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ON THE OCCURRENCE OF GENITAL HERPES SIMPLEX VIRUS INFECTION

Clinical and Virological Findings and Relation to Gonorrhoea

Stig Jeansson and Lars Molin

From the Department of Clinical Microbiology, Section of Virology, and the Department of Dermatology, Karolinska sjukhuset, Stockholm, Sweden

Abstract. In an investigation of genital herpes simplex virus (HSV) infection and gonorrhoea at the Venereal Disease (VD) clinic of Karolinska sjukhuset, 2 506 patients, males and females, were investigated. HSV was isolated from genital excretions of 4.4 % of the males and 5.2 % of the females. From males 96.7 % and from females 88.1 % of the HSV strains were of antigenic type 2. Within non-VD clinic controls, no virus was isolated from 131 males but HSV type 2 was isolated from 0.5 % of the females (2/401). Gonorrhoea was diagnosed in the VD group in 30.3 % of the males and 34.8 % of the females. In patients with gonorrhoea, HSV type 2 was isolated from 1.9 % of males and 5.1 % of females, whereas in those without gonorrhoea, HSV type 2 was isolated with frequencies of 5.3 % and 4.3 % respectively. In patients with simultaneous genital HSV type 2 infection and gonorrhoea the virus infection was inapparent in 63 % of the males and 65 % of the females whereas in cases with genital HSV type 2 infection only, the corresponding figures were 25.5 % and 31.3 %. It is suggested that genital HSV type 2 infection is usually subclinical in individuals with simultaneous gonorrhoea. After primary isolation of HSV type 2 from genital excretions, 44 patients were resampled at weekly intervals. An intermittent excretion of HSV type 2 was found to occur in some patients for several months after the primary isolation of HSV type 2.

Genital HSV infection was described as a venereal disease by Unna in 1883 (26), but it was not accepted as a sexually transmitted disease until the two types of HSV were recognized (22, 23). Clear evidence of sexual transmission has come from reports on patients attending VD clinics (3, 9, 10, 13, 16) and, in particular from studies on the development of infections in sexual contacts of HSV-infected consorts (15, 25).

An intermittent chronic excretion of HSV type 1 is well known to occur in eye and oral infections (7, 12). For the sexual transmission of genital HSV infection the duration and pattern of virus excretion are important, to some extent. These factors have been studied by Centifanto et al. (5, 6).

The purpose of the present investigation was to study genital HSV infection in patients attending a VD clinic, with special regard to inapparent genital HSV infection, the relationship between HSV infection and gonorrhoea, and the duration and pattern of HSV type 2 excretion after virologically verified genital infection.

MATERIALS AND METHODS

Clinical material studied

The specimens were obtained from 1 377 males and 1 129 females attending the Venereal Section of the Outpatient Department of Dermatology at Karolinska sjukhuset, Stockholm, at primary visits, during the period November 1969 to August 1971. The age and sex distribution are given in Table I. The relatively high number of males in the age group 20–24 years is due partly to the function of the VD clinic as a treatment center for military conscripts in addition to civilians.

No males studied were circumcised (1, 21).

It was not possible to investigate social structure and sexual behaviour in detail.

According to the rules applied at the clinic, cases with symptoms of genital infections, including HSV infections, have been taken care of at the VD section.

The age distribution of 532 controls in three different groups is given in Table II.

Group 1. 131 males attending the Outpatient Department of Dermatology at Karolinska sjukhuset, for various common dermatological diseases, but without history symptoms or signs of venereal disease including genital HSV infection.

Group 2. Females from a RFSU (Swedish Association for Sex Education) advisory clinic for birth control, 202 healthy females without history, symptoms or signs of venereal disease including genital HSV infection.

Group 3. 199 females, from the Outpatient Department of Gynaecology at Karolinska sjukhuset, consecutively attending the gynaecology clinic for various gynaecological disorders, representing a very heterogeneous group of patients with regard to state of health. No case of clinical genital HSV infection was found in this group.

All subjects were inspected for genital herpetic lesions and interviewed about the previous occurrence of such lesions.

Table I. *Age and sex distribution of patients from VD clinic*

Sex	Age group (years)						Total
	10–14	15–19	20–24	25–29	30–34	≥35	
Male	0	101	638	352	142	144	1 377
Female	6	331	441	224	64	63	1 129
Total	6	432	1 079	576	206	207	2 506

Collection of specimens

Samples for virus isolation were collected from all patients. In males the samples were taken from the urethra and in females, from the cervix. In addition viral cultures were also taken from suspected herpetic genital lesions. Repeated samples for virus isolation were collected from some of the patients from whom HSV was isolated. From all patients routine cultures for gonorrhoea were made, from the urethra in males and from urethra, cervix and rectum in females (8). In addition all patients were tested for syphilis by routine serological tests.

Virus isolation

The specimens for virus isolation were taken with a sterile, charred, cotton-tipped swab (8). Immediately after sampling the swab was placed in a tissue culture tube with GMK-AH 1 cells. The cells were maintained as previously described (11). The inoculated tissue culture tubes were transported to the virus laboratory within 1 to 5 hours. Maintenance medium was changed one day after inoculation. The tubes were incubated for one week at 35°C and observed at 1 to 2 day intervals for cytopathic effect. In preliminary experiments it was found that HSV was always isolated from specimens within one week after inoculation of GMK-AH 1 cells and that further incubation did not increase the frequency of isolation. Virus isolates were passaged 2–3 times in GMK-AH 1 cells and verified as HSV in complement fixation tests with anti HSV hyperimmune serum from guinea pigs and typed as HSV type 1 and type 2 by immunoelectro-osmophoresis against type specific rabbit sera (11).

Conventional statistical methods were used (4).

RESULTS

Frequency of genital HSV infection and gonorrhoea

The frequency of genital HSV infection as determined by virus isolation and of gonorrhoea in the VD clinic patients and in the controls is shown in Table III. HSV was isolated from 4.4% of the males and 5.2% of the females in the VD group. From 96.7 % of the males and from 88.1% of the females genital HSV strains were typed as HSV type 2.

No virus was isolated from the male controls. In group 2, HSV type 2 was isolated from one female of 51 years and in group 3, from one female of 26 years. Neither of these patients had genital herpetic lesions.

As shown in Table III, gonorrhoea was diagnosed in the VD group in 30.3% of the males and 34.8% of the females and from 1.5% of the controls in group 3. No case of gonorrhoea was found in control groups 1 and 2. The age distribution of the gonorrhoea cases is shown in Table IV and that of HSV type 2 cases in Table V.

In males the highest frequencies of HSV type 2 infection were observed in the age groups 20–24 years and 30–34 years. In females the highest frequency was observed in the age group 25–29 years.

Table II. *Age and sex distribution of controls*

Sex	Control group no.[a]	Age group (years)						Total
		10–14	15–19	20–24	25–29	30–34	≥35	
Male	1	0	33	43	28	15	12	131
Female	2	6	59	77	37	11	12	202
Female	3	0	26	34	29	17	93	199
Total		6	118	154	94	43	117	532

[a] Group 1: Males, patients from the Outpatient Department of Dermatology at Karolinska sjukhuset. Group 2: Females from RFSU (Swedish Association for Sex Education). Group 3: Females, patients from the Outpatient Department of Gynaecology at Karolinska sjukhuset.

Table III. *Frequency of genital HSV infection and gonorrhoea in patients from VD clinic and controls*

Source of specimens	Sex	Group	Percentage with HSV infection[b] Type 1	Type 2	Percentage with gonorrhoea[b]
VD clinic	M		0.1 (2/1 377)	4.3 (59/1 377)	30.3 (417/1 377)
	F		0.6 (7/1 129)	4.6 (52/1 129)	34.8 (393/1 129)
Controls[a]	M	1	0.0 (0/131)	0.0 (0/131)	0.0 (0/131)
	F	2	0.0 (0/202)	0.5 (1/202)	0.0 (0/202)
		3	0.0 (0/199)	0.5 (1/199)	1.5 (3/199)

[a] See footnote to Table II.
[b] Figures in parentheses represent the number of positive cases over the number of cases investigated.

Table IV. *Occurrence of gonorrhoea by age and sex in patients from VD clinic*

Sex	Percentage with gonorrhoea within each age group (years)[a] 10–14	15–19	20–24	25–29	30–34	⩾35	Total
Male	— (0/0)	39.6 (40/101)	32.9 (210/638)	27.8 (98/352)	23.9 (34/142)	24.3 (35/144)	30.3 (417/1 377)
Female	50 (3/6)	38.1 (126/331)	37.9 (167/441)	32.6 (73/224)	14 (9/64)	24 (15/63)	34.8 (393/1 129)
Total	50 (3/6)	38.4 (166/432)	34.9 (377/1 079)	29.7 (171/576)	20.9 (43/206)	24.2 (50/207)	32.3 (810/2 506)

[a] Figures in parentheses represent number with positive gonorrhoeal isolation over number tested.

Table V. *Isolations of HSV type 2 in VD clinic patients with and without gonorrhoea*

	Sex	Percentage positive within each age group (years)[a] 10–14	15–19	20–24	25–29	30–34	⩾35	Total
HSV type 2 infection with simultaneous gonorrhoea	M	—	5 (2/40)	1.9 (4/210)	2 (2/98)	0 (0/34)	0 (0/35)	1.9 (8/417)
	F	0 (0/3)	4.8 (6/126)	6.6 (11/167)	3 (2/73)	0 (0/9)	7 (1/15)	5.1 (20/393)
HSV type 2 infection without simultaneous gonorrhoea	M	—	2 (1/61)	6.1 (26/428)	4.7 (12/254)	6.5 (7/108)	4.6 (5/109)	5.3 (51/960)
	F	0 (0/3)	4.9 (10/205)	2.9 (8/274)	8.6 (13/151)	0 (0/55)	2 (1/48)	4.3 (32/736)
Average incidence of patients with HSV type 2 infection	M	—	3.0 (3/101)	4.7 (30/638)	4.0 (14/352)	4.9 (7/142)	3.5 (5/144)	4.3 (59/1 377)
	F	0 (0/6)	4.8 (16/331)	4.3 (19/441)	6.7 (15/224)	0 (0/64)	3 (2/63)	4.6 (52/1 129)

[a] In parentheses is given the number of cases with positive virus isolation over the number of cases investigated.

Table VI. *Clinical findings and occurrence of gonorrhoea in patients from VD clinic from whom HSV type 2 was isolated*

Clinical findings	Males		Females		Total	
	Number of patients[a]	%[b]	Number of patients	%	Number of patients	%
No herpes lesions / No anamnesis of herpes	18 (5)	31	23 (13)	44	41 (18)	36.9
Suspect herpes lesions / No anamnesis of herpes	15 (1)	25	7 (2)	13	22 (3)	19.8
Typical herpes lesions / No anamnesis of herpes	17 (2)	29	17 (3)	33	34 (5)	30.6
Typical herpes lesions / Anamnesis of herpes	9 (0)	15	5 (2)	10	14 (2)	12.6
Total	59 (8)	100	52 (20)	100	111 (28)	100.0

[a] Figures in parentheses represent number of patients with positive gonorrhoeal isolation.
[b] Percentage of total number of males or females from whom HSV type 2 was isolated.

Relationship between genital HSV infection and gonorrhoea

No single case of HSV type 1 infection was found when 417 males and 393 females with gonorrhoea were examined. Among 960 males and 736 females without gonorrhoea, 0.2% of the males and 1.0% of the females had a HSV type 1 infection.The difference between the occurrence of HSV type 1 in patients with and without gonorrhoea was not significant.

The frequencies of isolation of HSV type 2 from patients with and without simultaneous gonorrhoea are shown in Table V. A significantly higher frequency of HSV type 2 infection was found in males without gonorrhoea than with gonorrhoea ($0.01 > p > 0.001$). In females no significant difference was found in the corresponding groups.

The occurrence of HSV type 2 and gonorrhoea in relation to the age of VD clinic patients is also presented in Table V. In patients with gonorrhoea the highest frequencies of genital HSV type 2 infection were in males 15–19 years of age and in females over 35 years of age, whereas in patients without gonorrhoea, the highest frequencies were in males 30–34 years of age and in females 25–29 years of age. The difference in the frequencies of isolation of HSV type 2 from male patients over 30 years with and without gonorrhoea was not significant.

Relationship between clinical, virological and bacteriological findings

Table VI summarizes virological and clinical findings in VD clinic patients from whom HSV type 2 was isolated. The occurrence of gonorrhoea at the time of examination is also given. The occurrence of inapparent virus infection in patients with genital type 2 infection in relation to the occurrence of gonorrhoea can be calculated from the data given in Table VI. In patients with gonorrhoea the frequency of inapparent virus infection was 63% (5/8) in males and 65.0% (13/20) in females. The corresponding frequency of inapparent virus infection in patients without gonorrhoea is 25.5% (13/51) in males and 31.3% (10/32) in females. There is no significant difference in the frequency of inapparent genital type 2 infection between males with and without gonorrhoea, whereas in females this difference is almost significant ($p < 0.05$).

Duration and pattern of excretion of HSV type 2 from the genital tract

The pattern of virus excretion is illustrated in Fig. 1. Virus was recovered from 13 out of 34 patients sampled 1 to 2 weeks after primary virus isolation. Of patients sampled 5 weeks or more after the primary isolation, virus was isolated from 6 out of 16 cases. One female was continuously postive in 12 samples taken during a period of 7 weeks after the

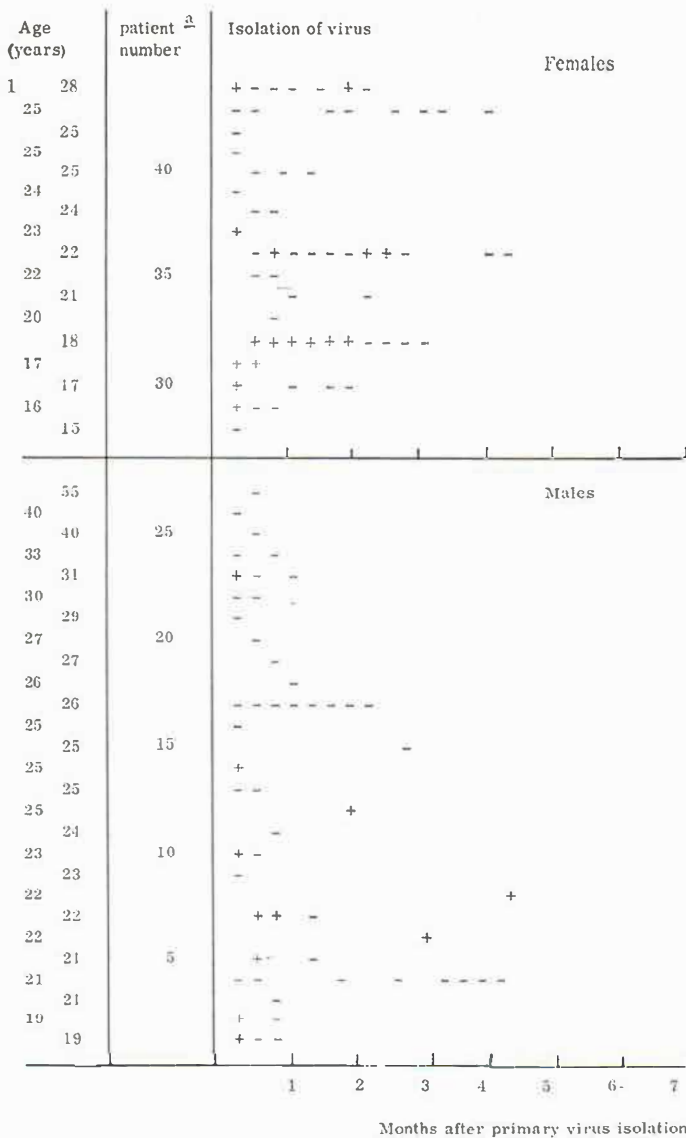

Fig. 1. Pattern of virus isolation from the genital tract of men and women after primary isolation of HSV type 2. Repeated samples for virus isolation were collected from some of the patients from whom HSV type 2 had been isolated. Samples were collected at intervals, often weekly. From a few patients more than one sample was collected within one week. In the figure this is only indicated as one sample. If virus was isolated from one or more of the samples collected within one week the result of virus isolation is illustrated as only one positive isolation. (*a*) The patient number identifies each individual patient. − = negative; + = HSV type 2 isolated.

initial virus isolation. This patient had simultaneously gonorrhoea, syphilis and hepatitis type B. From 4 out of 11 patients, 2 males and 2 females, an intermittent excretion of virus occurred 2 months or more after the primary virus isolation.

DISCUSSION

Nahmias & Roizman (17) reported that 96.5% (141/146) of HSV strains isolated from the genital tract from males and 91.0% (229/252) of these isolated from females were of antigenic type 2. These figures are in good agreement with the data from the present investigation (96.7% from males and 88.1% from females). In Table VII the frequencies of genital HSV infection found in several investigations are given. Rawls et al. (24) isolated HSV only from males with herpetic penile lesions. Jeansson & Molin (9) reported a high frequency of inapparent genital HSV infection in both sexes.

In a study of middle-aged and elderly males, Centifanto et al. (6) very frequently isolated HSV from urethral swabs; the virus infection was inapparent in all patients. However, a clear diagnosis was given only for 20 cancer patients, 4 of whom excreted HSV. In a study of a small group of patients

Table VII. *Frequency of genital HSV infection in different investigations*

Symbol: — = not recorded

	Percentage of HSV infection[a]		Typing of HSV strains	Clinic
	Males	Females		
Beilby et al. (3)	—	3.8 (8/209)	not typed	VD
Kleger et al. (13)	—	1.6 (8/494)	not typed	VD
Rawls et al. (24, 25)	2.0 (4/198)	1.8 (4/220)	typed	VD
Nahmias et al. (15)	0.3 (17/5 537)	5.7 (31/548)	typed	VD
Jeansson & Molin (9)	5.4 (7/130)	8.0 (10/125)	not typed	VD
Nahmias et al. (16)	—	5.3 (7/132)	typed	VD
Present investigation	4.4 (59/1 377)	5.2 (52/1 129)	typed	VD
Morriseau et al. (14)	17 (2/12)	—	typed	Urology
Centifanto et al. (6)	7.6 (11/144)	—	some strains typed	Urology
Nielsen & Vestergaard (19)	0 (0/48)	—	—	Urology

[a] Figures in parentheses represent number with positive virus isolation over number tested.

with acute prostatitis and or epididymitis, Morriseau et al. (14) isolated some HSV type 2 strains. Attempts by Nielsen & Vestergaard (19) to isolate virus from males with chronic prostatitis and/or chronic urethritis yielded negative results.

The inapparent genital HSV infection in males visiting VD clinics has been studied by Jeansson & Molin (9) and in 4 out of 7 cases the infection was found to be in-apparent. In the present study 30.5% of type 2 infections in males were non-apparent. In males with simultaneous gonorrhoea and HSV type 2 isolation, the virus infection was inapparent in 63% (5/8).

Jeansson & Molin (9), and Ng et al. (18) have suggested that type 2 infection is often in-apparent in females. In a study of 209 females from a VD clinic, most of them without external genital herpetic lesions, Beilby et al. (3) found that 13% (7/54) with and 0.6% (1/155) without gonorrhoea had genital HSV infection. Nahmias et al. (16) studied 7 females with type 2 infection. The virus infection was in-apparent in 4 with gonorrhoea, while 3 without gonorrhoea had herpetic lesions. Our data suggest that in females with simultaneous type 2 infection and gonorrhoea the virus infection is to major a extent inapparent (Table VI). In our study of males the available data were insufficient for any conclusions to be drawn.

In females, inapparent genital infection with HSV has been studied by Centifanto et al. (5). In their study, 3 females from whom genital HSV had been isolated but not typed were followed for a period of about 6 months with repeated cultures for virus isolation. HSV was isolated from all 3 patients and in 7.7% (47/614) of the specimens. In a similar investigation of 44 patients with verified type 2 infection we could demonstrate in 4 individuals an inapparent excretion of virus 2 to 5 months after the first virus isolation. These data seem to indicate that after primary infection, a chronic inapparent and intermittent excretion of HSV type 2 occurs in both sexes.

For HSV type 1 it is well known that a latent virus infection can be established in sensory nerve cells (20) and virus has been isolated from the trigeminal nerve ganglion (2). As an explanation of the chronic intermittent excretion of virus from genitalia a similar type of mechanism might be postulated.

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S. Jeansson, M.D.
Department of Clinical Microbiology
Section of Virology
Karolinska sjukhuset
S-104 01 Stockholm 60
Sweden