

# Herpes Simplex Virus (HSV) Infection in Men with Genital Ulcer Disease (GUD)—as Observed in Dhaka Medical College Hospital

Mamun SA<sup>1</sup>, Chowdhury MAH<sup>2</sup>, Khan RM<sup>3</sup>,  
Sikder MAU<sup>4</sup>, Hoque MM<sup>5</sup>.

## Abstract

*Genital herpes clinically underestimated because symptoms or sign occur only in some infection detected serologically. Prevalence of HSV subtypes in microbial etiology of Genital Ulcer Disease (GUD) in men, their association with clinical sign, complex of GUD and high-risk behavior were assessed. One hundred men with first episodes of genital ulcers were prospectively studied for serological evidence of syphilis (RPR and TPHA; T.pallidum IgM and IgG antibodies) and Polymerase Chain Reaction (PCR) proven chancroid and herpes. Demographic and epidemiological data were obtained in a standard interview. Positive syphilis serology observed in 11 cases, H. ducreyi detected in 65 cases and Herpes Simplex Virus in 13 cases. Among the PCR proven infections HSV type-2 detected in 7 cases, HSV type-1 in 4 cases and both HSV type-1 & 2 in 2 cases. Most of the HSV infections (92.3%) found as mixed infection with H. ducreyi. There was one PCR detected genital herpes case that was clinically undetermined. Among the PCR proven HSV infections clinical sign complex of genital herpes observed in one case, which had mixed microbial etiology. HSV infection was more prevalent in married than unmarried men (25.0% vs. 8.3%; P<0.05) and associated with early age promiscuous activity, multiple sexual partner, and sex with commercial sex workers and past infection with STDs. Presence of underdiagnosed HSV infection in men with GUD stress on the need for clinical suspicion of multiple infections. Patient with GUD should be carefully evaluated for HSV infection.*

## Introductions

Genital herpes is a contagious recurrent infection caused by the herpes simplex virus (HSV) of which there are two subtypes HSV-1 and HSV-2. The infection is usually acquired by sexual contact and like other genital ulcer diseases increases the risk of HIV transmission and acquisition<sup>1-2</sup>. Genital herpes clinically underestimated because symptoms or sign occur in less than 40% of the infections detected serologically<sup>3</sup>. Around 90-96% HSV-2 seropositive women cannot recognize the initial infection<sup>4</sup>. Although the virus spread through contact with lesion or secretion but individuals those never have any symptoms and do not know that they are infected with the HSV can also transmit the virus to others<sup>5</sup>. Because the asymptomatic shedding of small amount of virus at the site of infection, in genital or oral secretion or from inapparent lesion may be fairly common but is sufficient to infect a sex partner<sup>6-7</sup>. The genital herpes lesions appear as small red bumps that may develop into blisters. Over a period of days they become encrusted, form a scab and eventually heal without scarring. These manifestations are hardly present when patient seek treatment. Data collected in STD clinics in USA revealed that 80% of HSV-2 infections were unrecognized forms<sup>8</sup>.

Therefore the study was conducted to determine the prevalence of HSV subtypes in microbial etiology of GUD in men, their association with clinical sign complex of GUD and high-risk behavior.

## Methods

**Patients:** The study was carried out in the skin & VD outpatient department at Dhaka Medical College hospital and at the Laboratory science division of ICDDR, B, Dhaka. One hundred consecutive men with genital ulcers were included in the study. Each patient was ensured confidentiality and anonymity and asked to participate in the study. Demographic and epidemiological data were obtained in a standard interview.

**Clinical etiological diagnosis:** All patients underwent a physical examination of the external genitalia and inguinal

1. Shameem Al Mamun  
Skin and VD specialist, Dhaka Mohanagar General Hospital.
2. M Abu Hena Chowdhury  
Assistant Professor, Department of Dermatology and Venereology,  
Banga Bandhu Sheikh Mujib Medical University.
3. Rashed Mohammad Khan  
Associate Professor, Department of Skin and VD  
Sir Salimullah Medical College.
4. Md. Akram Ullah Sikder  
Professor, Department of Dermatology and Venereology,  
Banga Bandhu Sheikh Mujib Medical University
5. M Mujibul Hoque, Professor and Ex. Head of the Department,  
Department of Skin and VD, Dhaka Medical College Hospital.

region before sample for laboratory analysis were taken. The clinical, etiological diagnosis was made prior any knowledge of the laboratory result. Following criteria were used-a painless indurated clean based ulcer was considered as syphilis, a deep undermined purulent tender ulcers as Chancroid. Genital herpes was clinically diagnosed when multiple grouped vesicles were present or when ulcers were very superficial and tender. Genital scabies pyogenic ulcer when lesions were extremely itchy, multiple, purulent, tender and/or burrow present especially on the genitalia and adjacent area and characteristics distribution of scabies lesion elsewhere in the body. Donovanosis was considered when the lesion had a beefy aspect. When clinical feature did not corresponds to one of these criteria the clinical diagnosis was classified as undetermined. No attempts were made to clinically identified other causes of GUD or mixed infection.

**Laboratory diagnosis:** Samples were collected from the ulcer base after cleaning the lesion with sterile gauge and back of the haemostylet and 5ml of venous blood were collected for syphilis serology. Total and differential WBC counts were performed in all cases.

Detection of *H. ducreyi* by PCR technique: a dacron tipped swab was subsequently rubbed against the ulcer base and stored in a sterile 1 molar phosphate buffered saline solution (PBS; 50mM sodium phosphate, 0.15M sodium chloride; pH 7.5), but without sodium chenodeoxycholate<sup>9</sup> and stored at -70°C until shipped to the laboratory. Three different primers were used for the detection of the *H. ducreyi* genome<sup>10-12</sup>. Only sample giving a positive result with three primers were considered as being positive.

Detection of herpes simplex virus by PCR technique: detection and typing of herpes simplex virus was performed with two different<sup>13</sup> primers. Only samples giving a positive result with two primers were considered as being positive and further typing of herpes simplex virus was done according to Kimura<sup>14</sup>.

Serology for syphilis: for the diagnosis of syphilis, serum specimens were tested with the rapid plasma reagin test (RPR nosticon, Organon Teknika, Turnhoul, Belgium) which was titrated till the end point, as well as with the *T.pallidum* haemoagglutination assay (TPHA nosticon, Organon, Teknika). *T. pallidum* IgG and IgM antibodies were detected with an ELISA technique (Treponema pallidum IgM EIA, Treponema pallidum IgG comfort EIA, Meddens Diagnostics, Brummen, The Netherlands). Patients were considered as having primary syphilis if the RPR was reactive in the presence of a positive TPHA test and in all

cases where IgM antibodies to *T. pallidum* were diagnosed. All patients with a positive IgM test were screened for the presence of the rheumatoid factor (serodia-RA, Fujinebio, Tokyo, Japan).

Detection of *C. donovani*: with the back of the haemostylet smear from the ulcer was prepared for specific staining and microscopy.

HIV testing: HIV testing was unlinked and anonymous. Serum samples were tested with an enzyme-linked immunosorbent assay (ELISA) (Vironostika HIV-mixt, Organon Teknika) and positive results were confirmed by a line immunoassay (LIA tek HIV III, Organon Teknika). Quality control was carried out using standard quality control sera for HIV provided by the National Reference Centre for HIV/AIDS, Christian Medical College, Vellore, India.

Microscopic detection of the scabies mite was not performed since it is not expected to be fruitful in scraping of heavily infected lesion.

#### Statistical Analysis

The Pearson chi-square and the Yate's corrected chi-square test (when appropriate) were applied to assess the differences in proportions for statistical significance. Independent t test was done to measure difference between the means for significance. The statistical analysis was performed using the statistical software SPSS version 10.1.

#### Results

**Socio-demographic Characteristics:** summarized in Table-1. The average age of the sampled GUD population was  $24.7 \pm 6.8$  years and most of them belonged to 16-25 years age group, on the other hand HSV more frequently detected in 21-25 years age group and the mean age of the HSV infected GUD patient was  $23.9 \pm 5.0$  years. Most of the GUD patients were unmarried (72%) where most of the HSV infected men were married (53.8%) and HSV prevalence was also high in married than unmarried GUD patients (25.0% vs. 8.3%). Civil status and HSV infection showed significant relation ( $P=0.046$ ). A large number of GUD patients (54) were least educated (0-5 years) but HSV detected more in educated persons and HSV prevalence was also high in those have 11-15 years education (29.4%). As like GUD patients many HSV infected patients were worker or laborer but HSV infection was more prevalent in businessmen (4/16, 25.0%). Most of the GUD patients belonged to poor socioeconomic class (61%), in contrary a large proportion of HSV infected GUD patients represented

**Table-1:** Socio- demographic Characteristics of GUD Patients and patients harboring HSV infection in DMCH, Dhaka, Bangladesh.

Characteristics	GUD Population		HSV harboring Infection		HSV Prevalence in GUD Population (%)
	N = 100	%	N = 13	%	
Age group					
16 – 20 Years	30	30.0	3	23.1	3.3
21 – 25 Years	38	38.0	6	46.2	15.8
26 – 30 Years	19	19.0	3	23.1	15.8
> 30 Years	13	13.0	1	7.7	7.7
Average Age (Mean ± SD)	24.7 ± 6.8 Years		23.9 ± 5.0 Years		P = 0.693
Civil Status					
Single	72	72.0	6	46.2	8.3
Married	28	28.0	7	53.8	25.0
Education (Years)					
0	27	27.0	3	23.1	11.1
1 - 5	27	27.0	2	15.4	7.4
6 - 10	27	27.0	3	23.1	11.1
11 - 15	17	17.0	5	38.5	29.4
> 15	2	2.0	0	0.0	0.0
Occupation					
Govt. / Non Govt. Service	20	20.0	2	15.4	10.0
Business	16	16.0	4	30.8	25.0
Worker/Labourer	51	51.0	6	46.2	11.8
Student	8	8.0	1	7.7	12.5
Unemployed	5	5.0	0	0.0	0.0
Socio-economic status					
Lower	61	61.0	5	38.5	8.2
Middle	34	34.0	6	46.2	17.6
Higher	5	5.0	1	7.7	20.0

from middle socioeconomic class (46.2%) and HSV prevalence was more in higher socioeconomic class (20%).

**Clinical diagnosis:** Illustrated in Figure-1. The diagnosis based on clinical sign complex was genital scabies (n=49), chancroid (n=30), primary syphilis (n=12), genital herpes (n=3) and undetermined (n=6).

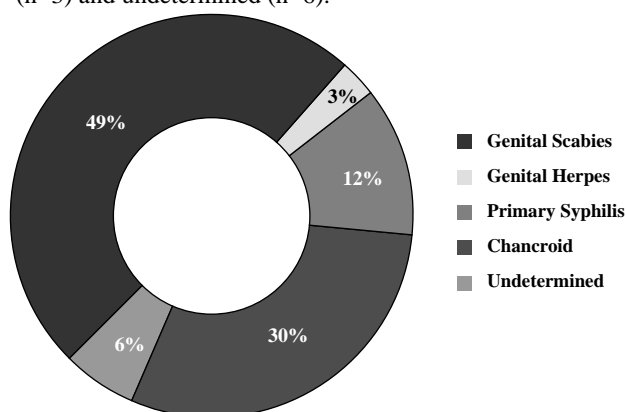


Fig. 1 Clinical diagnosis of 100 GUD Patients in DMCH, Dhaka, Bangladesh.

**Laboratory diagnosis:** presented in Table 2. A definite microbial etiology of 89 infections was found in 73 patients. Single infection observed in 58 cases and mixed infection in 15 cases. There were 11 serology proven syphilis, single syphilis infection was detected in 7 cases. H. ducreyi

detected in 65 cases, among them 50 had single chancroid infection. Herpes simplex virus was discovered by PCR in 13 cases but single herpes infection observed in one case. The 27 cases of GUD with no laboratory diagnosis included the 17 cases of clinically diagnosed genital scabies. These 17 cases had the highest eosinophil count (8.6±1.2) compared to others (4.1±1.7).

Table-2: Laboratory diagnosis of 100 GUD Patients in DMCH Dhaka.

Diagnosis	Microbial etiology		Single infection N = 58	% N = 100
	N=89	%		
Syphilis	11	11.0	7	7.0
Chancroid	65	65.0	50	50.0
Genital herpes	13	13.0	1	1.0
Mixed infection N = 15				
Syphilis, chancroid			3	3.0
Chancroid, herpes			11	11.0
Syphilis, chancroid, herpes			1	1.0
No diagnosis			27	27.0
<b>Total</b>			<b>13</b>	<b>100.0</b>

**HSV infection in GUD patients:** Presented in Figure-2. Among the 13 PCR proven HSV cases Herpes simplex virus type-2 detected in 9 cases and type-1 in 6 cases (two patients harbored both type -1 and type-2 infection).

**Etiological Presentation of HSV infection:** Summarized in Table-3. Most of the HSV infection (92.3%) found as mixed infection, *H. ducreyi* was present in all HSV mixed infections and positive syphilis serology in single case.

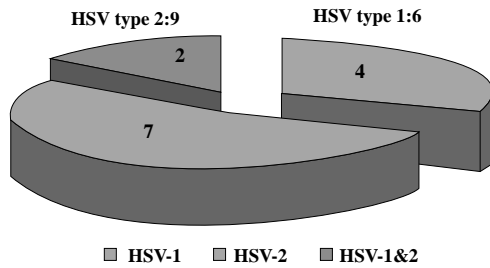


Figure-2: HSV type detected in genital ulcers, DMCH, Dhaka.

Finally the distribution of HSV infection in GUD patients were single genital herpes type-1 (n=1), HSV type-1 and chancroid (n=3), HSV type-2 and chancroid (n=6), HSV type-1 and type-2 and chancroid (n=2) and HSV type-2, chancroid and syphilis (n=1).

Table-3. Etiological Presentation of HSV infection in DMCH.

Diagnosis	N	%
<b>Single Infection N = 1</b>		
HSV type-1	1	7.7
<b>Mixed Infection N = 12</b>		
HSV type-1 and Chancroid	3	23.1
HSV type-2 and Chancroid	6	46.1
HSV type-1 & 2 and Chancroid	2	15.4
HSV type-2, Chancroid and Syphilis	1	7.7
<b>Total</b>	<b>13</b>	<b>100.0</b>

**Clinical Presentation of Genital Ulcers in Patient harboring HSV infection:** Presented in Table-4. Deep undermined purulent ulcers observed in 5 cases, these patients were clinically diagnosed as chancroid. In concordance with clinical diagnosis chancroid as a mixed infection was present in all cases, moreover positive syphilis serology observed in one case. The distribution of HSV was HSV type-2 (n=4) and HSV type-1 (n=1). Multiple purulent tender ulcers with scabies lesion in adjacent area and elsewhere in the body were observed in 4 cases.

Table-4. Clinical Presentation of Genital Ulcers in Patient harboring HSV infection.

Clinical Presentation	N	%
Deep undermined purulent ulcer	5	38.5
Purulent tender ulcers with scabies lesion	4	30.8
Clean based indurated ulcer	1	7.7
Very superficial erosion	1	7.7
Undetermined *	2	15.4
<b>Total</b>	<b>13</b>	<b>100.0</b>

These patients were diagnosed as genital scabies pyogenic ulcers but concomitant HSV infection and chancroid was observed in all cases. The distribution of HSV was HSV type-1 (n=2), HSV type-2 (n=1) and HSV type-1&2 (n=1). Clean-based indurated ulcer was observed in one case, which was clinically diagnosed as primary syphilis but laboratory diagnosis confirmed mixed infection of HSV type-2 and *H. ducreyi* and syphilis serology was negative. The clinical presentation of genital herpes ulcers (very superficial ulcers) was present in one case, this patient harbored concomitant HSV type-2 and *H. ducreyi* infection. The only single HSV type-1 infection was clinically undetermined.

**HSV infection and high-risk behavior:** Depicted in Table-5.

The sampled GUD populations have had their first sexual experience at age average  $19.2 \pm 3.1$  years. Most of the HSV infected GUD patients gained their first sexual experience before aged 20 years (92.3%) and average age at first sexual experience was  $17.9 \pm 1.8$  years. HSV prevalence was also high among this age group (15-20 years) of sampled GUD populations. Like sampled GUD populations (74%) comparatively a large proportion of HSV infected GUD patients (92.3%) have had multiple sexual partners and HSV was more prevalent in sampled GUD populations having multiple sexual partners. Only one HSV infected patient that had single sexual partner was unmarried and harboring HSV-1 infection presented with clinically undetermined genital ulcer. In previous year the sampled GUD population purchased sex averaged  $3.4 \pm 4.3$  times while HSV infected GUD patients purchased sex averaged  $5.9 \pm 5.0$  times, the difference was not statistically significant ( $P=0.053$ ). An ascending HSV prevalence observed among the sampled GUD patients in relation to number of purchasing sex in previous year. Comparatively a large proportion of the HSV infected GUD patients indicated sex workers as source of infection (76.9%) and 46.2% gave history of sexually transmitted infections. Previous STDs showed significant association with genital herpes infection (Corrected chi-square: 4.887,  $P = 0.027$ ).

Table-5: High-risk Characteristics of GUD Patients and patients harboring HSV infection in DMCH, Dhaka, Bangladesh.

Characteristics	GUD Population		HSV harboring Infection		HSV Prevalence in GUD Population (%)
	N = 100	%	N = 13	%	
<b>Age at First Sexual Experience</b>					
15 – 20 Years	76	76.0	12	92.3	15.8
21 – 25 Years	21	21.0	1	7.7	4.8
> 25 Years	3	3.0	0	0.0	0.0
Average Age (Mean ± SD)	19.2 ± 3.1 Years		17.9 ± 1.8 Years		P = 0.138
<b>Sexual Partner</b>					
No	15	15.0	0	0.0	0.0
Single	11	11.0	1	7.7	9.1
Multiple	74	74.0	12	92.3	16.2
<b>Sex in Exchange of Money</b> (Times in Previous Year)					
0	38	38.0	2	15.4	5.3
1 – 5	40	40.0	6	46.2	15.0
6 – 10	15	15.0	2	15.4	13.3
> 10	7	7.0	3	23.1	42.9
Average (Mean ± SD)	5.9 ± 5.0 Times		3.4 ± 4.3 Times		P = 0.053
<b>Sex Worker as Source of Infection</b>	47	47.0	11	84.6	23.4
<b>History of STDs</b>	16	16.0	6	46.2	37.5

#### Discussion:

HSV type-1 is primarily a non-genital pathogen account for the etiology of twenty percent of the herpes genitalis while HSV-2 as a predominant type of genital HSV responsible for 80% of the genital herpes. Although HSV-2 remains predominant type of genital herpes but an increasing proportion of HSV-1 is observed in this study, which is in contrast to the western text but full concordance with the six-year study of the incidence of herpes in a central Kentucky medical center where a trend toward increasing proportion of HSV-1 genitalis was observed<sup>15</sup>.

The clinical diagnostic outcome of genital herpes was very poor. None but one had the clinical presentation of HSV lesion and almost all had another microbial etiology. In these cases herpes simplex virus may secondarily invade the preexisting genital ulcers without altering the clinical sign complex of primary lesion. On the other hand the spontaneous resolution of genital herpes ulcers prevents many patients from seeking treatment. Perhaps these patients come to the OPD with concomitant infection that does not heal inherently and difficult to recognized clinically. It should be noted that high prevalence of chancroid was found in the study and all HSV harboring mixed infection contained chancroid etiology, it might be that chancroid ulcer enhance HSV transmission or vice versa. The strong association between HSV infection and chancroid clearly indicate that if chancroid exists predominantly, unrecognized form of genital HSV infection

would persist concurrently. As we have no previous data on microbial etiology of GUD, it is difficult to conclude whether the relative prevalence of genital HSV infection is increased or not.

The number of single herpes infection was too small for evaluation, so association of genital herpes with high-risk variables cannot be assessed independently. Early age promiscuous activity, multiple sexual partner, sex with commercial sex workers and past infection with STDs; all these high-risk variables were more prevalent in HSV harboring GUD patients when their prevalence in total GUD sample compared. In this study comparatively high prevalence of HSV infection was observed in married men. Women married to these men are at high risk for acquiring HSV infection. The consequence of HSV infection on reproductive health is well documented. Evidence suggests that cervical intraepithelial dysplasia (CIN 3) is more frequent among women who had a past history of herpes genitalis<sup>16</sup>. Recently it was found that HSV-2 infection in conjunction with human papillomavirus (HPV) infection increase the risk of cervical carcinoma in women<sup>17</sup>. HSV-2 modulates the susceptibility of human bladder cells to uropathogenic bacteria, thus increase the chance of superinfection of lower urinary tract<sup>18</sup>. HSV infection in pregnant women showed worst outcome with increased risk of miscarriage, decreased fetal growth, and preterm labor. Of infants infected with herpes at birth, 30-60% die within the first month and survivors may have long term complication such as mental retardation and seizure<sup>19</sup>.

Apparently underdiagnosis of genital herpes is a minor problem, since adequate treatment is not available or is too expensive for routine use in developing country like Bangladesh but case suspicion and counseling of the patient regarding risk of HSV transmission as well as HIV acquisition is extremely desirable as many continue unprotected sexual activity despite symptoms when the risk for transmission is highest. Genital herpes like other GUDs facilitate heterosexual transmission of HIV; which is a persistent global health problems. Bangladesh is a high prevalent country for STDs and is still a low prevalent country for HIV therefore the total absence of HIV that observed in the study is not unlikely though it contrasts strongly to the high HIV prevalence among patients with GUD in other parts of the world<sup>20-25</sup>. However we should kept in mind Bangladesh is sitting on the epicenter of high HIV prevalent countries (India, Myanmar and Thailand) on the other hand social factors active in the spread of HIV and STDs are consistently present, HIV/AIDS if break out in epidemic form this contrast will not exist anymore. As herpes is recurrent in nature and there is no cure for it, the chance of HIV acquisition is several times high in patients with genital herpes. Increased awareness of genital herpes along with public health efforts to implement prevention in high-risk populations will be critical to prevent ongoing transmission of genital herpes.

- 1 Clinical diagnosis of genital herpes is difficult because of lack of manifestation to satisfy the diagnostic criteria.
- 1 Presence of underdiagnosed HSV infection in men with GUD stress on the need for clinical suspicion of multiple infections.
- 1 Patient with GUD should be carefully evaluated for HSV infection.

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