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MOLECULAR DETECTION OF HERPES SIMPLEX VIRUS 1 AND 2 FROM MUCOCUTANEOUS LESIONS AMONG ADULTS ATTENDING TERTIARY CARE HOSPITAL

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ABSTRACT

Background: Herpes Simplex Virus (HSV) infections, caused by HSV-1 and HSV-2, are among the most common sexually transmitted infections, causing oral and genital lesions. Timely and accurate diagnosis is critical for managing outbreaks and reducing transmission. Objectives: This study aimed to determine the prevalence of HSV-1 and HSV-2 in adults presenting with mucocutaneous lesions, using Tzanck smear and Real-Time Polymerase Chain Reaction (RT-PCR), and to analyze associated socio-demographic and clinical factors. Materials and Methods: A cross-sectional study was conducted on 75 clinically suspected HSV cases attending Government Medical College Hospital from July 2021 to June 2022. Samples were collected from mucocutaneous lesions and analyzed using Tzanck smear microscopy and RT-PCR for HSV DNA detection. Demographic data, clinical features, and sexual behavior history were also collected and analyzed. Result: Out of 75 cases, 48 (64%) were positive for HSV by RT-PCR. HSV-1 was detected in 35 (46.6%), HSV-2 in 9 (12%), and 4 (5.3%) had co-infections. The majority of patients were aged 19-30 years (49.3%) and female (57.3%). A statistically significant association was found between HSV-1 and oral lesions (p=0.01), and between HSV-2 and genital lesions (p=0.017). Tzanck smear had limited sensitivity (26.7%). Most patients reported unprotected sexual practices (89.3%) and a history of multiple sexual partners (62.7%). Conclusion: HSV-1 is now a prominent cause of genital infections. RT-PCR proved to be superior in diagnosis over Tzanck smear. Public health strategies must address unprotected sexual behavior and promote awareness, especially in younger, less educated populations.

INTRODUCTION

Herpes Simplex Virus (HSV) infections impose a considerable burden on worldwide public health due to their high frequency, chronicity, and potential for asymptomatic spread.^[1,2] HSV has two major serotypes, HSV type 1 (HSV-1) and HSV type 2 (HSV-2), both of which cause recurring mucocutaneous infections.^[3] While HSV-1 has traditionally been associated with oral lesions and HSV-2 with genital lesions, this difference is becoming increasingly muddled as sexual habits change, particularly with the expanding prevalence of oral-genital contact.^[4,5] As a result, HSV-1 has

in many regions of the world.^[6] According to the World Health Organization, an estimated 3.7 billion persons under the age of 50 are infected with HSV-1, whereas roughly 491 million people aged 15 to 49 are infected with HSV-2.^[6] The burden is especially significant in low- and middle-income countries, where diagnostic services and awareness of sexually transmitted infections (STIs) are restricted.^[7,8] Underreporting and a lack of broad molecular diagnostic facilities in India are likely to reduce the true frequency of HSV infections.^[9] HSV infections are distinguished by periods of latency and recurrence. The virus remains dormant in nerve ganglia but can reawaken, resulting in symptomatic or asymptomatic shedding.^[10] Asymptomatic viral

shedding helps to spread the virus, especially among sexually active people. Many people are unaware of their infection, increasing the danger of transferring the virus to sexual partners.^[11,12] HSV has typically been diagnosed using clinical symptoms and cytological procedures such as the Tzanck smear, multinucleated large cells.^[13] which reveals However, this method lacks sensitivity and specificity. Serological testing can help detect previous infections, but they cannot diagnose active lesions.^[14,15] Molecular approaches, such as Real-Time Polymerase Chain Reaction (RT-PCR), provide higher sensitivity, specificity, and the capacity to distinguish between HSV-1 and HSV-2.[16,17] RT-PCR has thus emerged as the gold standard for confirming HSV infections, particularly in cases when the clinical diagnosis is equivocal.^[18] Despite diagnostic advances, there is a scarcity of information on the molecular incidence of HSV types 1 and 2 in India, particularly in resource-constrained tertiary care settings.^[19,20] Furthermore, sociobehavioral factors such as multiple sexual partners, a lack of condom use, and a low educational level continue to influence the transmission of HSV infections.^[21,22] Understanding how these factors interact with clinical and molecular findings is critical for developing effective public health measures. The purpose of this study was to investigate the prevalence and distribution of HSV-1 and HSV-2 among adults presenting with mucocutaneous lesions at a tertiary care hospital, using both conventional (Tzanck smear) and molecular approaches (RT-PCR). In addition, this project will examine the demographic and behavioral characteristics associated with HSV infections in order to provide better diagnostic, therapeutic, and preventive treatments in clinical and community settings.

MATERIALS AND METHODS

Study Design and Setting

This was a cross-sectional observational study conducted over a period of one year, from July 2021 to June 2022, at the Department of Microbiology in collaboration with the Department of Skin, STD, and Leprosy, Government Medical College and Hospital. The hospital caters to a large population, making it a suitable setting to study the epidemiology and molecular characteristics of Herpes Simplex Virus (HSV) infections.

Study Population

A total of 75 adult patients (aged 18 years and above) presenting with clinically suspected mucocutaneous lesions suggestive of herpes simplex virus infection were enrolled in the study. All participants attended the outpatient or inpatient services of the Dermatology and STD department during the study period.

Inclusion Criteria

- Adults (≥18 years) with clinically suspected HSV lesions on skin or mucocutaneous surfaces (oral or genital).
- Patients providing informed written consent to participate in the study.

Exclusion Criteria

- Patients already on antiviral treatment for HSV.
- Patients unwilling to provide consent.
- Lesions with a clear alternative diagnosis (e.g., aphthous ulcers, impetigo, syphilitic chancre).

Data Collection

Each participant provided a complete clinical and epidemiological history using a pre-structured questionnaire administered by an interviewer. This questionnaire collected key demographic information such as age, gender, marital status, and occupation. The educational background was documented to determine the amount of awareness about sexually transmitted illnesses. Participants were also questioned about their sexual history, including the occurrence of premarital or extramarital sexual interactions, the number of sexual partners, and the form of sexual contact (peno-vaginal, oral, or anal). Condom usage data was collected to determine whether sexual conduct was protected or unprotected. The clinical appearance was meticulously noted, including lesion type (ulcer, vesicle, crusting), duration of symptoms, and anatomical location (oral or genital).

Clinical Examination

All recruited patients had a thorough clinical evaluation performed by a certified dermatologist. The lesions were inspected and classified into four major clinical categories: single ulcers, multiple ulcers, multiple vesicles, and crusted erosions. The distribution and shape of lesions were carefully examined to aid in clinical diagnosis. The lesion's anatomical location was identified as either oral (suggesting herpes labialis) or genital (suggesting herpes genitalis). This clinical evaluation was critical in determining lesion features, laboratory findings, and the type of HSV infection diagnosed.

Sample Collection

Under stringent aseptic conditions, two types of samples were obtained from the base of vesicular or ulcerative lesions with sterile swabs. The first sample was used to make a Tzanck smear for cytological analysis,^[23] and the second sample was deposited in a viral transport medium (VTM) for molecular testing using real-time polymerase chain reaction (RT-PCR).^[24] To avoid degradation, all samples were labeled and documented before being swiftly sent to the microbiological laboratory. The prompt processing of these samples was important to ensuring diagnostic accuracy, particularly for molecular testing.

Laboratory Methods

Tzanck Smear Examination

Lesion material was equally distributed onto a clean glass slide before cytological investigation. The

slides were air dried, fixed, and then stained with Giemsa stain.^[25] The dyed slides were examined under a light microscope at 400x magnification for the presence of multinucleated giant cells (MNGCs), which are associated with herpes simplex virus infection. Although not specific, the presence of MNGCs provides a quick first indication of HSV and encourages further confirmation testing.

Real-time polymerase chain reaction (RT-PCR)

For molecular validation, DNA was extracted from the swab samples stored in VTM using a commercially available nucleic acid extraction kit and the manufacturer's instructions. RT-PCR was then carried out with a validated multiplex detection kit designed to detect both HSV-1 and HSV-2 serotypes. The assay used TaqMan probe technology to target conserved areas of the HSV DNA polymerase gene. A high-throughput real-time thermocycler, such as the Rotor-Gene Q, was used for amplification and detection. Internal controls were included in each run to confirm the test results. According to the manufacturer's standards, a cycle threshold (Ct) value of less than 38 indicated the presence of HSV DNA.

Statistical Analysis

All data was collated and imported into Microsoft Excel for preliminary analysis. The statistical analysis was carried out using SPSS software version 25.0. The data was summarized using descriptive which included frequencies statistics. and percentages for categorical variables. The Chi-square test was used to determine the relationship between clinical and demographic factors and HSV positive. The relationships between characteristics such as site of lesion and HSV type, gender and HSV positive and sexual behavior and HSV serotype were investigated. A p-value of less than 0.05 was judged statistically significant, showing a meaningful association between the variables studied.

Ethical considerations

The Government Medical College Institutional Ethics Committee evaluated and approved the study protocol prior to its beginning. All participants were given thorough information regarding the study's objectives, procedures, potential risks, and benefits. Each subject provided written informed consent prior to sample collection. The study was conducted in strict confidentiality and secrecy. Personal identifiers were removed or encrypted from the database, and the data was managed in accordance with institutional and national biomedical research ethics requirements.

RESULTS

Age-wise Distribution of Cases

The age distribution of the 75 clinically suspected herpes simplex virus cases showed that nearly half of the patients (49.3%) belonged to the younger age group of 19 to 30 years. This indicates that HSV infections are more common among young adults. The second most affected age group was 31 to 40 years, which accounted for 26.6% of the cases. A smaller proportion of cases were observed in older age groups, with 13.4% of patients in the 41 to 50 years range and 10.7% in the 51 to 60 years range.



Figure: 1 Age wise distribution of cases under study (n=75)

Gender-wise Distribution of Cases

In terms of gender, females represented a higher percentage of the study population, comprising 57.3% (43 out of 75 cases), while males accounted for 42.7% (32 cases) (Fig. 2). When analyzing the confirmed HSV positive cases, 19 of the 32 males tested positive, whereas 29 of the 43 females were positive for HSV infection. This indicates a slightly higher infection rate among females compared to males in the sample group. The predominance of females in the study and among positive cases may reflect differences in healthcare-seeking behavior or exposure risks in the population studied.



Figure: 2 Gender wise distribution of cases under study (n=75)

Sociodemographic and Clinical Characteristics of Study Participants

Among the 75 clinically suspected cases of herpes simplex virus infection, the distribution based on education showed that one-third of the participants (33.3%, 25 cases) had only primary school education, followed by 28% (21 cases) with middle school education. Those who had completed a degree accounted for 22.7% (17 cases), while 16% (12 cases) had education up to high school. This indicates a predominance of lower to moderate educational attainment among the affected individuals. Regarding occupation, labourers formed the largest group at 34.7% (26 cases), followed by skilled workers at 22.7% (17 cases), agricultural workers at 21.3% (16 cases), students at 13.3% (10 cases), and housewives comprising the smallest proportion at 9.3% (7 cases). This occupational distribution suggests that HSV infections may be more common among those engaged in manual and skilled labour, potentially reflecting socioeconomic factors influencing exposure and healthcare access. In terms of marital status, the majority of cases were married (62.7%, 47 cases), with unmarried individuals representing 21.3% (16 cases) and divorced or separated participants making up 16% (12 cases).

This pattern indicates that HSV infections are prevalent across different marital categories but are notably higher among married individuals, which may be related to sexual behavior dynamics within stable relationships. Clinically, multiple vesicles were the most frequent presentation, seen in 37.33% (28 cases) of the patients, closely followed by crusted erosions in 36% (27 cases). Multiple ulcers were observed in 20% (15 cases) and single ulcers in 6.7% (5 cases), demonstrating that HSV infections often manifest as multiple lesions with vesicular and crusted erosive features predominating. [Table 1]

Table 1: Demographic Characteristics and Clinical Presentation of Cases under Study (n=75)				
Characteristic Category		Frequency (n=75)	Percentage (%)	
	Primary School	25	33.3	
Education	Middle School	21	28	
Education	High School	12	16	
	Degree	17	22.7	
	Labourer	26	34.7	
Occupation	Skilled Worker	17	22.7	
	Agriculture	16	21.3	
	Student	10	13.3	
	Housewife	7	9.3	
	Married	47	62.7	
Marital Status	Unmarried	16	21.3	
	Divorced / Separated	12	16	
	Multiple Vesicles	28	37.33	
Clinical Presentation	Crusted Erosions	27	36	
	Multiple Ulcers	15	20	
	Single Ulcer	5	6.7	

Distribution of Cases by Site of Lesion, Sexual History, and Behavior

Among the 75 cases studied, 45.4% presented with oral herpes lesions, while 54.6% had genital herpes lesions. Regarding sexual behavior, a majority (62.7%) reported having multiple sexual partners, compared to 37.3% with a single partner. History of premarital or extramarital sexual contact was common, with 46.7% reporting extramarital contact, 22.7% premarital, and 4% both, while 26.6% denied any such history. The predominant mode of sexual

contact was peno-vaginal (53.3%), followed by combined peno-oral and peno-vaginal contact (29.3%). Ano-receptive and oro-receptive contacts were reported less frequently (9.4%), with anoreceptive and oro-receptive alone each reported by 4% of cases. Most participants identified as heterosexual (80%), with 20% identifying as homosexual. Notably, the vast majority (89.3%) practiced unsafe sexual practices, with only 10.7% reporting consistent use of protection. [Table 2]

Table 2: Distribution of Cases by Site of Lesion, Sexual History, and Behavior (n=75)				
Parameter	Category	Frequency	Percentage (%)	
Site of Losian	Oral (Herpes labialis)	34	45.4	
Site of Lesion	Genital (Herpes genitalis)	41	54.6	
Number of Sexual Portners	Single	28	37.3	
Number of Sexual Farthers	Multiple	47	62.7	
	Premarital	17	22.7	
Premarital/Extramarital Contact	Extramarital	35	46.7	
	Both	3	4	
	None	20	26.6	
	Peno-vaginal	40	53.3	
	Peno-oral & Peno-vaginal	22	29.3	
Mode of Sexual Contact	Ano-receptive & Oro- receptive	7	9.4	
	Ano-receptive	3	4	
	Oro-receptive	3	4	
Samal Oniantation	Heterosexual	60	80	
Sexual Orientation	Homosexual	15	20	
Safa Sarual Draatiaan	Protected	8	10.7	
Safe Sexual Practices	Unprotected	67	89.3	

Detection of Herpes Simplex Virus Cases by Tzanck Smear Study

In this study, the Tzanck smear examination detected multinucleated giant cells indicative of herpes simplex virus infection in 20 out of 75 cases, accounting for 26.7% positivity. However, the majority of cases, 55 (73.3%), showed negative results on the Tzanck smear. (Table 3) This indicates that while Tzanck smear can identify HSV infection in some cases, its sensitivity is relatively low compared to more advanced molecular methods.

Table 3: Detection of Herpes Simplex Virus Cases by Tzanck Smear Study (n=75)					
Tzanck Smear Result	Frequency	Percentage			
Positive	20	26.70%			
Negative	55	73.30%			

RT-PCR Analysis of HSV-1, HSV-2, and Coinfection among Study Participants

The Real-Time PCR analysis conducted in this study revealed that out of 75 clinically suspected cases of herpes simplex virus infection, 35 cases (46.6%) tested positive for HSV Type 1, while 40 cases (53.3%) were negative. For HSV Type 2, the RT-PCR detected viral DNA in 9 cases (12%), whereas the remaining 66 cases (88%) were negative. Overall, molecular detection identified a total of 48 positive cases of HSV: 35 cases were exclusively positive for HSV-1, 9 for HSV-2, and 4 cases (5.3%) were positive for both HSV-1 and HSV-2, indicating coinfection. These findings highlight the higher prevalence of HSV-1 compared to HSV-2 in mucocutaneous lesions in the study population and demonstrate the utility of RT-PCR as a sensitive method for accurate viral detection.

Table 4: RT-PCR Analysis of HSV-1, HSV-2, and Co-infection among Study Participants (n = 75)					
HSV Type Detected	Frequency	Percentage (%)			
HSV-1 Only	35	46.6			
HSV-2 Only	9	12			
Both HSV-1 and HSV-2	4	5.3			
Negative for HSV-1 & 2	27	36.1			
Total	75	100			

Association between Site of Lesion and Herpes Simplex Virus Types (HSV-1, HSV-2, and Coinfection) (n = 75)

In the present study, a statistically significant association was found between the site of lesion and HSV-1 detection. Among 34 cases presenting with oral lesions (Herpes labialis), 23 (67.7%) tested positive for HSV-1, compared to 16 (39.1%) of the 41 genital lesion cases. This difference was statistically significant with a chi-square value of 6.101 and a p-value of 0.01 (p < 0.05), indicating a stronger association of HSV-1 with oral mucocutaneous lesions. Conversely, the analysis of HSV-2 detection revealed a higher prevalence in

genital lesions. Of the 41 patients with genital herpes (Herpes genitalis), 11 (26.9%) were positive for HSV-2, whereas only 2 (5.8%) of the oral cases tested positive. This association was also statistically significant with a chi-square value of 5.692 and a p-value of 0.017, suggesting that HSV-2 is significantly more common in genital lesions. Regarding HSV-1 and HSV-2 co-infections, 4 cases (5.3%) were found to be positive for both serotypes. Interestingly, co-infection was evenly distributed between oral and genital sites, with 2 cases each, indicating that although co-infection is less frequent, it can occur in both anatomical regions. [Table 5]

Fable 5: Distribution of HSV Types Based on Lesion Site							
Site of Lesion	HSV-1 Positive (%)	HSV-1 Negative (%)	HSV-2 Positive (%)	HSV-2 Negative (%)	HSV-1 & HSV-2 Co- infection	Total Cases (%)	
Oral	23 (67.7%)	11 (32.3%)	2 (5.8%)	32 (94.2%)	2	34 (45.3%)	
Genital	16 (39.1%)	25 (60.9%)	11 (26.9%)	30 (73.1%)	2	41 (54.7%)	
Total	39 (52.0%)	36 (48.0%)	13 (17.3%)	62 (82.7%)	4 (5.3%)	75 (100%)	

HSV-1 and HSV-2 Serotypes among Tzanck Smear Positive and Negative Cases

Out of the total 75 cases analyzed in this study, 20 cases (26.7%) were positive on Tzanck smear, while 55 cases (73.3%) were negative. Among the Tzanck smear positive group, 11 cases were confirmed to have HSV-1, 5 cases had HSV-2, and 4 cases were found to have co-infection with both HSV-1 and HSV-2. In contrast, among the 55 Tzanck smear negative cases, 24 cases were still found to be

positive for HSV-1 and 4 cases for HSV-2 when tested with RT-PCR, highlighting that RT-PCR is more sensitive in detecting herpes simplex virus DNA even when cytological findings are absent (Table 6). Notably, no co-infections were detected among the Tzanck smear negative group, indicating that Tzanck smear may only identify co-infections when a high viral load or characteristic cytopathic effect is present. These results demonstrate that while Tzanck smear can provide preliminary cytological evidence of HSV infection, molecular diagnostics like RT-PCR remain essential for definitive

identification and typing, especially in smearnegative patients.

$-1 a D C V_{1} H D V = 1 a H U H D V = 2 D C V V D C A H U H Z I Z A H C C D H C A H C C A S C M (1 - 7)$	Table 6: HSV-1 and HSV-2 Serotypes	Among Tzanck Smear 1	Positive and Negative Cases	(n = 75)
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Tzanck Smear Result	HSV-1	HSV-2	Both HSV-1 & HSV-2	Total Cases
Positive $(n = 20)$	11	5	4	20
Negative $(n = 55)$	24	4	0	55
Total	35	9	4	75

DISCUSSION

The present study was conducted in the Department of Microbiology in collaboration with the Department of Skin, STD and Leprosy at Government Medical College and Hospital. A total of 75 patients with clinically suspected Herpes Simplex Virus (HSV) infections were enrolled over a one-year period. Detailed sociodemographic data, clinical history, and sexual behavior patterns were collected. Samples from mucocutaneous lesions were analyzed using both cytological (Tzanck smear) and molecular (RT-PCR) diagnostic techniques. Out of the 75 clinically suspected cases, 48 (64%) were confirmed as HSV positive using RT-PCR. Among them, 35 (46.6%) were positive for HSV-1, 9 (12%) for HSV-2, and 4 (5.3%) showed co-infection with both HSV-1 and HSV-2. The majority of patients (49.3%) belonged to the 19-30 years age group, followed by 31-40 years (26.6%). These findings align with studies conducted by Munawwar et al. (2018),^[26] and Krishna Ray et al. (2006),^[27] which demonstrated that HSV is more prevalent among young adults, a population typically at the peak of sexual activity and vulnerability to sexually transmitted infections (STIs). A notable female predominance (57.3%) was observed in this study. This correlates with findings from Cowan et al. (2003),^[28] which showed a higher prevalence of HSV-1 among females, possibly due to biological susceptibility and transmission dynamics favoring male-to-female transmission during vaginal intercourse. Regarding occupational and educational background, laborers (34.7%) constituted the largest group, followed by skilled workers (22.7%) and agricultural workers (21.3%). Educational status revealed that 61.3% had only primary or middle school education, reflecting a population that may have limited awareness of STI transmission and prevention. Similar associations between low educational level and higher STI risk were observed by C. Shendre et al. (2002), emphasizing the need for targeted health education.^[29] In terms of marital and sexual behavior, 62.7% were married, and a significant proportion had a history of extramarital (46.7%) or premarital (22.7%) sexual contact. Only 10.7% reported consistent use of protection, underscoring the role of unprotected sex as a major risk factor, consistent with findings by Shaw et al., who associated low condom use with higher HSV-2 prevalence among married individuals. Clinically, multiple vesicles (37.3%) and crusted erosions (36%)were the most common presentations, followed by

ulcers. Regarding lesion location, 45.4% had oral herpes (HSV-1) and 54.6% had genital herpes (HSV-2). While traditionally HSV-2 is associated with genital infections, a growing trend of HSV-1 causing genital infections has been documented, supported by studies like those by Bernstein et al. (2013), Nieuwenhuis et al. (2006), and Mathew et al. (2018).^[30-32] This shift may be attributed to increasing oro-genital sexual practices, particularly among young adults. Although Tzanck smear was positive in only 20 cases (26.7%), RT-PCR confirmed HSV in 48 cases, including 28 smear-negative cases. This clearly demonstrates the low sensitivity but moderate specificity of the Tzanck test, which may vary based on lesion stage and location. While a study by Folkers et al. (1988) reported higher sensitivity (79%),^[33] our findings support the superiority of molecular diagnostics, especially RT-PCR, in confirming HSV infections. The increasing detection of HSV-1 in lesions highlights а genital significant epidemiological shift, also reported in global studies. The 4 co-infection cases (5.3%) in this study align with findings from Xu et al. (2006),^[34] and Munawwar et al. (2018),^[26] who reported higher coinfection rates among HIV-positive populations, suggesting immunosuppression may facilitate dual infection. Furthermore, RT-PCR's diagnostic advantage over virus culture and serology has been well documented. Navidad et al. (2019),^[35] and Arshad et al. (2019),^[36] emphasized RT-PCR's higher sensitivity, speed, and cost-effectiveness. While serology may be useful for chronic or asymptomatic infections, its performance can be limited by false negatives or latency. New platforms like Solana HSV1+2/VZV, compared by Jevsnik et al. (2020),^[37] may offer even more accurate and user-friendly alternatives to traditional RT-PCR machines like LC480, though further validation is required. In this study reaffirms that HSV infection remains a public health concern, especially among sexually active voung adults with low awareness and poor protective practices. Despite global progress in STI prevention and control, HSV prevalence remains unchanged, as noted by Mao et al. (2021).^[38] Our findings advocate for increased accessibility to RT-PCR, targeted sexual health education, and routine screening, particularly among high-risk groups, to improve early detection and reduce transmission.

CONCLUSION

This study highlights key epidemiological and clinical patterns of HSV infection among adults

attending a tertiary care hospital. The highest burden of HSV was observed among young adults (19-30 years) and females, emphasizing their vulnerability. Low educational status and occupational backgrounds such as labor work were found to be significant risk factors, reflecting the need for targeted health education and awareness programs in these populations. Clinically, multiple vesicular lesions were the most common presentation. While genital herpes remained more prevalent than oral herpes, a noticeable rise in oral herpes cases was observed, particularly associated with oro-genital sexual practices. HSV transmission was significantly associated with unprotected sexual behavior, multiple sexual partners, and extramarital sexual contacts. The presence of homosexual behavior in 20% of the study population also points to evolving sexual practices and underlines the need for inclusive sexual health interventions. Molecular analysis using RT-PCR revealed that HSV-1 was three times more common than HSV-2 in oral lesions, while HSV-2 was five times more prevalent in genital lesions. Additionally, HSV-1 was more frequently detected among oro-receptive individuals, indicating changing patterns in the epidemiology of HSV infections. The findings underscore the importance of molecular diagnostics like RT-PCR for accurate detection and highlight the necessity for strengthened sexual health education, risk-reduction strategies, and improved access to STI testing, especially among high-risk groups.

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