study. The incidence of gonococcal infection in men was three to four times higher than that in women, and the trend is clearly different from that of chlamydial infection. We still cannot explain exactly why the incidence of male gonococcal infection is higher than that of female infection. The traditional explanation of the mild nature of the infection in women may be valid, because some screening programs for high-risk groups have a nonnegligible detection rate of gonococcal infection.[13,14] Screening programs for gonococcal infection may reveal higher incidences of the infection. In our study, only symptomatic patients were asked to be reported, so that nonsymptomatic patients with the infection would have been excluded from the data provided by institutes.

The incidence of genital herpes infection and condyloma acuminatum were low in this study. The incidence of latent or subclinical infections with herpes simplex virus (HSV) and human papillomavirus (HPV) has been reported to be higher than that of symptomatic infection.[15,16] Indeed, we already reported that HPV was detected in healthy men and men with urethritis. In particular, 18% of those with urethritis had HPV DNA on their external genitalia.[17] It is noteworthy that more than 80% of positive patients had high- or intermediate-oncogenic-risk HPV DNA. However, the detection seems to be transient so that symptomatic infection may be less prevalent, as found in our study.

Our study had several limitations in design. Although there were consistently high response rates throughout the 4 years of the study, variation in the institutes that participated may have affected the number of patients with STDs being reported, so that the total numbers might vary somewhat. The second is that diagnostic procedures for STDs, in particular for gonococcal or chlamydial infections, might not be the same in different clinics and hospitals. Some nongonococcal infections with chlamydia not determined may have been chlamydial infections because a specific detection test for chlamydia was not done. Furthermore, some detection procedures such as Gram staining for gonococcus have a definitely lower sensitivity than PCR or LCR. Each

institute that participated in the study had its own detection policy for the causative agent of STDs. This might also have affected the results. Nevertheless, the study is the first comprehensive one that allows us to estimate much more precisely the incidence of STDs. Thus, we now clearly understand that the incidence of STDs in our prefecture is higher than previously anticipated, in particular, those of gonococcal and chlamydial infections in younger people. These results emphasize the need to establish effective countermeasures for prevention, such as robust health education about STDs.

In conclusion, we conducted a prefecture-wide survey for STDs. High response rates for reporting the number of patients from each institute enabled us to estimate the incidence of STDs in Hokkaido. Incidences of gonococcal and chlamydial infections were prominently high, especially in younger people. The results clearly indicate the need for prompt establishment of practical countermeasures for prevention of these diseases.

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IS SEMINAL VESICULITIS A DISCRETE DISEASE ENTITY? CLINICAL AND MICROBIOLOGICAL STUDY OF SEMINAL VESICULITIS IN PATIENTS WITH ACUTE EPIDIDYMITIS

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ABSTRACT

Purpose: To our knowledge direct evidence of inflammatory involvement of the seminal vesicles has not previously been reported in patients with acute epididymitis. We verified the discrete disease entity of seminal vesiculitis associated with acute epididymitis.

Materials and Methods: The study included 13 patients who were clinically diagnosed with acute epididymitis. We report imaging, cytological and bacteriological findings in the seminal vesicles of patients with acute epididymitis.

Results: On transrectal ultrasonography 12 of the 13 patients (92.3%) had dilatation of the seminal vesicle on the side ipsilateral to epididymitis. Dilatation of the contralateral side was found in only 4 patients. Seminal vesicle fluid from the ipsilateral side showed inflammatory findings in all patients. In patients 40 years and younger *Chlamydia trachomatis* was detected in seminal vesicle fluid in 7 of the 8 patients with epididymitis with results positive for the microorganism on first voided urine.

Conclusions: Inflammatory responses were found in the seminal vesicles of patients with acute epididymitis. *Chlamydia trachomatis* was the causative pathogen most frequently detected in seminal vesicle fluid. Seminal vesiculitis is clearly associated with acute epididymitis and it may be a discrete disease entity.

KEY WORDS: testis, seminal vesicles, epididymitis, Chlamydia trachomatis, inflammation

Recent advances in imaging modalities such as transrectal ultrasonography (TRUS) have enabled us to investigate the external and internal architecture of the prostate and seminal vesicles. Christiansen and Purvis reported that 68% of patients with chronic abacterial prostatitis had inflammatory findings in the prostate and seminal vesicles on TRUS.[1] In addition, 72% of their patients had a history of epididymitis or a simultaneous association of inflammation in the epididymitis. When inflammatory findings were unilaterally found in 1 seminal vesicle, patients had epididymitis exclusively on the ipsilateral side. In a study of Littrup et al similar results were found and 62% of patients with "chronic prostatitis syndrome" had abnormalities of the seminal vesicles, characterized by elongation, dilatation and thickening of the septa.[2] Finally, Krishnan and Heal reported that the seminal vesicle on the side ipsilateral to epididymitis was enlarged in 13 of 18 patients who were evaluated by TRUS and 92% of enlarged seminal vesicles returned to normal size by 12 weeks after treatment.[3] These studies suggest that the seminal vesicle was involved in the infection of epididymitis and prostatitis. However, no cytological and bacteriological analyses of fluid in the dilated or elongated seminal vesicles were done in the studies.[1–3] Thus, direct evidence of inflammatory involvement of the seminal vesicles has not been extensively studied in patients with acute epididymitis.

In this context we studied seminal vesicle involvement by imaging, and by cytological and microbiological examinations of seminal vesicle fluid in patients with acute epididymitis. We determined whether seminal vesiculitis is a discrete disease entity.

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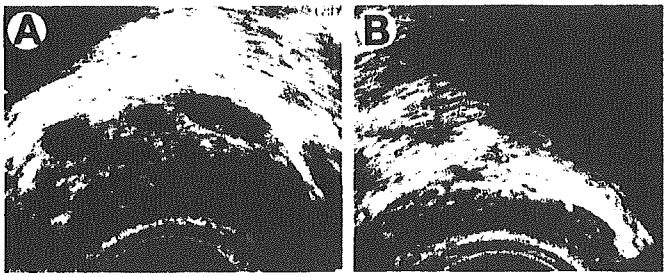
PATIENTS AND METHODS

Patient evaluation. All patients who presented to us had typical symptoms and clinical signs of acute epididymitis, characterized by fever a few days in duration and markedly swollen scrotal contents with severe tenderness. Testicular torsion was carefully differentiated by skilled urologists. All patients had 10 or more white blood cells in the urinary sediment of first voided or midstream urine. Patients were evaluated by TRUS before and after treatment, and bacteriological and cytological studies before treatment. After diagnostic evaluations they were treated with appropriate antimicrobial chemotherapy for an adequate duration.

TRUS imaging and TRUS guided seminal vesicle puncture. TRUS was performed using an Aloka SSD-5000 ultrasound apparatus (Aloka Co., Ltd., Tokyo, Japan) with a biplanar, high resolution 5.0 to 7.5 MHz transrectal transducer. The standard method was used for TRUS. Briefly, a probe covered by a rubber sack with a lubricant was gently inserted into the patient anus. In the transverse view of TRUS maximum right and left seminal vesicle areas were calculated automatically by instrument software. Seminal vesicle dilatation was defined by at least 1 finding on TRUS examination, namely 1) the area was 0.3 cm^2 larger than that of the opposite side according to the definition of Krishnan and Heal,[3] 2) the anteroposterior dimension was greater than 1.5 cm according to the definition of Littrup et al[2] or 3) an obvious cystic change was found (see figure). To evaluate the efficacy of antimicrobial treatment maximum seminal vesicle areas examined by TRUS were calculated again after treatment.

The seminal vesicle was punctured in a standard manner 2



TRUS reveals seminal vesicle involvement in patient with left acute epididymitis. *A*, left seminal vesicle is enlarged and dilated with multicystic changes. *B*, right seminal vesicle is not dilated.

to 3 hours after the initial administration of an antimicrobial agent to eliminate the possibility of microorganism dissemination. Briefly, each patient was placed in the lithotomy position under caudal anesthesia. The puncture attachment, an Aloka MP-2451 (Aloka Co., Ltd.) equipped with a transrectal transducer to guide the needle, was used to puncture the seminal vesicle. With careful observation of the longitudinal section of the seminal vesicle being monitored by TRUS an 18, 20 or 22 gauge needle was introduced into the seminal vesicle through the perineum and bilateral seminal vesicle fluid was aspirated. The aspirated fluid from the seminal vesicle was then subjected to microbiological and cytological examinations.

Microbiological study. All patients underwent standard microbiological examinations, including urinalysis of first voided and midstream urine specimens, gram staining of urethral discharge and detection. *Chlamydia trachomatis* and *Neisseria gonorrhoeae* of the first voided urine and seminal vesicle fluid were detected by a commercially available polymerase chain reaction method, Amplicor STD-I (F. Hoffmann-La Roche, Ltd., Basel, Switzerland). Aerobic bacterial pathogens of the midstream urine and seminal vesicle fluid were detected with a standard method. Briefly, specimens were incubated with 5% sheep blood agar, mannitol salt agar and bromthymol blue lactose agar at 36C for 24 hours. Significant colonies were identified by the analytical profile index procedure using the MicroScan Walk/Away system (Dade Behring, Inc., Dade MicroScan, Inc., West Sacramento, California) according to manufacturer instructions. In the case of aerobic culture positive culture was defined as 10^4 cfu/ml or greater for any bacteria that grew in urine.

Cytological analysis of seminal vesicle fluid. For smear samples preparation seminal vesicle fluid was applied to the surface of a microscopic slide just after puncture and dried. It was fixed by methanol and stained by Giemsa, dried again and used for microscopic examination. On cytological examination the seminal vesicle was considered to show inflammation if white blood cells (neutrophils) were consistently found in the smear sample in a 400× high power field, in accordance with our previous study.[4] The number of white blood cells is shown as the mean.

Treatment. When patients were tentatively diagnosed with acute epididymitis associated with chlamydial urethritis, in particular patients around 40 years and younger, they were treated orally with 100 mg levofloxacin 3 times daily or 200 mg clarithromycin twice daily for 2 to 3 weeks. When patients were older than 40 years or had a clinical history that excluded the possibility of sexually transmitted disease and they were diagnosed with an associated urinary tract infection, they were treated orally with 100 mg cefcapene pivoxil hydrochloride 3 times daily for 2 weeks.

Statistical analysis. The paired t test was used to assess the difference between the calculated area of the seminal vesicle before and after treatment.

Informed consent. Written informed consent was obtained from all patients who agreed to participate in this study.

RESULTS

Patient profiles and clinical outcomes. The study included 13 patients with acute epididymitis. Median patient age was 28 years (range 19 to 76). Epididymal involvement was on the right side in 5 patients and on the left side in 8 (table 1). All 13 patients showed improvement in symptoms and signs after appropriate treatment with antimicrobial agents at the last visit. We did not count the number of patients approached who did not agree to be studied.

Seminal vesicle findings. Dilatation of the seminal vesicle on the side ipsilateral to epididymitis was found in 12 of the 13 patients (92.3%) on TRUS examination. Only 4 patients (30.8%) had dilatation on the contralateral side with (3) or without (1) simultaneous ipsilateral dilatation. Seminal vesicle area on the ipsilateral side was significantly larger than on the contralateral side when it was evaluated before treatment (table 2).

Of the 13 patients 11 were evaluated again by TRUS status of the seminal vesicles after treatment. Although the time of evaluation after treatment varied from 6 to 96 days, vesicle size on the ipsilateral side was markedly reduced after treatment when compared in 11 patients with data available before and after treatment. However, no significant reduction was found between vesicle area on the contralateral side before and after treatment in 11 patients with data available. Cystic lesions of the seminal vesicle were found in 8 of the 13 patients with seminal vesicle involvement on the side ipsilateral to epididymitis. One patient also had these cystic lesions on the contralateral side. All visible cystic lesions disappeared after treatment.

The seminal vesicle on the side ipsilateral to epididymitis was successfully punctured in all patients but on the contralateral side in only 5 (38.5%). The mean volume of aspirated seminal vesicle fluid was 1.8 ml (range 0.2 to 4.0) on the ipsilateral side and 1.1 ml (range 0.1 to 2.0) on the contralateral side. Inflammation was found in the seminal vesicle ipsilateral to epididymitis in all 13 patients. However, only 2 of the 5 patients with successful vesicle puncture on the contralateral side showed inflammatory findings. No patients experienced any bleeding or infectious complications associated with seminal vesicle puncture, although there might have been bleeding from the prostate or subcutaneous tissue, or high grade fever due to sepsis as potential complications. We never tried to puncture the seminal vesicles of patients with shock status due to sepsis as a contraindication.

Microbiological findings. C. trachomatis was detected in patients 1 to 8 of the 13 patients (68.3%) in first voided urine. These 8 patients were younger than 40 years. Seven of them were also positive for this organism in seminal vesicle fluid from the ipsilateral side. In the remaining patient neither *C. trachomatis* nor bacteria were detected. *N. gonorrhoeae* was

TABLE 1. *Age, epididymitis side and seminal vesicle dilatation on imaging*

Pt No.—Age	Epididymitis Side	Seminal Vesicle Dilatation		Antimicrobial Treatment
		Ipsilat	Contralat	
1—19	Lt	Yes	No	Levofloxacin
2—22	Rt	Yes	Yes	Levofloxacin
3—22	Lt	Yes	No	Clarithromycin
4—23	Rt	Yes	No	Levofloxacin
5—26	Lt	Yes	No	Levofloxacin
6—26	Lt	Yes	No	Clarithromycin
7—28	Lt	Yes	No	Levofloxacin
8—37	Lt	No	No	Clarithromycin
9—40	Rt	No	Yes	Cefcapene pivoxil hydrochloride
10—43	Rt	Yes	Yes	Cefcapene pivoxil hydrochloride
11—57	Rt	Yes	No	Cefcapene pivoxil hydrochloride
12—74	Rt	Yes	No	Cefcapene pivoxil hydrochloride
13—76	Lt	Yes	Yes	Cefcapene pivoxil hydrochloride

TABLE 2. *TRUS of seminal vesicles*

Pt No.	Pretreatment Area (cm^2)		Posttreatment Area (cm^2)		Days
	Ipsilat	Contralat	Ipsilat	Contralat	Before + After Treatment
1	3.09	2.16	2.28	0.91	21
2	6.80	3.63	3.09	3.57	21
3	2.85	1.74	2.03	2.11	19
4	3.08	2.77	No data available	No data available	No data available
5	2.60	1.36	2.35	1.68	6
6	1.58	1.36	No data available	No data available	No data available
7	3.32	2.34	2.10	2.23	24
8	3.03	2.98	2.14	2.49	7
9	2.59	3.02	1.95	2.24	96
10	2.46	2.07	1.46	1.75	23
11	4.75	2.81	2.81	1.58	63
12	3.04	2.33	2.33	2.04	81
13	2.41	2.92	2.07	2.01	19
Totals	Mean ± SD 3.2 ± 1.3	Mean ± SD 2.4 ± 0.7	Mean ± SD 2.2 ± 0.4	Mean ± SD 2.1 ± 0.5	Median 24

Patients 1 and 8 were excluded from statistical analysis because of no available data after treatment.
Pretreatment ipsilateral vs contralateral and vs posttreatment ipsilateral, and pretreatment vs posttreatment contralateral paired t test $p = 0.015$, 0.003 and 0.053, respectively.

not detected in any of the 13 patients. *Escherichia coli, Streptococcus agalactiae* or *Flavobacterium indologenes* was isolated from the urine of 4 of the 13 patients. Patients 9 to 13 were 40 years old and older. Only 1 patient showed positive isolation of the same bacterium, *E. coli*, from urine and from fluid of the seminal vesicle on the side ipsilateral to epididymitis. One patient in the study did not have any microorganisms, probably because of antimicrobial treatment given elsewhere before his visit. No patients had any microorganism in fluid from the seminal vesicle on the contralateral side. Eradication of microorganisms was confirmed by urine examination after antimicrobial treatment in all 13 patients.

DISCUSSION

The results of our study clearly indicate that seminal vesiculitis is a discrete disease entity associated with acute epididymitis. The first evidence to support our interpretations was that abnormal dilatation of the seminal vesicle was clearly found more frequently on the side ipsilateral to epididymitis than on the contralateral side (92% vs 31%). Also, puncture fluid from the seminal vesicle on the ipsilateral side in all patients contained many white blood cells, indicating inflammation. In addition, the frequency of inflammatory findings was significantly higher in fluid from the ipsilateral seminal vesicle than from the contralateral one (100% vs 40%) when the puncture was successfully accomplished. As discussed, clinically significant microorganisms were detected in the fluid of more than 60% of patients. When these microorganisms were detected in the fluid, they were identical to those detected in urine. Furthermore, the reduction in the ipsilateral seminal vesicle paralleled the clinical outcome after appropriate antimicrobial treatment, also indicating the inflammatory nature of the infectious process. Thus, our results not only confirm the findings of previous studies,[1–3] but also add direct evidence that the seminal vesicle was strongly involved in the inflammation process.

It was intriguing that in our study *C. trachomatis* was detected in seminal vesicle fluid in younger patients with acute epididymitis, in whom the same organism was also detected in urine. Berger et al reported that *C. trachomatis* was isolated from the urethra and puncture fluid of epididymis in young patients with acute epididymitis.[5] It is well-known evidence that *C. trachomatis* can cause epididymitis. To our knowledge the results of our study provide for the first time evidence that *C. trachomatis* is involved in the development of seminal vesiculitis as well as epididymitis. In fact, in heterosexual men younger than 35 years bacteriuria is uncommon, whereas urethritis caused by *N. gonorrhoeae* or *C. trachomatis* is common.[6]

The last and most important question is whether seminal vesiculitis precedes acute epididymitis. In our study several patients with epididymitis already had dilatation and inflammation of the seminal vesicle on the contralateral side despite the finding that epididymitis was unilaterally limited, although the frequency of this abnormality was clearly lower than that on the ipsilateral side. Krishnan and Heal postulated in their study that epididymitis originated from seminal vesiculitis but the opposite did not occur.[3] If seminal vesiculitis precedes acute epididymitis, the seminal vesicle might be an infectious site of microorganisms, especially in the case of *C. trachomatis* infection. If this interpretation is correct, what is the origin of seminal vesiculitis? In the 1930s Hyams et al had already postulated 3 ways by which the disease might develop, namely direct extension from the posterior urethra with the highest probability, followed by extension from tuberculous epididymitis and blood-borne dissemination.[7] Since we have direct evidence that *C. trachomatis* is the causative microorganism of seminal vesiculitis, it is valid to speculate that the microorganism induced urethral infection caused seminal vesiculitis to develop, followed by epididymitis in some patients. However, we are still unable to explain why not all patients with chlamydial urethritis have seminal vesiculitis and epididymitis. Additional study is needed to establish the time courses.

A limitation of our study is that it is technically difficult to puncture seminal vesicles without dilatation. In this series 8 of the 13 patients could not undergo puncture successfully. Thus, we could not completely obtain inflammatory status results on the side opposite epididymitis. In addition, this puncture technique cannot be used as a routine diagnostic procedure because it is somewhat invasive for patients and the treatment for seminal vesiculitis would be the same as that for acute epididymitis.

CONCLUSIONS

Imaging, cytological and microbiological studies revealed a clear association of seminal vesiculitis with acute epididymitis. Dilatation of the seminal vesicle with inflammatory findings on the side ipsilateral to epididymitis was frequently found in patients with acute epididymitis. *C. trachomatis* was most frequently detected in fluid of dilated seminal vesicles, especially from patients 40 years and younger. The associated abnormal dilatation of the seminal vesicle disappeared in parallel with improvement in symptoms and signs

of acute epididymitis after treatment with an appropriate antimicrobial agent. This study suggests that seminal vesiculitis can be regarded as a discrete disease entity.

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NOTE

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Efficacy of an RNA detection test kit in the diagnosis of genital chlamydial infection

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Abstract A nucleic acid amplification method based on DNA detection, the current standard method for the diagnosis of genital infection by *Chlamydia trachomatis*, has been shown to potentially yield false-positive results after treatment in the clinical setting. RNA detection methods are more appropriate because viable organisms have multiple RNA copies that are surely detected by the method. In this study, we evaluated the efficacy of a new RNA detection test kit, the VIDAS PROBE CT test, in the diagnosis of genital chlamydial infection. For comparison, the standard DNA detection method, Amplicor STD-I, was also used in the study. First voided-urine samples and urethral smears from male patients with urethritis, and first voided-urine samples and cervical smears from female patients with cervicitis served as samples for the detection of *C. trachomatis*. Of the 60 first voided-urine samples from male patients, 21 were positive and 39 negative with the VIDAS PROBE CT test. Amplicor STD-I achieved exactly the same result. In female patients with cervicitis, the two test kits produced the same result, with 2 positive cervical smears and 38 negative. These results suggest that the VIDAS PROBE CT test is as efficient as Amplicor STD-I in the detection of *C. trachomatis*. While studies including a greater number of patients will be needed for revealing the unique advantages of the new RNA detection test kit, VIDAS PROBE CT, we concluded from the current study that the test may be clinically useful in the diagnosis of genital chlamydial infection.

Key words *Chlamydia trachomatis* · Genital infection · RNA detection

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The current standard method for the diagnosis of genital infection by *Chlamydia trachomatis* is genomic DNA detection.[1,2] The method is based on the polymerase chain reaction (PCR) or the ligase chain reaction (LCR) that amplifies a portion of *C. trachomatis* nucleic acid. This method achieves a higher sensitivity and specificity than other tests.[3,4] However, the method has a pitfall in that it detects non-viable *C. trachomatis* when it is employed for the detection of organisms after patients receive appropriate antimicrobial agents at an adequate treatment dose and duration.[5] This may lead to a false-positive result which may be embarrassing for medical professionals and patients. If the genomic DNA method shows a positive result after appropriate treatment, it is difficult to know whether the result indicates that the infection is cured and only nonviable organisms are detected, or whether the infection is persistent because of treatment failure. Indeed, there has been a report that positive DNA detection had been found for as long as several weeks, although bacteriological cure was achieved.[6]

In this context, RNA detection is more desirable, because it detects only viable microorganisms that have multiple RNA copies. This detection method has been available for other infectious diseases, such as HIV and hepatitis C virus (HCV) infections.[7,8] Recently, two techniques of RNA amplification have been made commercially available in kits. One is nucleic acid sequence-based amplification (NASBA),[6] and the other is transcription-mediated amplification (TMA).[9,10] In this study, we evaluated the clinical efficacy of the RNA amplification test kit, VIDAS PROBE *Chlamydia trachomatis* (VIDAS PROBE CT; bioMerieux, Rockland, MA, USA), which uses TMA as its amplification method.

Urine and urethral smear samples were obtained from male patients with clinically diagnosed urethritis. First voided-urine samples were collected by standard procedures and urethral smear samples were obtained by wiping the distal urethra with a wet cotton swab. In female patients with cervicitis, first voided-urine samples were collected by standard procedures and cervical smear specimens were obtained by wiping the cervix with a wet cotton swab. Specimens were frozen and stored at −20°C until tested. *C. trachomatis* was detected with the VIDAS PROBE CT and Amplicor STD-I (F. Hoffmann-La Roche, Basel, Switzerland) tests. In the VIDAS PROBE CT test, a specific sequence of *C. trachomatis* 23S ribosomal RNA (rRNA) is amplified, and the amplification and detection of *C. trachomatis* is automatically performed in special instruments (the bioMerieux AMPstation and VIDAS or miniVIDAS instruments) utilizing unit-dose packaged reagents. Urine and swab specimens are processed and added to the VIDAS PROBE CT reagent strip according to the manufacturer's instructions. Amplification of the internal control and *C. trachomatis* rRNA are simultaneously performed for approximately 1 h and 45 min. After completion of the amplification, the strips are transferred to the VIDAS instrument for detection. *C. trachomatis* was automatically detected in the VIDAS instrument for approximately 1 h and 50 min.

PCR for the Amplicor STD-I was performed by using the GeneAmp PCR System 2400 (Applied Biosystems, Foster City, CA, USA). A positive result was defined as a reading above 0.5 a standard spectrophotometer at 450 nm, equivocal was defined as 0.2 to 0.5, and negative, below 0.2.

To clarify any differences in results between DNA and RNA detections, we detected *C. trachomatis* from the first-voided urine samples of male patients with urethritis before and after treatment when they visited several times. They received 100 mg of levofloxacin three times daily or 100 mg of minocycline twice daily for at least 7 days. Patients with gonococcal urethritis were excluded from this study.

This study was approved by the Institutional Review Board for Clinical Research of Sapporo Medical University (no. 97-29) and written informed consent was obtained from patients participating in the study.

Table 1. Detection of *Chlamydia trachomatis* in the first-voided urine samples and urethral smears from male patients with urethritis

		Amplicor	STD-I
		Positive	Negative
First-voided urine			
VIDAS PROBE CT	Positive	21 Patients	0
	Negative	0	39 Patients
		Amplicor	STD-I
		Positive	Negative
Urethral smear			
VIDAS PROBE CT	Positive	2 Patients	0
	Negative	0	15 Patients

First-voided urine samples were tested in 60 male patients with non-gonococcal urethritis. In a non-selected group of 17 of these 60 patients, urethral smear samples were also evaluated. The VIDAS PROBE CT test showed a positive result for 21 urine samples and negative result for 39, which agreed with the results by the Amplicor-STD-I (Table 1). In the urethral smear samples, the results by the VIDAS PROBE CT test agreed with those by the Amplicor-STD-I test.

In 40 female patients with cervicitis, 39 first-voided urine samples and 40 cervical smear samples were tested by the VIDAS PROBE CT and the Amplicor-STD-I tests. The VIDAS PROBE CT revealed 2 positive urine samples and 2 positive smear samples from the same 2 patients. All other samples tested were negative. The results of the Amplicor-STD-I test were exactly the same as those obtained by the VIDAS PROBE CT test.

In a follow-up study, nine male patients who were positive for *C. trachomatis* by the VIDAS PROBE CT and Amplicor-STD-I tests before treatment were tested by their first-voided urine (Table 2). All but one patient showed a negative result for the organism during and after treatment, with follow-up ranging from 5 to 17 days. In one patient, the organism was positive by the VIDAS PROBE CT and Amplicor-STD-I tests, when the first-voided urine samples were tested 2 days after the initiation of treatment. However, 10 days after treatment, both methods produced a negative result. No discrepancy was found in the results between the two test methods. All patients were cured with appropriate antimicrobial chemotherapy for urethritis.

In this study, both the VIDAS PROBE CT and Amplicor STD-I tests achieved the same result in terms of detection of *C. trachomatis* in male patients with urethritis and female patients with cervicitis. Unfortunately, in this study (in contrast to the results for urethritis) there were only a few patients with cervicitis who were positive for *C. trachomatis* by the VIDAS PROBE CT and Amplicor STD-I tests . Thus, additional studies may be necessary for confirming that both methods have basically no difference in their capacity for detection of the organism, particularly in female cervicitis. Nevertheless, our preliminary study clearly indicates that the VIDAS PROBE CT has equivalent efficacy, when compared with the current standard PCR test in detecting *C. trachomatis* in first-voided urine and smear samples. Evaluation of the VIDAS PROBE CT has been performed not only in Japan but also in Europe and the United States. The efficacy of the test method in larger populations will be established in the very near future.

We also evaluated the clinical efficacy of the VIDAS PROBE CT test for the detection of *C. trachomatis* from first-voided urine of male patients with urethritis after antimicrobial treatment, comparing it with that of the Amplicor STD-I test. This is because it may be expected that detection of the RNA of *C. trachomatis* would have a different result from that of the DNA of the organism. Indeed, Morré and colleagues[6] reported that, in female patients with cervicitis, there were discrepancies in the results post-treatment between the DNA and RNA detections, when swab

Table 2. Detection of *Chlamydia trachomatis* with VIDAS PROBE CT and Amplicor STD-I test kits before, during, and after treatment in male patients with urethritis

	Pretreatment		During treatment			After treatment		
	VIDAS	AMP	Days	VIDAS	AMP	Days	VIDAS	AMP
Case 1	Positive	Positive	7	Negative	Negative	17	Negative	Negative
Case 2	Positive	Positive	2	Positive	Positive	10	Negative	Negative
Case 3	Positive	Positive	12	Negative	Negative	–	–	–
Case 4	Positive	Positive	7	Negative	Negative	–	–	–
Case 5	Positive	Positive	5	Negative	Negative	–	–	–
Case 6	Positive	Positive	6	Negative	Negative	–	–	–
Case 7	Positive	Positive	8	Negative	Negative	15	Negative	Negative
Case 8	Positive	Positive	7	Negative	Negative	–	–	–
Case 9	Positive	Positive	7	Negative	Negative	–	–	–

VIDAS, VIDAS PROBE CT; AMP, Amplicor STD-I; days, days after start of treatment; –, not evaluated

samples from the cervix were considered. In terms of test results, we did not find any discrepancy between the two detection methods in this study. However, we may need a post-treatment test for the detection of *C. trachomatis* in patients with persistent infection or infection refractory to appropriate antimicrobial treatment.[11] In this situation, an RNA detection test would be useful.

In conclusion, the VIDAS PROBE CT test for *C. trachomatis* in patients with urethritis or cervicitis is as clinically useful as the Amplicor STD-I test for the detection of the organism. When we consider that the RNA detection test detects only viable cells, it may become a standard test for the detection of *C. trachomatis*.

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Detection of Human Papillomavirus DNA on the External Genitalia of Healthy Men and Male Patients with Urethritis

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Background: **Only a few studies have been done involving detection of human papillomavirus (HPV) DNA on the external genitalia of men without genital warts, although many have been done for women. We conducted HPV DNA detection among healthy male volunteers and men with urethritis, both having no visible lesions on their external genitalia.**

Goal: **The goal of the study was to determine the detection rate of HPV DNA in volunteers and patients with urethritis and to determine risk factor(s) for positive DNA.**

Study Design: **This was a prospective clinical study.**

Results: **HPV DNA was found in 1.3% of 75 volunteers and in 18.5% of 130 patients with urethritis. DNA of a high-intermediate oncogenic risk was more predominant than the low-risk type. Among various risk factors, only a history of STD was a significant factor for the positive detection of HPV DNA in multiple regression analysis.**

Conclusion: **HPV DNA was found in patients with urethritis more frequently than in volunteers, probably because the former had higher sexual activity.**

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WHILE HUMAN PAPILLOMAVIRUS (HPV) INFECTION is closely linked with development of genital warts and cervical and penile cancers, depending on the DNA type, asymptomatic infection by the virus is more prevalent. Peyton et al.[1] reported that asymptomatic infection by HPV in cervical specimens was found in 39.2% of 3863 women and that the infection was strongly associated with age and the number of lifetime and recent sex partners. Richardson et al.[2] reported similar findings; they noted 21.8% overall HPV detection in asymptomatic female university students. The study also indicated that lifetime frequency of sexual intercourse and lifetime number of oral sex partners were associated with high-oncogenic-risk HPV infection.

However, partly because of the extremely low incidence of penile cancer in developed countries, there have been few studies of asymptomatic HPV infection in men.[3] Another factor hampering such studies is that we have not established how and what specimens we need to collect to determine the rate of asymptomatic HPV infection.

Since asymptomatic infection with the virus in men is partly responsible for asymptomatic infection in women, who more frequently develop cancer of genital lesions, it is crucial to determine the prevalence of such infection and the DNA types of the virus that are involved in it. Thus, we tried to detect HPV DNA in the external genitalia of healthy men and male patients with urethritis, all of whom were free of visible lesions caused by the virus. We also studied the natural clinical course of men who were positive for HPV DNA at the first examination. Although our study may not necessarily reveal a substantial rate of asymptomatic HPV infection in men, the results will enhance our understanding of the natural history of the infection.

Patients and Methods

The study included 75 healthy male volunteers who were recruited from among university students for 1 month by advertisements that explained the study design and its clinical relevance. For 3 months, consecutive patients with urethritis (130 men) who agreed to participate were included in the study. They visited a clinic or hospital with which one of the authors is affiliated, reporting symptoms related to urethritis. The response rate of the patients was >90%. Since the clinical significance of detection of HPV DNA in the absence of genital warts was not established, we informed participants of the result of the examination only when they wanted to know.

The healthy male volunteers were asked to respond to a self-administered questionnaire for information about age, marital status, history of sexually transmitted diseases (STDs), average frequency of sexual intercourse in the previous 3 months, and number of current sex partners. The average frequency of intercourse was assessed as 3 to 4 times per week, 1 to 2 times per week, 3 to 4 times per month, 1 to 2 times per month, less than 1 time per month, and none. The participants were also asked to mark on the questionnaire their preputial status according to four illustrated types: type A, in which the prepuce completely covers the glans; type B, in which the prepuce covers half of the glans; type C, in which the prepuce is beyond the sulcus but can be extended to

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cover half of the glans without compressing it; and type D, in which the prepuce is completely absent.[4]

In addition, they were asked to confirm carefully by themselves that they did not have any visible genital warts on their external genitalia, including the glans, coronal sulcus, inner surface of the prepuce, or urethral meatus. The confirmation was made at the time of initiation of the study and then 3 and 6 months later, together with HPV DNA tests. The rationale for self-examination was based on the clinical experience of urologists that most patients with genital warts usually visit a clinic to report a visible tumor on the external genitalia that they find by themselves.

The healthy volunteers underwent an HPV DNA detection test three times, at the initial examination and then 3 and 6 months later. They were carefully guided by one of us (S.T.) on how to obtain test specimens for the virus DNA. Using a body model, the urologist instructed them at each examination how and where they should wipe with a cotton swab for detection of HPV DNA. The glans, coronal sulcus, and inner surface of the prepuce were extensively wiped with a wet cotton swab by the volunteers themselves. Then the cotton swab was put in a storage bottle of a Digene Swab Specimen Collection Kit (Digene Corporation, Gaithersburg, MD) containing buffer solution provided by a manufacturer, and the bottles were kept at −20 °C until the detection test.

Hybrid Capture II (Digene Corporation) was used to detect HPV DNA and to determine the DNA type. The method identified two types of DNA: that of high-intermediate oncogenic risk and that of low oncogenic risk. The former included DNA types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and the latter included DNA types 6, 11, 42, 43 and 44. The viral DNA detection was done according to the instructions of the manufacturer (Mitsubishi Kagaku Bio-Clinical Laboratories, Inc., Tokyo).

Male patients with urethritis were asked to respond to a self-administered questionnaire as the healthy volunteers did. They underwent diagnostic examinations by a urologist, including urinalysis of the first-voided urine, Gram staining of urethral discharge, and detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in the first-voided urine, both of which were done with a commercially available PCR assay (Amplicor STD-I; Hoffmann-La Roche, Ltd., Basel, Switzerland). They also underwent standard physical examination by a urologist, including careful inspection of the glans, coronal sulcus, inner surface of the prepuce, and urethral meatus of the external genitalia, with confirmation of no visible genital warts and of preputial status, as described for healthy volunteers at the initial visit.

If patients visited again, at that visit a urologist confirmed by inspection that there were no visible warts on the external genitalia as well as the urethral meatus. HPV DNA detection tests were done at the first and following visits; the latter were not uniform but variable, depending on patients' situations. The specimens for the viral DNA were obtained by urologists, who examined patients with the same methods as for healthy volunteers.

For 10 patients with urethritis who were positive for HPV DNA, including 8 with high-intermediate risk types and 2 with a low-risk one, the DNA types were confirmed by restriction fragment length polymorphism (RFLP) and a hybridization technique with type-specific probes, as reported previously,[5] at Mitsubishi Kagaku Bio-Clinical Laboratories, Inc., Tokyo.

Statistical analyses were done with the Fisher exact probability test, the Kruskal–Wallis test, and multiple regression, and StatView software for Windows, version 5 (SAS Institute, Cary, NC), was used for all analyses.

The protocol of this study was approved by the Institutional Review Board of Sapporo Medical University. Informed consent was obtained from all participants.

TABLE 1. Detection of HPV DNA and Clinical Backgrounds of Healthy Volunteers and Patients with Urethritis

Variable	No. (%) of Men	
	Volunteers (75)	Urethritis (130)
HPV DNA*		
Negative	74 (98.7)	106 (81.5)
Positive	1 (1.3)	24 (18.5)
HPV DNA type		
Low risk		4 (16.7)
High-intermediate risk	1	12 (50.0)
Both		8 (33.3)
Median age (range), y	22 (18–35)	28 (17–49)
Married		
Yes	0	37 (28.5)
No	75 (100)	93 (71.5)
History of STD		
Yes	0	62 (47.7)
No	75 (100)	68 (52.3)
Average frequency of sexual intercourse		
3 or 4 Times/week	3 (4.0)	13 (10.0)
1 or 2 Times/week	22 (29.3)	57 (43.8)
3 or 4 Times/month	8 (10.7)	22 (16.9)
1 or 2 Times/month	12 (16.0)	20 (15.4)
<Once/month	5 (6.7)	12 (9.2)
None	25 (33.3)	6 (4.6)
No. of current sex partners		
>4	0	3 (2.3)
4	0	1 (1.0)
3	0	13 (10.0)
2	2 (2.7)	23 (17.7)
1	40 (53.3)	75 (57.7)
None	33 (44.0)	15 (11.5)
Preputial status		
Type A	3 (4.0)	6 (4.6)
Type B	13 (17.3)	22 (16.9)
Type C	28 (37.3)	46 (35.4)
Type D	31 (41.3)	56 (43.1)

* Significant difference between healthy volunteers and patients with urethritis (Fisher's exact probability test).

Results

Detection of HPV DNA and DNA Types in Healthy Volunteers and Male Patients with Urethritis

Healthy male volunteers were clearly younger than patients with urethritis (Table 1). HPV DNA was detected on the genitalia of only one healthy volunteer (1.3%). The detection rate of HPV DNA was significantly higher among men with urethritis (18.5%) than among healthy volunteers (Table 1; $P < 0.0001$ by Fisher's exact probability test). Among the 130 men with urethritis, HPV DNA was detected in 6 (10%) of 60 patients with gonococcal infection, 11 (25%) of 44 with chlamydial infection, 3 (50%) of 6 with both gonococcal and chlamydial infections, and 4 (20%) of 20 with both nongonococcal and nonchlamydial infections.

The one positive healthy volunteer had HPV DNA of a high-intermediate-risk type (Table 1). Of 24 patients with urethritis who were positive for HPV DNA, the low-risk type was found in 4

TABLE 2. Clinical Backgrounds of Patients with Urethritis Who are Positive or Negative for HPV DNA Detection

Variable	Negative HPV DNA (106 patients)	Positive HPV DNA (24 patients)
Median age (range), y	28 (17–49)	25 (19–45)
Married: no. (%)		
Yes	36 (34.0)	1 (4.2)
No	70 (66.0)	23 (95.8)
History of STD: no. (%)		
Yes	45 (42.5)	17 (70.8)
No	61 (66.0)	7 (29.2)
Average frequency of sexual intercourse: no. (%)		
3 or 4 Times/week	9 (8.5)	4 (16.7)
1 or 2 Times/week	43 (40.6)	14 (58.3)
3 or 4 Times/month	21 (19.8)	1 (4.2)
1 or 2 Times/month	17 (16.0)	3 (12.5)
<Once/month	10 (10.6)	2 (8.3)
None	6 (5.7)	0
No. of current sex partners (%):		
>4	1 (1.0)	2 (8.3)
4	0	1 (4.2)
3	11 (10.4)	2 (8.3)
2	17 (16.0)	6 (25.0)
1	63 (59.4)	12 (50.0)
0	14 (13.2)	1 (4.2)
Preputial status: no. (%)		
Type A	5 (4.7)	1 (4.2)
Type B	16 (15.1)	6 (25.0)
Type C	40 (37.7)	6 (25.0)
Type D	45 (42.5)	11 (45.8)

(16.7%), the high-intermediate-risk type in 12 (50.0%), and both types in 8 (33.3%). Thus, of the 25 men positive for HPV DNA, 84% had HPV DNA of a high-intermediate risk type.

Sexual Activity, Preputial Status, and Significant Risk Factor(s) for HPV DNA Detection in Healthy Volunteers and Patients with Urethritis

Marital status differed for volunteers and patients with urethritis (Table 1). Patients with urethritis had sexual intercourse more frequently and more sex partners than healthy volunteers. Patients had a history of STD more frequently than did volunteers (Table 1). The history was more prominent for patients positive for HPV DNA than for those who were negative (Table 2). Almost 50% of patients negative for HPV DNA and more than 70% of those positive for HPV DNA had sexual intercourse one or two times per week or more frequently, which was in contrast to the finding that only 30% of volunteers had intercourse at such a frequency (Table 1).

As for sex partners, 20% of patients with urethritis positive for HPV DNA and 10% of those negative for HPV DNA had three or more current sex partners, while no volunteers had such a number (Tables 1 and 2). Status of the prepuce did not affect detection of HPV DNA. The four prepuce types were almost equally distributed among healthy volunteers and patients with urethritis who were positive or negative for HPV DNA.

Risk factors for positive detection of HPV DNA, including age, marital status, history of STD, average frequency of sexual intercourse, number of current sex partners, and type of prepuce, showed statistically significant differences among volunteers, patients with urethritis who were negative for HPV DNA, and

TABLE 3. Statistical Analysis Findings Among Three Groups—Healthy Volunteers, Patients with Urethritis Negative for HPV DNA and Those Positive—According to Risk Factors

Risk Factor for HPV DNA Detection	Significant Difference Among 3 Groups: *P* Value*
Median age	<0.0001
Marital status	<0.0001
History of STD	<0.0001
Average frequency of sexual intercourse	<0.0001
No. of current sex partners	<0.0001
Preputial status	0.9737

* Kruskal-Wallis test.

patients with urethritis who were positive for HPV DNA (except for preputial status; Table 3). However, multiple regression analysis of these factors revealed a history of STD to be a significant, independent risk factor for HPV DNA positivity ($P = 0.0122$).

In follow-up of the 75 healthy volunteers, all were examined for HPV DNA, with repeated instruction on obtaining specimens and confirmation that there were no visible warts at 3 and 6 months after the initial examination. The one volunteer positive for HPV DNA at the initial examination showed persistent infection with HPV of the same DNA type 3 months later. However, no HPV DNA was found 6 months later. No volunteers were found to become positive for HPV DNA during the 6-month follow-up. Development of genital warts was not noticed in any volunteers during this period.

Of the 130 patients with urethritis, 69 (including the 18 positive for HPV DNA at the first visit) underwent a detection test and were confirmed not to have genital warts by inspection again by a urologist at the second or third visit. The same type of HPV DNA was detected again at the second visit (median follow-up, 11 days; range, 3–30) in 16 of 18 who were positive at the first visit; two patients were negative at this second follow-up visit. Only 2 of 16 patients who were positive for HPV DNA at both the first and second visits had a third visit (at 35 and 145 days, respectively, after the diagnosis). They were persistently positive for the same type of HPV DNA. The remaining 51 patients negative for HPV DNA at the initial visit had second visits, with a median follow-up period of 10 days (range, 2–62) and one of these had a third visit as well (follow-up: 29 days). None of these 51 patients became positive for HPV DNA at the second or third visit.

Confirmation of HPV DNA Types with the PCR Method

We confirmed HPV DNA types for eight patients who were positive for high-intermediate-risk DNA and two of those positive for low-risk DNA with use of Hybrid Capture II. Their DNA types were analyzed by the RFPL and hybridization methods,[5] which revealed that specimens from eight patients who were positive for HPV DNA high-intermediate-risk types contained DNA types 16, 31, and 58. The method also revealed that swab specimens from the two patients positive for a low-risk type contained DNA type 6.

Discussion

The prevalence of asymptomatic HPV infection in men who were positive for HPV DNA on their external genitalia but had no visible lesion by pneoscopy was reported to be 7.2% in a study of

voluntary participants of the Finnish Army.[3] In addition, the study indicated that having many sex partners, a history of STD, and nonuse of condoms were significant risk factors for HPV infection of the genitalia. In a study of asymptomatic Mexican men, a higher HPV DNA detection rate, 43%, was noted,[6] although the backgrounds of controls and method of detection were not the same as those in our study. Unfortunately, the precise prevalence of asymptomatic infection in men has not been determined because the backgrounds of subjects recruited in studies and study designs are not always similar.

Indeed, there are no widely accepted standards for healthy volunteers. In our study, although we recruited university students, they might not have been ideal controls because their lifestyle, including sexual behavior, may have differed somewhat from that of ordinary workers. In addition, the most appropriate specimens for sampling have not been established for screening asymptomatic HPV infection. Iwasawa et al.[7] reported that urine samples could be used to detect HPV DNA in patients with genital warts and suggested that such samples might be applicable for screening asymptomatic subjects. However, a recent publication indicated that urine samples had very low sensitivity for such detection, in comparison with samples obtained by wiping the external genitalia.[6]

Obtaining urethral samples from the meatus by wiping with a swab is not more useful for subjects in the screening setting because of its painful and uncomfortable nature. Thus, our finding that the detection rate of HPV DNA for healthy volunteers was extremely low may stem from their background. We believe that the sampling method did not contribute to the rate, although a false-negative result might have been possible in the volunteers because they obtained specimens by themselves.

Nevertheless, what is important in the results of our study is that nearly 1 of 5 men with urethritis had asymptomatic HPV infection of their external genitalia that was revealed by HPV DNA detection with Hybrid Capture II, indicating that the asymptomatic infection might have been associated with other STDs such as urethritis.

High-intermediate-risk HPV DNA was detected more frequently than the low-risk type in the current study. Because there are reports of similar findings in female studies,[2,8] the HPV DNA types detected may have the same tendency in men and women. However, there is controversy about whether sex partners have the same type of HPV DNA.[9] In addition, while the efficacy of the Hybrid Capture method has been clinically established for the diagnosis of HPV infection,[10,11] undetermined HPV DNA types might not be detected by this method, since there are currently more than 80 types and the number is still growing. The detection of more types of HPV DNA is thus necessary to achieve greater accuracy.

A history of STD was determined by multiple regression analysis to be the only significant risk factor for HPV DNA positivity in our study. In addition, patients with urethritis who were positive for HPV DNA reported more sexual activity than those who were negative and volunteers. Thus, our results indicated that HPV DNA detection in men was closely linked with sexual behavior that was characterized by a history of STD and sexual frequency, which was consistent with the results of previous studies.

As for the association of preputial status in men with HPV infection and cervical or penile cancer, circumcision was reported to reduce the risk of penile HPV infection, and the absence of neonatal circumcision and potential resulting complications were associated with development of penile cancer.[12,13] Although phimosis is one of the risk factors of penile cancer, other factors such as sexual activity, smoking,[12] and inadequate hygiene may be involved.

In this study, no significant difference was found between the HPV infection rate and the status of the prepuce. Rather, multiple regression analysis showed that a history STD was the only significant risk factor for asymptomatic HPV infection. Because HPV prevalence differs among countries,[9] various environmental factors may be involved in genital HPV infection.

While the natural history of cervical cancer associated with HPV infection has been partly clarified,[14] the natural history of asymptomatic HPV infection has not been determined, particularly for men. A population-based cohort study that recruited young Swedish women who were asymptomatic revealed that HPV infection was transient, and a new HPV-type-specific infection was associated with a new sex partner or an abnormal smear after enrollment.[15] In our study, HPV infection persisted for the first 3 months in the one volunteer who was positive for HPV DNA at the initial evaluation. Patients with urethritis who were positive for HPV DNA at the initial evaluation were followed-up, although for a short interval, and most of them were positive for HPV at the second visit.

Because the number of patients enrolled in the follow-up study was small and their follow-up period was short and variable, depending on personal reasons, we could not fully elucidate the natural history of asymptomatic male HPV infection. However, HPV infection may persist, although the infection is transient, and reinfection is not excluded. What is important is that men positive for HPV DNA can transmit it to female partners during its persistent period. The natural history of asymptomatic infection by HPV will be clarified only with studies that provide longer follow-up and precise determination of HPV DNA types.

In conclusion, HPV DNA was detected in patients with urethritis more frequently than in healthy male volunteers. The high-intermediate-risk type was detected predominantly in men who were positive for HPV DNA. The risk factor for such detection was a history of STD, a circumstance suggesting association with the sexual activity of men.

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感染症

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72. 尖圭コンジローマ

72.1 病原体の性状

尖圭コンジローマ(condyloma acuminatum)は，human papillomavirus (HPV) より引き起こされる疣贅で，性感染症の1つである．HPVは，Polyomavirus, Simian virus 40 (SV 40) とともにPapovavirus科に属するDNAウイルスである．ウイルス粒子は，直径45～55 nmの小型のウイルスであり，72個のcapsomereで構成される正二十面体構造のカプシドと，約8000塩基対の環状二本鎖DNAを有する．HPVは，そのDNA homologyの相違により約80種類以上の型に分類される[1]．尖圭コンジローマの原因としては，6型 (HPV-6) と11型 (HPV-11) で約90%を占める．頻度は低いが16, 18, 52, 56型も原因となるが，この場合には，子宮頸癌や陰茎癌など悪性化する危険性がある[1,2] (表72.1)．HPVは，直接的接触によって皮膚の上皮細胞や粘膜に感染する．

72.2 国内外の流行状況

厚生省 (当時) 性感染症センチネル・サーベイランス研究班[3]によると，尖圭コンジローマの年間罹患率 (10万人・年対) は，男性で23.8，女性で27.8であり，女/男比は1.17で，やや女性の罹患率が高く，年齢別罹患率では，20歳代にピークがあった．罹患率としては，男性では性器ヘルペスよりもやや低く，女性では性器ヘルペスよりもかなり少なく淋菌感染症とほぼ同じであった．尖圭コンジローマは性器クラミジア感染症，性器ヘルペス，淋菌感染症，梅毒などの全性感染症の約5%を占めており，年間罹患数の推移としてはほぼ横這いであったが，15～24歳の若年女性では，1998年度と比較して1999年度では20%弱の増加が認められ，若年女性への広がりが注目される．

表72.1 尖圭コンジローマのHPV型別と悪性化の有無 (文献[1,2]を改変)

型	頻度	悪性化との関連
6	高	low or no oncogenic risk
11	高	low or no oncogenic risk
16	稀	oncogenic risk
18	稀	oncogenic risk
41	稀	low or no oncogenic risk
44	稀	low or no oncogenic risk
45	稀	oncogenic risk
52	稀	oncogenic risk
56	稀	oncogenic risk

72.3 臨床症状

男性では，亀頭，冠状溝，包皮，肛門周囲，外尿道口に好発し，まれに尿道や膀胱に発生する．女性では，腟，陰唇，肛門周囲，外尿道口に発生するが，子宮頸部にも感染している場合がある．16型などのoncogenic riskを有するHPV感染では，陰茎癌，子宮頸部異形成上皮や子宮頸癌の原因となる．

潜伏期間は1～6か月 (平均3か月) である．病変は，無痛性で，乳頭状あるいは鶏冠状に増殖し，集簇したり多発する傾向がある．接触により出血したり，二次感染にてびらん，壊死となることもある．

72.4 典型的な症例

30歳男性，冠状溝から包皮にかけての多発性の疣状の腫瘤を主訴に受診．明らかな自覚症状はないものの，尿沈渣検鏡で白血球を10/hpf認めたため，念のため*Chlamydia trachomatis*のPCR検査を提出した．PCR検査では陽性であり，後日抗菌薬による治療を行った．腫瘤の視触