Herpes Simplex Virus 1 and 2 in Herpes Genitalis: A Polymerase Chain Reaction-Based Study from Kerala

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Abstract

Background: Herpes genitalis is an ulcerating sexually transmitted infection, the clinical importance of which lies in its ability to produce painful and recurrent disease in addition to its potential role as a cofactor in acquisition and transmission of human immunodeficiency virus. In recent years, there are increasing reports of genital herpes due to herpes simplex virus (HSV)-1 from various parts of the world. Molecular diagnostic methods such as polymerase chain reaction (PCR) have got both diagnostic and prognostic significance in genital herpes. Aims: The present study was designed to identify the viral serotype in herpes genitalis patients in our locality, using PCR. Materials and Methods: The specimens from forty herpes genitalis patients were subjected to nested PCR and results were evaluated. Results: PCR was positive for HSV in 83% of cases, of which 58% were due to HSV-1. HSV-2 accounted for maximum number of recurrent herpes. Limitation: Higher sample size would have been more representative. Conclusion: A rising trend of type 1 HSV was observed in herpes genitalis in south India probably due to increasing practice of orogenital sex.

Key Words: Herpes genitalis, herpes simplex virus, polymerase chain reaction

Introduction

Genital herpes simplex virus (HSV) infection is a major public health problem with significant morbidity and transmission potential.[1] Historically, HSV-1 has been associated with oral mucocutaneous disease and HSV-2 with genital infection.[2] However, studies clearly indicate a change in epidemiology of genital herpes in recent years.[2-5]

In a seroprevalence study done among 135 patients with sexually transmitted diseases, from a referral hospital in south India, 82.9% had HSV-1 and 2 coinfection while 8.1% and 2.2% had HSV-1 and 2 infection alone, respectively.[6] By doing seroprevalence, we cannot categorically identify the viral strain of genital herpes due to the high seroprevalence of HSV-1 in normal population. Since the type of HSV infection affects the prognosis and subsequent counseling of patients, type-specific testing for HSV is always recommended.[5] Nucleic acid amplification test such as polymerase chain reaction (PCR) are now considered as the gold standard for detecting herpes virus genome.[6] PCR is a rapid and highly sensitive method for detecting HSV and in differentiating between HSV-1 and 2 genotypes.[7-9] PCR is even more sensitive than cell culture for detecting herpes virus.[1-7] It is also valuable in detecting asymptomatic viral shedding in genital herpes.[7]

Studies to detect virus serotype in herpes genitalis by PCR is lacking from southern India. We conducted this study to detect the prevalence of HSV-1 and 2 strains in herpes genitalis in our area using PCR.

Materials and Methods

It was a cross-sectional study conducted at a tertiary care center for a period of one year, after obtaining permission the Ethics Committee of the Institute. Forty herpes genitalis patients who attended our outpatient department during the period were included in the study. Genital herpes was diagnosed clinically
and using Tzanck smear. Patients with recurrent herpes genitalis on chronic suppressive therapy with antiviral drugs were excluded from the study. Relevant sociodemographic data were collected through personal interview with the patients, ensuring adequate privacy. Sexual history of spouses was obtained in relevant cases.

Samples were collected from the genital lesions using cotton-tipped sterile swabs soaked in viral transport media, provided by the National Institute of Virology (NIV) unit, which was situated in our hospital building complex. The vesicular fluid was obtained after rupturing the vesicles with a sterile needle. If there were no vesicles swabs were obtained from the base of erosions/ulcers. Swabs were immediately transferred to viral transport medium and sent under ice-cold temperature to NIV unit, where PCR was done.

Viral DNA amplification was done using nested PCR method. It involved two different consecutive PCRs which increased the sensitivity. DNA was extracted from the patient sample using QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s instruction. The DNA was isolated from 200 µL of the sample and the DNA was eluted in 50 µL of elution buffer. The first PCR reaction was performed in 50 µL reaction volume-containing 25 µL of 2xPCR master mix (Promega), 0.5 µM each forward TO1 and reverse TO2 primers and 10 µL of extracted DNA. The procedure for PCR was as follows: initial denaturation at 94°C for 3 min and then maximum 40 cycles of amplification including denaturation at 94°C for 30 s, annealing at 65°C for 30 s, extension at 72°C for 45 s, and a final extension at 72°C for 10 min. The nested PCR reaction was also performed in 50 µL reaction volume containing 25 µL of 2xPCR master mix (Promega), 0.5 µM each forward TO1 and two reverse primers T1B1 and T1B2, and 5 µL of the first-round PCR product. The amplification conditions for the nested PCR were initial denaturation at 94°C for 3 min followed by 30 cycles of denaturation (94°C for 30 s), annealing (60°C for 30 s), and extension (72°C for 45 s). After completion of the cycles, a final step of extension at 72°C for 7 min was also performed. The appropriate positive and negative internal controls were included in each experiment. The PCR products were analyzed in 2% agarose gel. The HSV-1 and HSV-2 were identified according to their size, 109 bp and 143 bp for HSV-1 and HSV-2, respectively. Data were entered into Microsoft Excel and analyzed. Association between sociodemographic factors and HSV-1 and HSV-2 was done using Chi-square test and value of $P<0.05$ was considered as statistically significant.

**Results**

We studied forty patients with herpes genitalis, majority of whom (75%) were aged between 20 and 40 years. Females outnumbered males in the study. Primary herpes genitalis was diagnosed in 31 (78%) patients while the rest had recurrent herpes. Three patients suffered from more than six episodes of recurrences during the last year. Thirty (75%) patients practiced orogenital sex. Only one patient claimed to use condom regularly. Two patients, both males, were human immunodeficiency virus (HIV) seropositive (Table 1).

PCR was done in all forty patients with herpes genitalis. A positive test result was obtained in 33 (83%) patients. Of the 33 PCR positive cases, 19 (58%) were HSV-1 and 14 (42%) were HSV-2. None was positive for both strains. Gel image of PCR-amplified products is shown in Figure 1.

Table 2 shows the age and sex distribution of PCR-positive patients which clearly shows maximum HSV-1 positivity in females below 40 years. However, the differences among the groups were not significant.

A total of 20 (61%) PCR-positive patients presented after 5 days of the onset of symptoms. HSV was isolated 2 weeks after the onset of symptoms in two patients who were HIV positive.

Of the seven PCR-negative patients, three had taken acyclovir tablets for $>5$ days. Two patients presented about 1 week after the onset of symptoms.

In the present study, primary herpes genitalis was mostly associated with HSV-1 virus. In recurrent herpes genitalis, HSV-2 was the common isolate as shown in Table 3. This result was statistically significant.

Among the 14 HSV-2 patients, 11 (79%) had multiple sexual partners during the past 1 year. Eighteen (95%) of HSV-1 genome positive cases had sexual exposure with a single partner only. This observation was statistically significant ($P<0.05$).
Table 1: Sociodemographic features of our patient (n=40)

| Characteristic                      | n (%) |
|-------------------------------------|-------|
| Age (years)                         |       |
| <20                                 | 4 (10)|
| 20-40                               | 30 (75)|
| >40                                 | 6 (15)|
| Sex                                 |       |
| Male                                | 19 (48)|
| Female                              | 21 (52)|
| Educational status                  |       |
| <10th standard                      | 4 (10)|
| 10th                                | 12 (30)|
| 12th                                | 10 (25)|
| Degree                              | 10 (25)|
| Postgraduate                        | 4 (10)|
| Marital status                      |       |
| Married                             | 28 (70)|
| Unmarried                           | 12 (30)|
| Sexuality                           |       |
| Heterosexual                        | 37 (93)|
| Homosexual                          | 2 (5)|
| Bisexual                            | 1 (2)|
| History of orogenital contact       |       |
| Present                             | 30 (75)|
| Absent                              | 10 (25)|
| History of previous episode         |       |
| Present                             | 9 (22)|
| Absent                              | 31 (78)|
| HIV status                          |       |
| HIV positive                        | 2 (5)|
| HIV negative                        | 38 (95)|
| Number of sexual partners in last year |       |
| Single                              | 26 (65)|
| Multiple                            | 14 (35)|

Table 2: Age and sex distribution of polymerase chain reaction positive patients (n=33)

| Age (years) | HSV-1 | HSV-2 | Chi-square test | P       |
|-------------|-------|-------|-----------------|---------|
| <40         | 17 (52)| 11 (33)| 0.138*          | >0.05   |
| ≥40         | 2 (6)  | 3 (9)  |                 |         |
| Sex         |       |       |                 |         |
| Male        | 6 (18) | 9 (27) | 3.477           | >0.05   |
| Female      | 13 (40)| 5 (15) |                 |         |

Discussion

PCR detected HSV in 83% of clinically diagnosed herpes genitalis cases. About 58% and 42% of PCR-positive cases were due to HSV-1 and HSV-2 virus, respectively. A similar study by Muralidhar et al.[9] conducted between 2010 and 2011 in New Delhi using real-time PCR showed HSV-1 positivity in 32.2% cases. In contrast to their study, our project clearly indicated a change in the epidemiology of herpes genitalis in favor of HSV-1. This change was in accordance with the various studies from developed industrialized parts of the world.[2-5] Cowan et al. observed an increase in genital herpes due to HSV-1 in the United Kingdom, particularly among young people.[4] Proportion of genital herpes due to HSV-1 increased from 31.4% to 42.8% in British Columbia, according to Gilbert et al. during their study period (1997–2005).[5] Studies from Scotland and Australia also showed a rising prevalence of HSV-1 in genital herpes.[2,3] Possible explanations for this changing trend included change in sexual practices and/or change in viral pathogenicity.[6] Decreased acquisition of HSV-1 in early childhood rendering young adults more susceptible to infection was also proposed.[4]

None of our patients had coinfection of HSV-1 and HSV-2. The study from New Delhi revealed HSV-1 and 2 coinfections in 25% of PCR-positive herpetic ulcers.[9]

HSV-1 virus was most commonly isolated from females in the present study. This was in accordance with previous reports.[12] Genital herpes in reproductive age group women is significant due to the risk of neonatal herpes infections.

We observed Type 1 HSV to be more prevalent than Type 2 in primary herpes genitalis which was well documented.[10,11] About 87.5% of PCR-positive recurrent genital herpes in our study was due to HSV-2. Most of the literature reported HSV-2 as the common cause of recurrent herpes genitalis.[7,9,10,12] Lower tendency for HSV-1 to reactivate is of prognostic significance as genital herpes is often associated with high degree of psychological distress.

All patients with HSV-1 infection admitted orogenital contact with their partners of which 18 had only a single partner. Similar findings were observed in other studies as well.[10,11] Increasing acceptability and practice of orogenital sex could explain the rising trend of HSV-1 in genital herpes. Orogenital transmission also explained the occurrence of herpes genitalis within sexually stable relationships where there had been no other partner. Subclinical shedding of HSV-1 from the oral cavity had been well documented.[10] This emphasized the need for educating youth regarding the potential for oral-labial to genital transmission of HSV-1.
About 79% of patients with HSV-2 infection had multiple sexual partners. A higher number of sexual partners is suggested as a risk factor for HSV-2 virus.\(^{[10]}\)

Of the 25 PCR-positive primary herpes genitalis cases, 18 (72%) had HSV-1. This was at par with other studies which showed an increase in HSV-1 isolates in primary herpes genitalis.\(^{[3,4,10]}\)

PCR significantly increases HSV-1 and 2 detection in both early (<5 days) and late (>5 days) presentations of herpes infection.\(^{[9]}\) By the time, the lesions have crusted, the viral culture will be positive in about 25% of cases only.\(^{[11]}\) About 61% of our PCR-positive cases presented after 5 days of onset of symptoms. HSV virus was positive for an average of 6.8 days, by PCR, in a study by Cone et al.\(^{[12]}\) We were able to detect HSV even after 2 weeks of disease onset, in two patients, who were HIV-positive. Persistence of lesions and prolonged shedding of virus due to HIV infection could be the reason for the same.

PCR was negative in seven cases of clinically diagnosed herpes genitalis. Three of these patients were on acyclovir therapy when they presented to us. Antiviral drugs might have decreased viral shedding in these patients. Two patients presented late (>7 days) after the onset of symptoms. Inappropriate collection or processing could have been the reason for negative PCR in other two cases.

**Conclusion**

Our study clearly showed a change in the epidemiology of herpes genitalis in India. Increasing isolation of HSV-1 genome in genital herpes could be multifactorial. We observed a greater frequency of orogenital sex in our patients as a part of safer sex programs. Increasing proportion of HSV-1 was identified in patients with primary herpes genitalis, in females and in those practicing oral sex. In recurrent herpes and in people with multiple partners, HSV-2 continued to be the major etiological agent. Knowledge of regional epidemiology of HSV infection is important for clinical and public health practice. Large-scale studies using PCR is needed to further substantiate the changing trends in genital herpes in India.

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Nil.

**Table 3: Relation between clinical type of herpes and herpes simplex virus genome (n=33)**

| Clinical type                  | Number of patients (%) | Total number of patients | Chi-square test | P     |
|-------------------------------|------------------------|--------------------------|-----------------|-------|
|                               | HSV-1 | HSV-2 | | | |
| Primary herpes genitalis      | 18     | 7     | 25 | 6.517* | <0.05 |
| Recurrent herpes genitalis    | 1      | 3     | 8  |       |      |

*Fisher’s exact test. HSV: Herpes simplex virus

**Conflicts of interest**
There are no conflicts of interest.

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