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Original Research Article

Occurrence of genital ulcer due to herpes simplex virus type-1 (HSV-1) in attendees of a regional centre for sexually transmitted infections, central India: how disturbing is it?

Thenmozhi P.[1], Jayesh I. Mukhi[2], Manisha K. Sharma[1], Kalindi S. Deogade[1], Sonali S. Gosavi[1], Vandana A. Agarwal[1]*

[1]Department of Microbiology, [2]Department of Skin and VD, GMCH, Nagpur, Maharashtra, India

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***Correspondence:**
Dr. Vandana A. Agarwal,
E-mail: agarwal.gmc@gmail.com

ABSTRACT

Background: Genital herpes is caused predominantly by Herpes simplex virus type 2 (HSV-2) and less commonly by HSV-1. Genital HSV-1 infection results from oral sex, with fewer recurrences, mild symptoms, less asymptomatic shedding and poor genital transmission. The present study was undertaken to identify frequency of occurrence of HSV-1 and HSV-2 in genital herpes by microscopy, serology and Real Time Polymerase Chain Reaction (RT-PCR).
Methods: Genital ulcer swabs and serum were collected at regional STI centre, Govt Medical College and Hospital, Nagpur. A total of 53 patients of Genital Ulcer Disease from December 2020 -22 were examined for etiology by microscopy, serum IgM and IgG against HSV-1 and 2 by ELISA and HSV-1 and 2 DNA by RT- PCR.
Results: Out of 53 genital swabs processed, 6 (11.3%) and 28 (52.8%) samples were positive for HSV-1 and 2 DNA respectively. Of the 6 HSV-1 DNA positive samples, seropositivity for HSV-1 IgM was in 2 (33.3%) samples and for HSV-1 IgG in 4 (66.7%) samples. Of the 28 HSV-2 DNA samples, HSV-2 IgM was positive in 4 (14.3%) samples and HSV-2 IgG was positive in 7 (25%) samples, multi nucleated giant cells were seen in 2 (7.14%) samples. The remaining 15 (53.6%) HSV-2 DNA positive samples were seronegative.
Conclusions: HSV-1 was detected in 6 (11.3%) samples. Though these genital ulcers may be mild, it is important to counsel the patients for abstinence or safe sex practices to prevent their partners from acquiring painful non-genital ulcers due to HSV-1.

Keywords: Genital ulcer, HSV-1, HSV-2, RT-PCR

INTRODUCTION

The acquisition of herpes simplex virus type-1 (HSV-1) in genital herpes with prior herpes simplex virus type-2 (HSV-2) infection is unusual, but HSV-2 acquisition in the occurrence of previous HSV-1 infection is common.[1-2] Initially there was a credence that HSV-1 causes oro labial lesions and HSV-2 causes genital lesions. However now studies suggest that not only has the prevalence of HSV-1 increased in genital lesions but it has even exceeded that of HSV-2 in certain regions.[3] HSV 1 and 2 both can cause clinically indistinguishable multiple lesions or ulcers at oro genital and oro-labial sites. The recent increase in isolation of HSV-1 from genital lesions of herpes is probably because of greater frequency of sexual practices of fellatio and cunnilingus.[4]

The classical clinical indicators of recurrent genital herpes by HSV-2 include multiple trivial, clustered, neuropathic painful, vesicular or erythematous lesions in the genital region also appreciated in the extensive genital area from recurrent itchiness, redness or burning sensation to painful blisters, sores and including the groin, buttocks, and thighs; the lesions may recur at the same or different sites.[5-7] Genital HSV-1 infection results from oral sex, with fewer recurrences, mild symptoms, less asymptomatic shedding and poor genital transmission.[8-9] This is in contrast to primary oro facial HSV-1 infections which are recurrent, painful and highly infective.

Hence the present study was undertaken to find the frequency of occurrence of HSV-1 and HSV-2 as etiological agents of genital herpes in our region.

METHODS

The present study was conducted at Government Medical College (GMC) and Hospital, Nagpur which is a regional centre for the diagnosis, treatment, control, training and research for STI in central India. The centre is sponsored by Maharashtra States AIDS Control Society (MSACS), Mumbai. The Institute is running STI clinics in the Departments of Dermatology and Venereology and Obstetrics and Gynaecology. The RSTRRL (Regional STI Training, Research and Referral Laboratory) in the Department of Microbiology is receiving samples for etiological diagnosis of STIs.

A cross sectional study was conducted from December 2020 to December 2022, a total of 53 genital ulcer swabs were processed for detection of HSV-1 and 2 DNA by Real Time Polymerase Chain Reaction (RT- PCR) in RSTRRL, GMCH, Nagpur. Male and female patients presented with genital lesions and extra genital lesions (on buttocks, thighs or penile fissures/atypical lesions) were included as study participants in our study. The only exclusion criteria was the patients who were not willing to give blood samples.

Ethical clearance for the study was obtained from the Institutional Ethics Committee. Permission to conduct the study was sought from the respective departments. Informed consent was obtained from the patients before enrolment into the study.

Sample collection, transportation and storage

Two swabs from the genital ulcer/lesion and 5mL of blood were collected as per standard protocol.[10-11] One genital ulcer swab was transported in a sterile tube for immediate processing in the laboratory. The second swab was transported in viral transport medium maintaining cold chain for processing. The second swab and the serum samples were both stored at -20^0 C for further processing.

Processing of the sample

Microscopy

Smear was prepared from the first swab, Giemsa stained and examined microscopically for the presence of multi nucleated giant cells.[12]

Serological test

IgM and IgG antibodies to HSV-1 and HSV-2 were detected in serum by HSV-1 and HSV-2 ELISA based kits (Nova Tec Imunodiagnostica GmbH, Germany) using the corresponding recombinant antigens gG1 for HSV type 1 and gG2 for HSV type 2.

HSV-1 and 2 DNA detection by real time -polymerase chain reaction (RT- PCR)

Patho Detect™ Real time PCR (qPCR) protocol of My Lab discovery solutions was used for an *in vitro* detection of HSV 1 and 2 DNA in clinical samples. One step real time PCR with Taqman fluorogenic probe chemistry (uses 5´nuclease activity of Taq DNA polymerase) enabled the detection of a specific PCR product during PCR cycles, viz FAM - fluorescein amidities, important synthetic equivalents of fluoroscein dye used in oligonucleotide synthesis. VIC - Victoria. VIC has been developed after the modifications of Aequorea victoria green fluorescent protein, an asymmetric xanthene dye with fluorescence in the yellow-green part of the spectrum, for labelling real-time PCR probes.

RESULTS

Out of 53 swabs collected, 42 (79.2%) were from male and remaining 11 (20.8%) were from female patients (Table 1). The age group distribution of 53 patients was 30 (56.6%) patients in the age group 20-29 years, 11 (20.7%) in 30-39 years, 8 (15.1%) in 40-49 years, and 2 (3.8) patients in both 10-19 years and more than 50 years (Table 1). Among the study population farmers and daily wagers were 34 (64.2%), 10 (18.9%) were professional workers and 9 (16.9%) were students (Table 1).

Table 1: Demographic characters in GUD patients.

Demographic characters		N (%), n = 53
Gender	Male	42 (79.2)
	Female	11 (20.8)
Age distribution in years	0-9	0 (0)
	10-19	2 (3.8)
	20-29	30 (56.6)
	30-39	11 (20.7)
	40-49	8 (15.1)
	>50	2 (3.8)
Occupation	Farmers+ daily wagers	34 (64.2)
	Students	9 (16.9)
	Professionals	10 (18.9)

In the 53 swabs tested for HSV DNA by PCR, 34 swabs were positive for HSV DNA. Table 2 shows the results and comparison of HSV-1 and 2 DNA by PCR and IgM and IgG for HSV-1 and 2 by ELISA. In 2 samples positive for multi nucleated giant cells by microscopy were also positive for HSV-2 DNA but were negative for HSV-2 antibodies by ELISA. Overall HSV DNA PCR was negative in 19 samples in our study participants of which 5 samples were IgG positive of which two were HSV-1 IgG positive and three were HSV-2 IgG positive.

Table 2: Detection of HSV-1 and HSV-2 genital infection by PCR and ELISA in 53 patients of genital ulcer disease.

Test	Positive	Negative
HSV DNA	34	19
HSV-1 DNA	6	47
HSV-2 DNA	28	25
HSV-1 DNA and ELISA	6 (IgM-2, IgG - 4)	47
HSV-2 DNA and ELISA	11 (IgM-4, IgG -7)	42
Only HSV-1 and ELISA	2 (IgG)	51
Only HSV-2 and ELISA	3 (IgG)	50

DISCUSSION

A predictable 3752 million people have been reported to have HSV-1 infection equivalent to a global prevalence of 67% in 0-50 years old being upmost among women in the WHO African region.[13]

Durukan et al have reported that the first episode of ano genital herpes due to HSV-1 was the leading cause of ano genital herpes in younger women population and Men having sex with men (MSM).[14] Younger women's inherent susceptibility to HSV infections compared with men is because of the thin mucosal lining of the female external genitalia making them vulnerable than the keratinized skin of male genitalia. However, in the present study, male patients accounted for 79.2% and the remaining 20.8% were female patients (Table 1). This may be due to inhibitions of female patients in attending the STI clinic in our region as the ulcer tends to sort out in 2 to 3 weeks.

Stanberry et al and Langeland et al have documented that initially HSV-2 sero prevalence was considered as a predictor of genital herpes but with recent trend towards increasing sexual acquisition of HSV-1 in adolescence and early adulthood, both HSV-1 and 2 needed to be diagnosed to get a clearer picture of genital herpes.[15] We found 56.6% of the patients were in the age group 20-29 years (Table 1). Probably this changing trend included change in psychosexual practices or might be viral pathogenicity. [5] Individuals with genital HSV-1 are still at risk of HSV-2 acquisition but it is still a dilemma whether previous genital HSV-1 infection modifies the risk of genital herpes due to HSV-2 acquisition than the prior oral HSV-1.[16] In our study, majority were farmers and daily wagers (64.2%) by occupation who presented with painful genital ulcer and least followed by students (16.9%). This was similar to the study of Jain et al.[17] Epidemiological studies from United States (US) have shown that HSV-2 antibody prevalence (sero prevalence) continues to decline and thus genital herpes due to HSV-2 is declining and there is an emerging data suggesting that genital herpes due to HSV-1 is increasing. This emphasizes the need for testing both HSV-1 and 2 DNA in genital herpes infection. Cowan et al reported 60.4% prevalence of HSV-1 antibody in UK and that HSV-1 was associated with early age of first sexual intercourse that reflected the sexual practices of people initiating sex in that age group.[18]

Almukdad et al reported that the proportion of HSV-1 (versus HSV-2) detection in genital herpes in Australia and New Zealand was relatively high (30.5%) and comparable to levels observed in other Western countries (37% in Canada and 34% in Europe) but considerably higher than those in other regions (19% in Asia, 11% in LAC).[19-23] The proportion of HSV-1 detection in genital herpes was also increasing year by year, as in other Western countries.[24] These studies highlight the changing trends from HSV-2 to HSV-1 in genital herpes.

In the present study RT- PCR detected HSV-1 and HSV-2 DNA in 6 (11.3%) and 28 (52.9%) samples respectively (Table 2). However Mathew et al from Kerala, Muralidhar et al and Brijwal et al from Delhi reported HSV-1 DNA in 58%,32 % and 25% samples from genital herpes respectively.[25-27] Also, in a meta-analytic study by Khadr et al which included published data from Asian countries, 19% of genital herpes cases were due to HSV-1 (as divergent to HSV-2).· Recent data from some developed countries showed that a significant proportion of first-episode genital herpes is caused by HSV-1, though HSV-1 is transmitted most commonly through non sexual route.[24]

PCR detected 19 negative samples of which 5 samples were ELISA IgG positive of which two were HSV-1 IgG positive and three were HSV-2 IgG positive (Table 2). These findings indicate that these patients of non herpetic ulcer with past HSV-1 and HSV-2 infection respectively. Co infection of HSV-1 and HSV-2 in genital ulcer has been reported by Muralidhar et al, however in the present study we did not find such a co infection.[26]

The study was not without limitations. Out of the many patients who presented with painful genital ulcer in the STI clinic during study period, only 53 samples were randomly selected for DNA detection by RT-PCR due to cost restraints. RT-PCR on larger number of samples can perhaps corroborate the changing trends of increasing HSV-1 in genital herpes.

CONCLUSION

Although genital ulcers due to HSV-1 are known to be mild, patients need counselling for abstinence or safe sex practices to prevent their partners from acquiring painful non-genital ulcers. Both HSV-1 and HSV-2 DNA should be tested in painful genital ulcer as the strengthening of an STI programme-management relies heavily on evidence-based interventions which is empowered by PCR studies.

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