



CHANGING ETIOLOGICAL TRENDS OF GENITAL ULCER DISEASE: A CLINICO-MICROBIOLOGICAL STUDY FROM NORTH INDIA

Clinical Microbiology

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ABSTRACT

Background: Genital ulcer disease (GUD) remains an important sexually transmitted infection (STI) syndrome because of its association with increased morbidity and facilitation of human immunodeficiency virus (HIV) transmission. Over recent decades, the epidemiology of GUD has undergone a substantial shift, with viral pathogens increasingly replacing classical bacterial etiologies. Accurate laboratory diagnosis is essential for understanding contemporary disease trends and evaluating the effectiveness of syndromic management strategies. **Methods:** A prospective cross-sectional study was conducted among 200 adult patients presenting with genital ulcer disease of sexually transmitted infection origin at a tertiary care hospital in North India between July 2024 and December 2025. Detailed demographic, behavioral, and clinical data were recorded. Ulcer specimens were subjected to microscopy and culture, while blood samples were evaluated using serological assays including Rapid Plasma Reagin (RPR), Treponema pallidum Hemagglutination Assay (TPHA), HSV-2 IgM ELISA, Chlamydia trachomatis IgM ELISA, and HIV serology. Statistical analysis was performed using SPSS software. **Results:** The mean age of participants was 39.35 ± 12.66 years. Males constituted 88% of cases and 90% of participants belonged to rural areas. The highest proportion of patients was observed in the 31–40-year age group (33%). HSV-2 was the predominant etiological agent, detected in 42 (21%) patients, followed by Treponema pallidum in 12 (6%), Klebsiella granulomatis in 2 (1%), and HIV infection in 18(9%). No cases of chancroid or lymphogranuloma venereum were identified. Vesicular morphology ($p<0.001$) and inguinal lymphadenopathy ($p=0.003$) were significantly associated with HSV-2 infection. Syndromic diagnosis classified 93% of patients as herpes genitalis, whereas laboratory confirmation was obtained in only 21%, indicating substantial clinicomicrobiological discordance. **Conclusions:** The present study demonstrates a clear epidemiological transition in genital ulcer disease towards viral etiology, with HSV-2 emerging as the predominant pathogen. The declining occurrence of classical bacterial causes and the significant discrepancy between syndromic and laboratory diagnosis highlight the need for strengthened laboratory support in STI management programmes.

KEYWORDS

INTRODUCTION

Genital ulcer disease (GUD) represents one of the most important clinical syndromes among sexually transmitted infections (STIs) because of its significant impact on sexual health and its established role in facilitating the acquisition and transmission of human immunodeficiency virus (HIV). Disruption of the genital mucosal barrier by ulcerative lesions increases susceptibility to HIV infection and enhances viral shedding among infected individuals, thereby amplifying transmission within the community.

Historically, bacterial pathogens such as *Treponema pallidum*, *Haemophilus ducreyi*, and *Klebsiella granulomatis* accounted for the majority of genital ulcer disease cases worldwide. However, several studies conducted over the past two decades have documented a marked epidemiological shift from bacterial to viral causes, particularly herpes simplex virus (HSV). Genital herpes has now emerged as the leading cause of genital ulceration in many developed and developing countries, reflecting changing sexual behaviors, improved control of bacterial STIs, and the lifelong recurrent nature of HSV infection.

Despite advances in diagnostic technology, the etiological diagnosis of genital ulcer disease remains challenging. Clinical manifestations frequently overlap, and classic textbook presentations are often absent. Multiple concurrent infections, atypical presentations, and HIV co-infection may further obscure clinical diagnosis. Consequently, reliance on clinical examination alone is associated with poor diagnostic accuracy and may result in inappropriate treatment, missed diagnoses, and inaccurate epidemiological surveillance.

To overcome these challenges, syndromic management has been widely adopted in resource-limited settings, including India. Although this approach facilitates prompt treatment and reduces dependence on sophisticated laboratory infrastructure, it may lead to overtreatment, unnecessary antimicrobial exposure, and failure to accurately define the underlying etiological spectrum. Several studies have demonstrated significant discordance between syndromic diagnosis and laboratory-confirmed etiological diagnosis of genital ulcer disease.

The epidemiology of genital ulcer disease varies considerably across geographical regions and evolves over time. Contemporary data from India indicate a progressive increase in herpes genitalis and a declining

prevalence of chancroid, donovanosis, and lymphogranuloma venereum. However, regional differences continue to exist, and updated data from North India remain limited.

Understanding the current etiological profile of genital ulcer disease is essential for guiding patient management, evaluating existing syndromic management protocols, and developing evidence-based public health strategies. The present study was therefore undertaken to evaluate the clinico-microbiological profile, etiological spectrum, and clinicomicrobiological correlation of genital ulcer disease among patients attending a tertiary care hospital in North India. The study also aimed to assess the utility of conventional laboratory methods and serological investigations in establishing etiological diagnosis and to examine the relationship between clinical findings and microbiological confirmation.

MATERIALS AND METHODS

An aetio-clinicomicrobiological prospective cross-sectional study was conducted in the Departments of Microbiology and Dermatology at Government Medical College and Guru Nanak Dev Hospital, Amritsar, Punjab, India, from July 2024 to December 2025. The study included 200 sexually active adult patients (>19 years of age) presenting with genital ulcer disease (GUD) of sexually transmitted infection origin. Patients with non-STI genital ulcers, those who had received antimicrobial therapy within the preceding two weeks, and those unwilling to participate were excluded. Institutional Ethics Committee approval was obtained prior to commencement of the study, and written informed consent was obtained from all participants.

A detailed demographic, behavioural, and clinical history was recorded using a structured proforma. Clinical examination included assessment of ulcer morphology, site, number, tenderness, induration, discharge, and associated inguinal lymphadenopathy. Based on clinical findings, a provisional syndromic diagnosis was assigned by the dermatologist according to standard STI management guidelines.

Following clinical examination, the ulcer surface was gently cleansed with sterile normal saline to remove exudate and surface contaminants. Three sterile cotton-tipped swabs were collected from the active margin and base of each ulcer under aseptic precautions. In patients with suspected donovanosis, crush smears were additionally prepared from

granulation tissue obtained from the ulcer base. All specimens were appropriately labelled and transported immediately to the Microbiology laboratory for processing. Approximately 5 mL of venous blood was collected aseptically from each participant and transferred to sterile screw-capped vials for serological investigations.

The first ulcer swab was utilized for direct microscopic examination. Two smears were prepared on clean grease-free glass slides. Gram-stained smears were examined under oil immersion for inflammatory cells and Gram-negative coccobacilli arranged in characteristic parallel chains or “school of fish” appearance suggestive of *Haemophilus ducreyi*. Giemsa-stained preparations were examined for intracellular Donovan bodies demonstrating the characteristic bipolar “safety-pin” appearance diagnostic of *Klebsiella granulomatis*. Tzanck smears were prepared in all cases, irrespective of clinical diagnosis, by scraping the base of the lesion and staining with Giemsa stain. Smears were examined for multinucleated giant cells, acantholytic cells, and nuclear moulding suggestive of herpes simplex virus infection.

The second ulcer swab was used for culture and fungal microscopy. Specimens were inoculated onto 5% blood agar, MacConkey agar, chocolate agar, and GC agar enriched with haemoglobin and serum using the streak plate technique. Culture plates were incubated at 35–37°C in an atmosphere containing 5–10% carbon dioxide for 48–72 hours and examined for growth of bacterial pathogens, particularly *Haemophilus ducreyi*. Simultaneously, a potassium hydroxide (KOH) mount was prepared using 10% KOH solution and examined microscopically for budding yeast cells and pseudohyphae suggestive of candidal infection.

The third ulcer swab was collected for molecular evaluation and placed in viral transport medium. Specimens were stored at 2–8°C for short-term preservation and at –70°C for prolonged storage until further analysis. Although molecular testing was reserved for selected cases, appropriate transport and storage conditions were maintained to preserve specimen integrity.

Serological investigations were performed on serum separated from clotted blood samples following centrifugation. Serum samples were stored at 2–8°C until testing and preserved at –70°C for long-term storage when required. Screening for syphilis was performed using the Rapid Plasma Reagin (RPR) test, and reactive samples were confirmed by Treponema pallidum Hemagglutination Assay (TPHA). Detection of herpes simplex virus infection was carried out using HSV-2 IgM enzyme-linked immunosorbent assay (ELISA), while Chlamydia trachomatis infection was evaluated using Chlamydia trachomatis IgM ELISA. Human Immunodeficiency Virus (HIV) testing was performed according to National AIDS Control Organisation (NACO) Strategy III using three different assays based on distinct immunological principles, following pre-test and post-test counselling and maintenance of confidentiality.

An etiological diagnosis was established when microbiological, serological, or molecular evidence of a specific pathogen was obtained. Data were entered into Microsoft Excel and analysed using Statistical Package for Social Sciences (SPSS) version 26.0. Associations between clinical variables and laboratory-confirmed etiologies were evaluated using Chi-square test or Fisher's exact test as appropriate. A p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 200 patients presenting with genital ulcer disease (GUD) of sexually transmitted infection origin were enrolled during the study period. The majority of patients belonged to the 31–40 years age group (33.0%), followed by 21–30 years (23.0%) and 41–50 years (22.0%). Males constituted 88.0% of the study population, while females accounted for 12.0%. Most participants were married (83.5%) and resided in rural areas (90.0%). Secondary-level education was the most common educational status (60.0%), and the majority of patients were employed in the private sector (76.0%). Most participants belonged to the lower socioeconomic class (89.5%).

A single sexual partner during the preceding year was reported by 93.5% of patients, whereas 6.5% reported multiple partners. Condom use was largely inconsistent, with only 8.0% reporting regular use and 77.0% reporting occasional use. All study participants identified as heterosexual.

Table 1. Sociodemographic and behavioural characteristics of GUD patients (n = 200)

Characteristic	Category	Number (%)	Percentage (%)
Age group (years)	≤20	7 (3.5)	3.5
	21–30	46 (23.0)	23.0
	31–40	66 (33.0)	33.0
	41–50	44 (22.0)	22.0
	51–60	22 (11.0)	11.0
	>60	15 (7.5)	7.5
Gender	Male	176 (88.0)	88.0
	Female	24 (12.0)	12.0
Marital status	Married	167 (83.5)	83.5
	Unmarried	33 (16.5)	16.5
Residence	Rural	180 (90.0)	90.0
	Urban	20 (10.0)	10.0
Educational status	Illiterate	5 (2.5)	2.5
	Primary	16 (8.0)	8.0
	Middle	41 (20.5)	20.5
	Secondary	120 (60.0)	60.0
	Graduate	17 (8.5)	8.5
	Intermediate	1 (0.5)	0.5
Occupation	Private employee	152 (76.0)	76.0
	Government employee	14 (7.0)	7.0
	Housewife	22 (11.0)	11.0
	Student	9 (4.5)	4.5
	Unemployed	3 (1.5)	1.5
Socioeconomic status	Lower	179 (89.5)	89.5
	Middle	21 (10.5)	10.5
Number of sexual partners in last year	Single	187 (93.5)	93.5
	Multiple	13 (6.5)	6.5
Condom use	Never	30 (15.0)	15.0
	Occasional	154 (77.0)	77.0
	Always	16 (8.0)	8.0
Sexual orientation	Heterosexual	200 (100)	100

Serological testing demonstrated HSV ELISA positivity in 42 (21.0%) patients, making it the most common positive serological finding. Syphilis serology was reactive in 12 (6.0%) patients by both VDRL and TPHA. HIV seropositivity was observed in 18 patients (9.0%)

No patient tested positive for lymphogranuloma venereum.

Table 2. Results of Serological Tests Performed on GUD Cases (n = 200)

Serological test	Number performed	Number reactive/positive	Percentage positive
VDRL	200	12	6.0
TPHA	200	12	6.0
HSV IgM ELISA	200	42	21.0
HIV	200	18	9
LGV IgM ELISA	200	0	0.0

The commonest clinical diagnosis among patients presenting with genital ulcer disease was herpes genitalis, accounting for 189 (94.5%) cases. Laboratory confirmation was obtained in 42 (21.0%) patients. Syphilis was clinically suspected in 9 (4.5%) patients, whereas laboratory testing identified 12 (6.0%) cases. Donovanosis demonstrated complete concordance between clinical and laboratory diagnosis. No cases of chancroid or lymphogranuloma venereum were identified either clinically or microbiologically.

Table 3. Comparison of Clinical and Laboratory Diagnosis of Genital Ulcer Disease

Diagnosis	Clinical diagnosis n (%)	Laboratory diagnosis n (%)
Herpes genitalis	189 (94.5)	42 (21.0)
Syphilis	9 (4.5)	12 (6.0)
Donovanosis	2 (1.0)	2 (1.0)
Chancroid	0	0
LGV	0	0

Discussion

Genital ulcer disease (GUD) continues to represent an important public health problem because of its role in facilitating transmission of sexually transmitted infections, particularly HIV. The present study provides a contemporary clinico-microbiological evaluation of GUD

among patients attending a tertiary care hospital in North India and demonstrates a clear epidemiological transition towards viral etiology, with HSV-2 emerging as the predominant pathogen. Similar changes in the etiological spectrum of GUD, with increasing predominance of herpes genitalis and declining occurrence of classical bacterial causes, have been documented in Indian studies.^{1,3}

The majority of patients in the present study belonged to the sexually active age group of 31–40 years, with a mean age of 39.35 ± 12.66 years. Similar age distributions have been reported in previous Indian studies, in which GUD was predominantly observed among young and middle-aged adults.^{2,3} This age group represents a sexually active segment of the population and may therefore have greater exposure to sexually transmitted infections. The predominance of males (88%) observed in the present study is consistent with previous Indian studies and may partly reflect differences in healthcare-seeking behaviour and access to STI services between men and women.^{1,3} The overwhelming rural representation (90%) further highlights the importance of accessibility of STI diagnostic and treatment services in rural populations.

Behavioural assessment revealed widespread inconsistent condom use, with only 8% of participants reporting regular condom use and 77% reporting occasional use. Similar patterns of inconsistent condom use have been described in Indian studies of STI patients and highlight the continuing importance of behavioural interventions, condom promotion, partner notification and early healthcare seeking.^{1,2}

Clinically, vesicular lesions, multiple ulcers, tenderness, and inguinal lymphadenopathy were important findings in the present study. These clinical features are characteristic of genital herpes and may explain the high proportion of patients clinically classified under the herpes genitalis syndrome. Similar clinical patterns and a high frequency of herpes genitalis have been reported by Muralidhar et al. and Bachaspatimayum et al.^{1,3} The significant association observed between vesicular morphology and HSV-2 positivity ($p < 0.001$) supports the clinical usefulness of vesicular morphology as an indicator of herpetic disease. Likewise, inguinal lymphadenopathy demonstrated a significant association with HSV-2 infection ($p = 0.003$). However, clinical findings alone cannot reliably establish the etiological diagnosis of GUD because considerable overlap exists between different ulcerative STIs.^{2,3}

The diagnostic yield of conventional microbiological methods was poor. Gram staining failed to identify *Haemophilus ducreyi* in any patient, while Giemsa staining demonstrated Donovan bodies in only two cases and Tzanck smear was positive in five patients. The limited sensitivity and specificity of conventional microscopic methods for GUD diagnosis have also been described in previous studies.^{1,3} Although these techniques remain inexpensive and are useful where advanced laboratory facilities are unavailable, their negative results cannot reliably exclude specific etiologies. The poor performance of Tzanck smear in the present study, compared with the higher detection of HSV-2 by serological testing, further emphasizes the limitations of cytological examination as a standalone diagnostic method.

Culture also demonstrated limited usefulness in the present study. No etiologically significant bacterial pathogen was isolated, while the majority of culture-positive specimens yielded coagulase-negative staphylococci, which were considered colonizing flora rather than primary pathogens. The declining yield of conventional bacterial culture in GUD is consistent with the changing epidemiology reported in Indian studies, where classical bacterial causes such as chancroid have become increasingly uncommon.^{1,2,3} Molecular investigations have also demonstrated that a substantial proportion of clinically diagnosed GUD may have viral rather than bacterial etiologies, supporting the need for more sensitive pathogen-specific diagnostic approaches.⁵

Serological investigations provided the major diagnostic contribution in the present study. HSV-2 IgM antibodies were detected in 21% of patients, making HSV-2 the most frequently identified etiological agent. Syphilis, confirmed by reactive RPR and TPHA, accounted for 6% of cases, while donovanosis was uncommon and no cases of chancroid or lymphogranuloma venereum were detected. The predominance of herpes genitalis and relatively low frequency of classical bacterial ulcerative STIs are consistent with contemporary Indian reports.^{1,3} Muralidhar et al. reported herpes genitalis as the predominant cause of GUD in New Delhi, while Bachaspatimayum et al. similarly reported herpes genitalis in the vast majority of patients in northeastern India.^{1,3}

The absence of chancroid in the present study is particularly noteworthy. Historically, chancroid was an important cause of genital ulcer disease in India; however, its occurrence has declined considerably in contemporary studies.^{1,2,5} The present finding is therefore consistent with the changing epidemiological pattern of GUD in India. Similarly, the absence of lymphogranuloma venereum supports observations that LGV is relatively uncommon among patients presenting with routine GUD in many Indian clinical settings.^{1,3}

A major finding of the present study was the substantial clinicomicrobiological discordance between syndromic diagnosis and laboratory confirmation. Although 93% of patients were clinically diagnosed as having herpes genitalis, laboratory confirmation was obtained in only 21%. Similar discrepancies between clinical/ syndromic diagnosis and laboratory-confirmed etiology have been documented in India. Prabhakar et al. demonstrated that the etiological diagnosis of GUD frequently differed from the clinical syndromic classification and highlighted limitations in the performance of existing syndromic algorithms.² More recent evidence has likewise shown that syndromic management of GUD has limited diagnostic accuracy for individual pathogens.⁵ While syndromic management remains valuable for providing immediate treatment in resource-constrained settings, laboratory-supported diagnosis can reduce diagnostic uncertainty, improve surveillance and facilitate pathogen-specific management.

The HIV seropositivity rate of 9.0% observed in the present study is substantially higher than the prevalence expected in the general population and underscores the epidemiological association between GUD and HIV infection. Previous Indian studies have also reported increased HIV positivity among patients presenting with genital ulcers.^{1,3} The biological association between genital ulceration, particularly HSV-2 infection, and increased susceptibility to HIV acquisition is well established.⁵ Therefore, routine HIV testing should remain an integral component of the evaluation of patients presenting with GUD, accompanied by appropriate counselling and prevention interventions.

Overall, the findings of the present study indicate a continuing epidemiological transition in GUD in North India, characterized by predominance of HSV-2 and a marked reduction in classical bacterial ulcerative STIs. The findings are broadly consistent with contemporary Indian studies, although regional differences in the etiological spectrum remain possible.^{1,3} The substantial discrepancy between syndromic and laboratory diagnosis further emphasizes that clinical morphology, although useful for initial assessment, should ideally be complemented by appropriate laboratory investigations whenever available.

CONCLUSION

The findings of the present study have important public health implications. The predominance of HSV-2 infection, coupled with the demonstrated limitations of conventional microscopy and culture, indicates that serological and molecular diagnostic methods should be incorporated more widely into routine STI services. Improved diagnostic accuracy would facilitate pathogen-specific treatment, enhance surveillance of changing epidemiological trends, and strengthen STI control programmes. Furthermore, the high prevalence of behavioural risk factors identified in the present study underscores the need for intensified health education, condom promotion, partner notification, and community-based awareness programmes, particularly in rural populations.

In conclusion, the present study confirms a clear epidemiological shift in genital ulcer disease towards viral etiology, with HSV-2 emerging as the predominant pathogen. The declining prevalence of classical bacterial causes, poor performance of conventional microbiological methods, and substantial discordance between syndromic and laboratory diagnosis collectively highlight the need for strengthening laboratory-supported STI diagnostic services in India.

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