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Original Article

Microbiology Section

Prevalence of Herpes Simplex Virus in Genital Ulcer Patients: A Cross-sectional Study from South Assam, India

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ABSTRACT

Introduction: Herpes Simplex Virus (HSV) causes an ulcerating Sexually Transmitted Infection (STI). It produces painful and recurrent genital ulcers. In recent years, there have been increasing reports of genital herpes due to HSV-1 from various parts of the world. There is a lack of documented HSV Polymerase Chain Reaction (PCR) studies in this part of the country. So, this study was planned to estimate the prevalence of HSV type 1 (HSV-1) and type 2 (HSV-2) in patients with genital ulcers.

Aim: To estimate the prevalence of HSV-1 and HSV-2 in patients with genital ulcers.

Materials and Methods: The present cross-sectional study was conducted at Virus Research Diagnostic Laboratory (VRDL), Department of Microbiology, Silchar Medical College & Hospital, Silchar, Assam, India, among all the samples consecutively collected from patients presenting with genital infection in the Sexually Transmitted Infections Clinic of the Department of Dermatology for the period from April 2022 to August 2023. A total of 86 genital swab samples were collected from the base of the ulcer of patients, placed in viral transport media and transported at 4°C to VRDL for further testing. Out of 86 samples tested for HSV, 44 were males & 42 were females. Viral genomic Deoxyribonucleic Acid (DNA) was extracted from all the samples. Furthermore, from the extracted DNA, Real-time PCR (qPCR) was performed to detect the presence of HSV-1 and HSV-2 genes in the samples. Statistical analysis was performed using Microsoft Excel.

Results: Out of 86 samples collected, DNA PCR was positive in 46 (53.5%) patients with genital ulcers. Herpes Simplex Virus type 2 (HSV-2) was detected in 27 (31.4%) samples, making it more prevalent than HSV-1, which was found in 16 (18.6%) samples. Co-infection with both HSV-1 and HSV-2 was observed in three patients (3.5%).

Conclusion: Although there is a change in the aetiology of genital herpetic ulcers from HSV-2 to HSV-1, however, the present study showed that there is still an increased prevalence of HSV-2 in genital ulcers found in patients in South Assam.

Keywords: Genital infection, Herpes simplex virus type 1 and 2, Polymerase chain reaction, Sexual transmission

INTRODUCTION

Genital herpes is a chronic Sexually Transmitted Infection (STI) characterised by recurrent, self-limited genital ulcers, caused by herpes simplex virus type 1 (HSV-1) or type 2 (HSV-2) [1]. Generally, HSV-1 causes oro-labial lesions and HSV-2 causes genital lesions [2]. HSV-1 and 2 both can cause clinically indistinguishable multiple lesions or ulcers at orogenital and oro-labial sites [2,3].

Genital HSV infection is extremely common throughout the world, with epidemiological surveys demonstrating rising infection rates in most countries [4]. According to the World Health Organisation (WHO), more than 500 million people of 15-49 years are estimated to have a genital infection with Herpes Simplex Virus (HSV or herpes) [1]. The Centres for Disease Control (CDC) also published a report on genital herpes in the year 2018, where they mentioned that an estimated 572000 new genital HSV infections were occurring in the United States of America (USA) among people of reproductive age [5]. However, there is a lack of nationwide data available on genital herpes infections in India.

Genital herpetic infection is mainly diagnosed on the basis of clinical symptoms, typically, with the presence of papular lesions progressing to vesicular and ulcerative lesions [6]. However, the clinical differentiation of genital HSV infection from other infectious and non infectious etiologies of genital ulceration is often difficult [7]. Thus, a clinical diagnosis of genital herpes should be confirmed with laboratory tests. Since, the type of HSV infection affects the prognosis and subsequent counselling of patients, type-specific testing for HSV is always recommended [4,6,8]. There are plenty of studies on genital Herpes detection by serological methods, but Polymerase Chain Reaction (PCR) are now considered a more reliable and new gold standard for detecting the herpes virus genome [9,10]. The sensitivity and specificity of PCR are even higher than viral culture methods [9,11]. It is also valuable in detecting asymptomatic viral shedding in genital herpes.

As there are too many undiagnosed cases of genital ulcer in this setting. So, the present study was planned to rule out the presence of HSV and its type, which are very common causes of genital ulceration. Also, in recent years, no such study has been reported from this part of the world and this is the first of its kind being done in South Assam.

The primary objective of the study was to detect the prevalence of HSV-1 and 2 viruses in genital ulcers in the South Assam region using real-time PCR and the secondary objective was to determine the demographic variables among the positive patients.

MATERIALS AND METHODS

The present cross-sectional study was conducted in the VRDL, Department of Microbiology, Silchar Medical College and Hospital, Silchar, Assam, India for the period from April 2022 to August 2023. Ethical clearance for the study was obtained from the Institutional Ethics Committee (IEC Number: SMC/ETHICS/3rd March, 2022/50. Permission to conduct the study was sought from the respective departments. Informed consent was obtained from all the participants in the study.

Inclusion criteria: This is a prevalence-based study, consecutively including all the patients with genital ulcer attending the STI Clinic of the Dermatology department of the study Institute.

Exclusion criteria: Patients on antiviral therapy were excluded.

Study Procedure

A total of 86 genital swab samples were consecutively collected from patients attending the STI clinic of the study Institute. All the samples were collected from the base of the ulcer of patients. In case of vesicles, the vesicular fluid was obtained after rupturing the vesicles with a sterile needle, placed in viral transport media and transported at 4°C to VRDL, Dept. of Microbiology, SMCH, for further testing. Out of 86 samples collected for analysis, 44 were males and 42 were females.

All the samples were subjected to the extraction of the viruses genomic DNA using the QIA amp DNA extraction kit (QIAGEN, Germany) following the manufacturer's instructions. The DNA was stored at -20°C until processing. The extracted DNA was then subjected to real-time PCR by using Real Star® HSV PCR Kit 1.0 (altona Diagnostics GmbH, Hamburg). The probe for HSV-1 DNA was labelled with FAM, the probe for HSV-2 DNA was labelled with Cy5, and the probe specific for Internal Control was labelled with JOE.

STATISTICAL ANALYSIS

Statistical analysis was performed using Microsoft Excel.

RESULTS

Out of 86 samples collected, 44 (51.2%) were males and 42 (48.8%) were females. Almost all the participants in this study were married. The demographic characteristics of the population are shown in [Table/Fig-1].

The duration of the genital ulcers varied from 5 days to about 1 month, but 60% had ulcers for more than 5 days.

Demographic characters	Category	n (%),
Gender	Male	44 (51.2)
	Female	42 (48.8)
Marital status	Married	84 (97.6)
	Unmarried	2 (2.3)
Age distribution (years)	≤20	6 (6.9)
	21-30	44 (51.1)
	31-40	30 (34.8)
	>40	6 (6.9)
Education	Illiterate	8 (9.3)
	Less than class 10	32 (37.2)
	Class 10	36 (41.8)
	Less than class 12	7 (8.1)
	Graduate	3 (3.4)

[Table/Fig-1]: Demographic characteristics of patients with genital ulcers. N=86

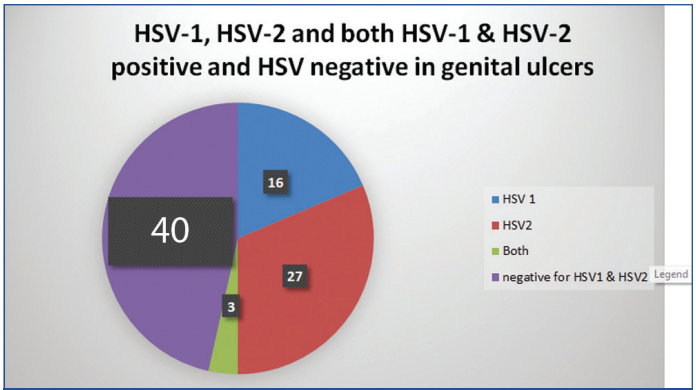
DNA PCR turned out to be positive in 46 (53.5%) patients with genital ulcers. HSV-2 (31.4%, 27/86) was more commonly isolated from the genital ulcers as compared to HSV-1 (18.6%, 16/86). Three patients (0.03%) had co-infection with HSV-1 and HSV-2 [Table/Fig-2].

With respect to gender, female preponderance was noted (56.5% females as compared to 43.5% males) among the positive patients [Table/Fig-3].

Also 50% (23/46) of positive patients belonged to the 21-30 years age group, followed by 41.3% positivity in the 31-40 year age group. HSV DNA positivity was 60% in those with a duration of ulcers less than 5 days.

The majority of the patients positive for HSV were in the age group from 21 to 30 years (50%). In the present study, patients presenting with genital ulcers for the first time were detected with HSV-1 infection. In recurrent cases, HSV-2 was the common isolate.

The HSV-2 was most commonly isolated from females in this study [Table/Fig-2]. Out of the 3 cases with co-infection, 2 (66.6%) were females and only 1 (33.3%) was male.



[Table/Fig-2]: HSV-1, HSV-2 PCR positive samples and HSV negative samples in genital ulcers.

Demographic features	Category	HSV DNA positive (Total =46), n (%)
Gender	Male	20 (43.5%)
	Female	26 (56.5%)
Age distribution	≤ 20	2 (4.3%)
	21-30	23 (50%)
	31-40	19 (41.3%)
	>40	2 (4.3%)
Duration	<5 days	28 (60.8%)
	>5 days	18 (39.1%)

[Table/Fig-3]: HSV positive cases among patients with genital ulcer.

DISCUSSION

The present study showed that 53.5% genital ulcers are caused by HSV. Similar prevalence was also observed in a study by Singh V et al., where the overall detection of HSV was 58.5% combining various methods like Tzanck smear, HSV IgM Enzyme-linked Immunosorbent Assay (ELISA), PCR and viral culture [9]. Only PCR led to the detection of 43.4 % cases of HSV, which is comparable to the present study [9]. In the study from Kerala by Mathew R et al., higher prevalence of 83% was detected by PCR of samples from genital ulcer cases [12]. Similarly, a study from Maharashtra by Thenmozhi P et al., found a prevalence of 64% by HSV DNA PCR [13]. Other studies from the Netherlands by Bruisten SM et al., found the established prevalence of 39% and a measured prevalence of 48% by PCR, which is comparable to the present study [14]. In other European countries and North American cities high prevalence of HSV was found in patients with genital ulcer. HSV persists in the genital tract and can be shed for many years, and can be reactivated. This may be the reason for the high prevalence of the virus in genital ulcer patients all over the world [15,16]. Also, it is important to keep in mind that a negative PCR does not exclude HSV infection because virus shedding is intermittent [15].

The HSV-2 (31.4%) was observed to be more prevalent than HSV-1 (18.6%) in genital ulcers. This is similar to a study in Nagpur by Thenmozhi P et al., where HSV-1 DNA and HSV-2 DNA detection were 11.3% and 52.9% respectively [13]. However, Mathew R et al., in a study from Kerala, reported 58% and 42% of HSV-1 and HSV-2 cases by PCR [12]. Similarly, Muralidhar S et al., from New Delhi reported HSV-1 DNA in 32% and 25% of genital herpes patients [16]. In the present study, results are consistent with other studies from Africa, where HSV-2 (91%) was found to be the dominant cause of genital ulcers [17]. In Western Europe and the USA, there is a change in the epidemiology of genital herpes and it is caused mainly by HSV-1 infection [17]. This shows the importance of using type-specific tests that clearly distinguish between HSV-1 and HSV-2. HSV-1 can be shed more easily and to higher titres than HSV-2, which is more intricately associated with the cytoskeletal matrix element actin [14]. Further, this will help to identify any change in the trends of the aetiology of genital ulcers in a particular geographical location.

In the present study, co-infection of HSV-1 and HSV-2 occurred in 6.5% cases. Co-infection has also been reported by Muralidhar S et al., whereas Thenmozhi P et al., did not report any co-infection [13,16].

The HSV-2 was more prevalent in females (59.26%; 16/27) as compared to HSV-1 (43.75%; 7/16) in the present study. This could be due to the reason that women are more susceptible to HSV-2 infection than men [15]. The risk factors for HSV-2 infection are the same as for other STIs: a high number of sexual partners throughout one's lifetime, young age at the onset of sexual activity, and previous history of STIs. This finding is in contrast with a study by Mathew R et al., where HSV-1 was most commonly isolated from females [12].

Majority of the patients in whom HSV DNA was detected were in the age group 21 to 30 years. This is similar to a study by Mathew R et al., where the majority of the HSV-positive patients were in the age group <40 years [12]. This indicates a positive outlook concerning the STI management programme in this part of Assam, as this means that the young population is aware of the need to avail healthcare facilities to get proper diagnosis and also has shed their inhibition to approach healthcare facilities in cases with genital ulcers. On the other hand, this also means that IEC activities need to be strengthened to prevent transmission of HSV in this reproductive age group.

Real-time PCR significantly increased HSV-1 and 2 detection in both early (5 days) presentation. The PCR-positive cases (60%) were found presenting after 5 days of the onset of ulcer, which is comparable to a study by Mathew R et al., where 61% presented with ulcer > 5 days. Cone RW et al., found HSV to be positive for an average of 6.8 days after onset of symptoms [12,18]. Laboratory diagnosis of HSV in genital ulcer cases will help manage the infection with proper antiviral treatment at the earliest and also differentiate from other organisms causing genital ulcers.

Limitation(s)

The limitation of this study is the relatively small sample size. The sample size was not calculated for this study. Also, it was difficult to elicit history regarding the sexual practices due to social inhibition among the patients attending the outpatient department. The viral load assay in case of HSV DNA-positive patients was not done in this study due to time constraints. Viral load could have provided a better insight with regard to HSV shedding from the genital tract of infected patients.

CONCLUSION(S)

Optimal management of genital ulcers (both bacterial and viral) following syndromic management, particularly in areas with high prevalence of HSV is important. In the present study, authors found that 53.5% genital ulcers are caused by HSV. Particularly, HSV-2 infection which is found to be more common in the present study, can cause epithelial disruption which leads to high risk of acquiring other STIs like Human Immunodefeciency Virus (HIV), therefore, it is necessary to carry out regular surveillance of genital ulcer disease aetiology and a major component of which must be type-specific PCR. Strengthening of IEC activities with patient counselling regarding safe practice should be emphasised to bring down the prevalence of genital ulcers in India.

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