

Genital ulcer disease: Study of factors associated and utility of rapid diagnostic methods in current pandemic

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ABSTRACT

Background: Genital ulcer disease (GUD) is regarded as the most difficult conundrum for clinicians, as valid and rapid methods to diagnose them are limited, especially in India. The sexual behavior of an individual plays a role in the causation of GUD, and it depends on psychological, behavioral, and social well-being, which was significantly affected during the COVID-19 pandemic. Hence, the present study was undertaken to determine the etiology of genital ulcers by using simple, rapid microbiological methods and to study various factors associated with them. **Materials and Methods:** Various rapid tests like staining (Giemsa, Gram, and Diff Quick) and serology for antibody detection were used to diagnose agents causing genital ulcers among 71 suspected patients during the study period of 1 year. After due informed consent, sociodemographic and behavioral factors were recorded in proforma and analyzed. **Results:** Rapid tests could aid in diagnosing 47.9% of cases of GUD. Turnaround time was less than 60 minutes for all tests carried out. Genital herpes was the most common GUD followed by syphilis. Male gender (74.6%), age group of more than 34 years old (62%), literate (71.8%), and earning (70.4%) constituted most of the population presenting with GUD. Behavioral factors like promiscuity with a known person (83.1%) had a significant association (P 0.04) with GUD. **Conclusion:** Rapid diagnostic tests could assist in the early treatment of GUD cases. Factors like literacy, financial independence, and promiscuity with known individuals were associated with GUD in our study during the COVID-19 pandemic.

Keywords: Factors associated, genital ulcer disease, rapid methods

Introduction

Genital ulcer diseases (GUD) can have a major impact on morbidity and mortality in many countries, including India, due to their ability to enhance the rate of sexual transmission of the human immunodeficiency virus (HIV).^[1] Recently, the Centers for Disease Control (CDC) released a fact sheet suggesting that the cases reported as sexually transmitted diseases (STDs) dropped during the beginning months of the pandemic, but

following that, there was a surge, indicating increased STD rates until January 2021. This was an added challenge for sexually transmitted infection (STI)-related programs, as the shift of resources for the pandemic led to a lack of manpower and materials.^[2] Although GUD can be due to infectious or non-infectious causes, infectious causes increase the burden not only on healthcare but also on society due to their potential to spread.^[3] The most common infectious causes of GUD in developing countries like India are herpes simplex virus (HSV)-2, *Treponema pallidum*, *Haemophilus ducreyi*, *Klebsiella granulomatis* and *Chlamydia trachomatis* (serovars L1-L3). There is significant clinical heterogeneity in the morphologic presentation of GUD, making the clinical diagnosis unreliable without confirmatory laboratory tests.^[4,5] Nucleic acid amplification techniques like polymerase chain reaction (PCR) have been developed to detect

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several etiological agents like viruses and bacteria, including HSV-2, *T. pallidum*, and *H. ducreyi*. If there is a clinical dilemma in diagnosis, then PCR offers confirmation of diagnosis. PCR for GUD diagnosis is costly, chances of contamination increase during transportation, and is done in those labs with good turnover in GUD samples.^[6] Very few laboratories in our country cater to the need for diagnosing STIs using both PCR and culture, which are time-consuming.^[7,8] As there is significant variability in the presentation of GUDs, which causes diagnostic dilemmas for clinicians, simple tests like microscopy, staining, and rapid kits were more cost-effective during the pandemic era compared to PCR.^[9,10] Decreased affordability of patients due to job losses, along with travel restrictions due to new variants emerging in COVID-19, still made it more difficult for the transportation of samples and patients to higher referral laboratories where NAAT was available.^[11]

Few studies are available in our country suggesting the use of rapid techniques, which can aid in diagnosing GUD, as most of the samples from suspected cases are sent to referral labs for PCR or diagnosed clinically and treated empirically. Thus, there is a need to know about simple techniques which can aid clinicians in prompt diagnosis and help switch precise therapy in patients with GUD. The COVID-19 pandemic not only affected the entire world economically but also contributed to disturbed psychological and social well-being in individuals.^[12] There was a need to study how these social, demographic, and behavioral factors will now contribute to the etiopathogenesis of GUD in this pandemic scenario. Hence, the present study was undertaken to determine the etiology using simple, rapid microbiological methods, and study the factors associated with GUD in patients attending the STIs clinic at our hospital.

Aims and objectives

The aims and objectives of this study were to assess various etiological agents using simple and rapid microbiological diagnostic tools available in our tertiary care setting and to determine various factors associated with GUD.

Materials and Methods

This was a prospective observational study carried out in the Department of Microbiology. Institutional Ethical Committee (IEC) clearance (IEC/IRB no: 01/2022) was obtained before the start of the study. The study was started initially as a short-term studentship (STS) project of the Indian Council of Medical Research (ICMR-STs, ref no: 2022-03261) and was further continued for a total duration of 1 year, from July 2022 to June 2023.

The study population consisted of all OPD patients attending the STI Clinic in the Dermatology Department. Criteria for inclusion were patients presenting with genital ulcers with an age ≤ 60 years. Children ≤ 15 years and elderly patients > 60 years of age were excluded from the study. Patients diagnosed with non-infectious causes of genital ulcers clinically; patients

diagnosed with STI clinically (other than genital ulcers); and patients clinically suspected or diagnosed with lymphogranuloma venereum (LGV) (a rapid test was not cost-effective) were also excluded.

Study tools

An informed, written consent was taken from each patient at the start of the study. Sociodemographic characteristics, along with clinical features, were inquired into and recorded (in a proforma).

Procedure

For syphilis

Darkfield microscopy was performed within twenty minutes of collection on the serous exudates collected from the ulcer to look for *Treponema pallidum*. A positive darkfield microscopy was taken to be positive for syphilis irrespective of the serological test results for syphilis.

Serological tests performed for syphilis were the rapid immunochromatographic test (Syphilis Card Test) (Immunopak) for the detection of IgM, IgA, and IgG antibodies and the rapid plasma reagin test (RPR) (Carbogen) for the detection of cardiolipin antibodies by qualitative and semiquantitative techniques for diagnosis.

For Genital herpes

A swab was collected from the ulcers' base and stained with Giemsa's stain to look for multinucleated giant cells (MNGCs) indicative of herpes infections by Tzanck smear. A serological test was also performed for the detection of HSV-2 IgM and IgG antibodies by a rapid immunochromatographic test kit (on-site rapid test by CTK Biotech).

For Donovanosis

A small tissue piece from the ulcer was crushed between two glass slides, and one slide was stained with Giemsa stain to look for the typical morphology of "safety pin appearance," which is indicative of donovanosis. Diff Quick stain was also performed on another slide with crushed tissue to find *Klebsiella granulomatis*.

For Chancroid

A swab was collected from the ulcer for smear and stained with Gram's stain, which was then observed under oil immersion objective for the presence of pus cells and Gram-negative coccobacilli in clusters or the "fish in stream" appearance seen in *Haemophilus ducreyi*.

Statistical analysis

The data was entered into a Microsoft Excel sheet. IBM SPSS software version 26, USA, for Windows, was used for analysis. The data were represented by frequencies and percentages. For the analysis of categorical data, the Chi square test was used. Differences were considered statistically significant if *P* value was < 0.05 . IBM SPSS Statistics for Windows, version 24 (IBM

Corp., Armonk, N.Y., USA) software program was used for statistical calculations.

Observation and Results

Total cases clinically diagnosed/suspected of infectious etiology in GUD were 71. Rapid tests (microscopy and serology together) came positive in 47.9% (34) cases.

Factors associated

Sociodemographic factors

Majority of GUD patients were males (74.6%) compared to females (25.4%). 62% of patients were of the age group of 34 years and above compared to those between 25 to 34 years' age group (15.5%). Most of the patients were literates (secondary school level and above) (71.8%) compared to illiterates (28.2%). 70.4% of patients were earning or had a source of income (most of them were daily wage laborers) compared to those who were not (29.6%). The association of these sociodemographic factors with GUD (by using rapid diagnostic tests) was not statistically significant (P value > 0.05) [Table 1].

Behavioral factors

Most patients had a history of promiscuity (83.1%). Promiscuity was seen with a known person (other than spouse in married individuals), e.g., a friend, a known acquaintance, or colleagues (83.1%) rather than with a paid sex worker (16.9%), and its association with GUD (by using rapid diagnostic tests) was statistically significant (P 0.04). Sexual orientation was heterosexual (94.4%) in most of the patients compared to homosexual (5.6%). Unprotected sexual intercourse was practiced in 88.7% of patients, compared to 11.3% of patients who practiced protected sexual intercourse by using barrier precautions (condoms). These precautions were mainly used when a paid partner was sought. [Table 2].

Demographic and clinical findings in relation to clinical diagnosis

The mean age of patients was 37.7 ± 12.1 years. The mean duration of symptoms was 17.12 days, with a range of 3–60 days. 15.5% (11/71) cases had h/o genital ulcers in the past. Multiple ulcers were present in 32.4% (23/71) cases. Painful ulcers were seen in 40.8% of cases, and 28.2% (20/71) of cases

had induration (a sign elicited on examination) in the ulcers examined. Vesicular lesions were noted in 23.9% (17/71) of cases and inguinal lymphadenopathy in 7.04% (5/71) of cases. Previous history of topical application of ointment (antiseptic or antibiotic) was noted in 15.5% (11/71) cases.

Among 42 clinically diagnosed or suspected syphilis cases, the mean age of patients was 39.4 ± 12.8 years, the mean duration of symptoms was 23.2 days, induration was present in 47.6% (20/42) of cases, and pain in the ulcer area was present in 4.8% (2/42) of cases. Among 23 clinically diagnosed herpes cases, the mean age of patients was 34.4 ± 10.2 years, the mean duration of symptoms was 7.6 days, multiple ulcers were present in 78.3% (18/23) of cases, pain was present in 95.6% (22/23) of cases, and vesicles were present along with ulcers in 69.6% (16/23) of cases [Image 1]. Among five clinically diagnosed or suspected chancroid cases, the mean age of patients was 36 ± 13.1 years, the mean duration of symptoms was 10 days, pain was present in 100% (5/5) of cases, and inguinal lymphadenopathy was present in 80% (4/5) of cases. Only one case was clinically diagnosed or suspected as donovanosis, as it was a beefy red ulcer with no pain, lymphadenopathy, or induration. [Table 3].

Utility and turnaround time (TAT) of rapid tests in diagnosing GUD

Among the 71 cases of genital ulcers included in the study, 59.2% (42/71) of cases had painless ulcers, and 40.8% (29/71) had painful ulcers. 42 cases were clinically suspected of syphilis. Of them, 21 cases were tested for darkfield microscopy (DFM), and none showed the presence of spirochetes; those samples positive by rapid immunochromatographic tests for syphilis were further tested for RPR qualitative and semiquantitative, and all 16 samples tested positive with titers of $>1:8$ dil. Thus, 38.1% of syphilis cases could be diagnosed by rapid methods with a TAT of 30–60 minutes for microscopy and serology. 78.3% (18/23) herpes cases could be diagnosed by rapid tests, including Tzanck smear (12 cases) with a TAT of 60 minutes, and on serology, six cases were IgG positive with a TAT of 60 minutes. None of the HSV-suspected cases were positive for chancroid by Gram stain or for IgM by serology. Of the five chancroid cases clinically suspected, gram stain, Tzanck smear, and herpes serology were also performed as differential diagnoses. None came back positive. Rapid tests could not aid in diagnosing chancroid and had a TAT

Table 1: Association of sociodemographic factors with GUD

Total (n=71)	Sociodemographic data	Number of patients	Percentage	GUD diagnosed by rapid methods (n=34)	Chi square value	P
Gender	Male	53	74.6	21	1.833	0.17
	Female	18	25.4	13		
Age (years)	<25	16	22.5	6	1.12	0.57
	25-34	11	15.5	8		
	>34	44	62	20		
Educational status	Literate	51	71.8	25	0.033	0.85
	Illiterate	20	28.2	9		
Financial dependency	Earning	50	70.4	20	1.39	0.23
	Nonearning	21	29.6	14		

Table 2: Association of behavioral factors with GUD

Total (n=71)	Behavioral factors	Number of patients	%	GUD diagnosed by rapid methods (n=34)	Chi square value	P
History of Promiscuity	Present	59	83.1	33	4.13	0.04*
	Absent	12	16.9	1		
Promiscuity with known/paid person (n=59)	Known	49	83.1	32	3.89	0.04*
	Paid	10	16.9	1		
Sexual orientation	Homosexual	4	5.6	2	0.002	0.959
	Heterosexual	67	94.4	32		
Barrier precautions (condoms)	Used	8	11.3	1	2.03	0.15
	Not used	63	88.7	33		

Note: *=statistically significant (P<0.05)

Table 3: Correlation of demographic and clinical findings with clinical diagnosis

n=71	All (n=71)	Syphilis (n=42)	HSV (n=23)	Chancroid (n=5)	Donovanosis (n=1)
Mean age and SD* (in years)	37.7±12.1	39.4±12.8	34.4±10.2	36±13.1	52
Mean duration of symptoms (days)	17.12	23.2	7.6	10	13
Previous history of genital ulcer (%)	11 (15.5)	1 (2.4)	10 (43.5)	0	0
Multiple ulcers (%)	23 (32.4)	3 (7.1)	18 (78.3)	1 (20)	1 (100)
Previous history of treatment (topical) (%)	11 (15.5)	7 (16.7)	1 (4.3)	2 (40)	1 (100)
SIGNS					
Vesicles present (%)	17 (23.9)	1 (2.4)	16 (69.6)	0	0
Pain (%)	29 (40.8)	2 (4.8)	22 (95.6)	5 (100)	0
Induration (%)	20 (28.2)	20 (47.6)	0	0	0
Inguinal lymphadenopathy (%)	5 (7.04)	0	1 (4.3)	4 (80)	0
Tender adenopathy (%)	4 (5.6)	0	0	4 (80)	0

Note: *=Standard deviation

of 30 minutes. For a single case of donovanosis suspected, Diff Quick also could not help in diagnosing this case, although the TAT was 30 minutes [Table 4 and Figure 1]. Thus, 78.3% of rapid tests confirmed HSV cases were noted, compared to 38.1% of cases of syphilis [Image 2]. 52.1% (37/71) of cases were negative with rapid tests for GUD diagnosis. The absence of multiple GUD pathogens was seen in every single patient in the present study.

Discussion

Genital ulcers have a varied clinical presentation. In the current study, the positivity rate of rapid tests in microbiology laboratory or at STD clinic was less than 50%. Genital herpes was the most common cause of GUD aided by rapid lab methods, followed by syphilis. Similar findings were observed in other studies in India and other countries too.^[13-15] Chancroid and donovanosis could not be diagnosed by rapid methods in our study as they are rarely encountered in developing countries like India now.^[16,17]

Men presented with GUD more commonly in the present study, as they have opportunities for practicing high-risk behavior compared to women.^[18] The mean age of patients in our study was 37.7 ± 12.1 years, which is similar to the study done by Looker KJ *et al.* in 2020,^[19] where they observed that the burden of GUD for HSV-2 increased with age. This age distribution in our study differs from a few recent studies where GUD was observed in a younger age group (less than 35 years).^[20-22] The mean duration of symptoms in patients with GUD in our study was an average of 17 days, which is like a study done in

2002.^[7] Multiple ulcers were commonly noted in herpes cases in the present study, like few studies.^[23,24] Induration, although a common finding in syphilis, was seen in only 47.6% of cases, as protean manifestations of syphilis are common.^[25,26]

Educational status was defined based on their ability to read, write, and understand any language based on article of the Constitution of India.^[27] GUD was seen more among literates in our study as they probably gained more information from peers or mobile phones on STIs, etc., which was also highlighted by a study done in New Delhi in 2013.^[18] In our study, GUD was encountered more in earning patients, which was a new finding, as low education and poverty autonomously have an influence on STI behavior and infections.^[28]

Promiscuous sexual behavior was the most important determinant of GUD in this study, which is similar to a study done in Tanzania in 2011.^[29] Patients never needed to pay a known person, leading to more promiscuous behavior, and this finding was similar to a study in 2012.^[6] Heterosexual orientation was predominant in the present study, similar to a study in New Delhi in 2013.^[18] Lack of barrier precaution used for sexual intercourse is itself an independent risk factor for any STI, which was also seen in our study. Barrier precautions were used when a paid partner was sought.

There was an interlapping of the GUD diagnosis when the patient complained of pain. A thorough history, along with general and local examinations, aided the further planning of rapid tests to be

Table 4: Utility and turnaround time (TAT) of rapid tests in diagnosing GUD

n=71	Clinical diagnosis (n)	Lab-confirmed GUD by Rapid methods (n)	%	Rapid Tests performed	Positives (%)	TAT* (mins)
Syphilis	42	16	38.1	Darkfield microscopy	0	30
				Rapid test-ICT [†]	16 (38.1)	60
				RPR [‡]	16 (38.1)	60
Herpes	23	18	78.3	Tzanck smear	12 (52.2)	60
				Rapid test-HSV-2 [§] IgM/IgG Ab	6 (26.1)	60
Chancroid	5	0	0	Gram stain	0	30
Donovanosis	1	0	0	Giemsa stain	0	60
				Diff Quick stain	0	30
Total	71	34	47.9			

Note: * = Turnaround time, [†] = Immunochromatographic test, [‡] = Rapid plasma reagin test, [§] = Herpes simplex virus-2

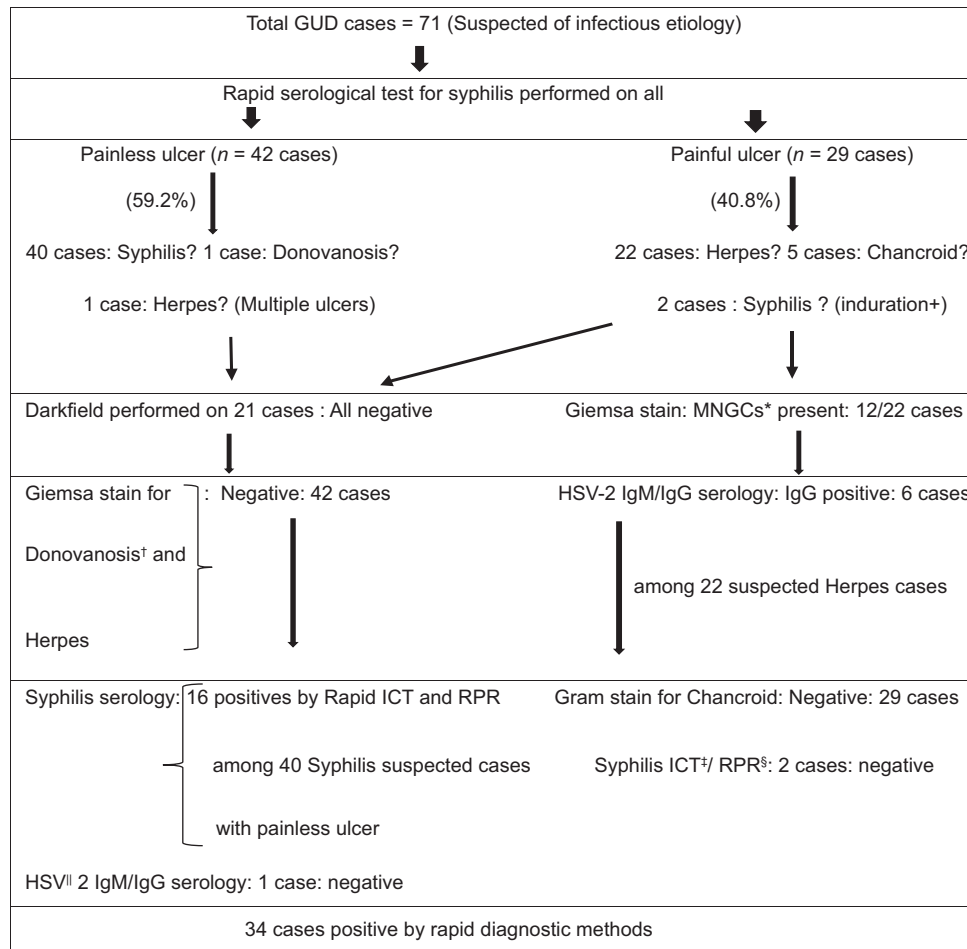


Figure 1: Algorithm for testing patients suspected with GUD. Note: * = Multinucleated giant cells, † = Diff Quick stain done simultaneously, ‡ = Immunochromatographic test, § = Rapid plasma reagin test, || = Herpes simplex virus

carried out. GUD needs to be evaluated by a lab-based or syndromic approach, as inaccuracies in clinical diagnosis can be seen even by the most experienced clinician in more than 50% of cases.^[30] DFM was carried out on 21 patients' samples only. The availability of serous exudate was a prerequisite at our setup as the presence of fibers, RBCs, or refractile elements (when collected in the absence of serous exudate) could result in misinterpretation during DFM. Purwoko *et al.* (2021),^[31] on laboratory diagnosis of syphilis, emphasized the drawbacks of DFM like false positivity, false negativity, and misinterpretation and suggested DFM for lesions

with serous discharge only. Thus, routine screening of all painless ulcers for DFM is not recommended due to practical reasons. The sensitivity of DFM is 74–79%, which is less and decreases with time. Few patients (7/21) gave a history of topical application of ointments on ulcers, which further decreased sensitivity leading to negative DFM in the present study.^[32] This finding contrasts with a study done by Murlidhar *et al.* in 2013,^[18] where the positivity rate of DFM was 4.76%. They carried out DFM in more cases (63 cases) compared to our study (21 cases). As genital ulcer presentation precedes positive serological tests in syphilis, as seen in the present



Image 1: Herpetic lesions on external genitalia of male patient

study, where 26 cases were negative of 42 tested, awareness of this fact has a crucial role during clinical practice.^[30]

The Tzanck smear showed better results compared to serology for HSV diagnosis. Our study had a better detection rate (52.2%) for Tzanck when compared to the study done in 2013 which was 30.3%.^[4] Although NAAT has more sensitivity and specificity compared to Tzanck, subjective errors can be mitigated by thorough history taking, local examination, and microscopic diagnosis by experienced personnel, as done by a recent study in 2022 where 84.62% of patients had complete concordance with respect to clinical and Tzanck smear diagnosis for herpes.^[33] For herpes, rapid tests were positive for IgG antibodies for HSV-2, which indicated previous acquisition of the herpes virus and development of antibodies to it. Some studies have highlighted the use of HSV-2 IgG antibodies for the detection of genital herpes in symptomatic patients.^[34,35] The IgM antibody for HSV-2 was absent in our cases, although it helps in screening and may indicate seroconversion or reactivation: It is considered a poor marker for new infection.^[36,37] Microscopy did not aid in the diagnosis of chancroid and donovanosis, as some of our patients (3/6) had visits to local registered medical practitioners (RMP), who advised them on topical treatment. Another reason could be that for the diagnosis of donovanosis, microscopy has a sensitivity of only 60 to 80%. Microscope sensitivity for chancroid ranges from 5 to 63%, which is also low.^[38,39] Although a study by Murlidhar *et al.* in 2013^[18] showed 3.4% positivity of gram stain for chancroid, it was carried out on 89 samples, which was high compared to our sample size of 29. Diff Quick or Rapi-Diff stain is also considered effective for diagnosing donovanosis, but it could not aid in diagnosis in the present study.^[40] This study was conducted with affordable, easily procurable kits and reagents.

As patients with GUD are treated on an outpatient basis, the availability of rapid tests (microscopic or serological) can not only aid GUD diagnosis but can also assist all hospitals in offering expeditious patient services. It can also indirectly decrease the STI burden on society when patients are counseled for abstinence and partner treatment.

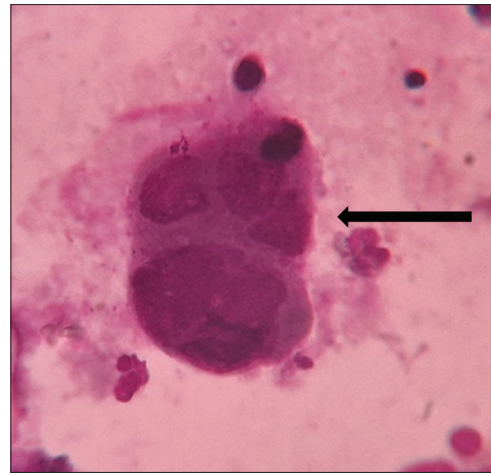


Image 2: Tzanck smear showing multinucleated giant cells (MNGCs) with nuclear molding (arrow)

Limitations

Certain factors, like the number of sexual partners, substance abuse, etc., were not studied. Rapid testing of patients for LGV was not cost-effective and was not used due to the non-affordability of patients. As this was a single-center study, the results cannot be extrapolated to the general population.

Conclusion

GUD was more common in middle-aged, earning, and literate patients. Behavioral factors like promiscuity with a known person and nonuse of barrier precautions during coitus were associated with GUD. Although rapid tests for herpes and syphilis could aid in GUD diagnosis, they were not effective for the diagnosis of chancroid and donovanosis. Rapid lab-based diagnostic methods aid in early diagnosis and prompt treatment of GUD.

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Conflicts of interest

There are no conflicts of interest.

References

1. Sousa JD, Müller V, Vandamme AM. The impact of genital ulcers on HIV transmission has been underestimated-A critical review. *Viruses* 2022;14:538.
2. Wright SS, Kreisel KM, Hitt JC, Pagaoa MA, Weinstock HS,

- Thorpe PG. Impact of the COVID-19 pandemic on centers for disease control and prevention-funded sexually transmitted disease programs. *Sex Transm Dis* 2022;49:e61-3.
3. Cunha Ramos M, Nicola MRC, Bezerra NTC, Sardinha JCG, Sampaio de Souza Morais J, Schettini AP. Genital ulcers caused by sexually transmitted agents. *An Bras Dermatol* 2022;97:551-65.
 4. Gomes Naveca F, Sabidó M, Amaral Pires de Almeida T, Araújo Veras E, Contreras Mejía Mdel C, Galban E, *et al.* Etiology of genital ulcer disease in a sexually transmitted infection reference centre in Manaus, Brazilian Amazon. *PLoS One* 2013;8:e63953.
 5. Loh AJW, Ting EL, Wi TE, Mayaud P, Chow EPF, Santesso N, *et al.* The Diagnostic accuracy of syndromic management for genital ulcer disease: A systematic review and meta-analysis. *Front Med* 2022;8:806605.
 6. Prabhakar P, Narayanan P, Deshpande GR, Das A, Neilsen G, Mehendale S, *et al.* Genital ulcer disease in India: Etiologies and performance of current syndrome guidelines. *Sex Transm Dis* 2012;39:906-10.
 7. Sanchez J, Volquez C, Totten PA, Campos PE, Ryan C, Catlin M, *et al.* The etiology and management of genital ulcers in the Dominican Republic and Peru. *Sex Transm Dis* 2002;29:559-67.
 8. Muralidhar S. Molecular methods in the laboratory diagnosis of sexually transmitted infections. *Indian J Sex Transm Dis* 2015;36:9-17.
 9. Ahmed J, Rawre J, Dhawan N, Dudani P, Khanna N, Dhawan B. Genital ulcer disease: A review. *J Family Med Prim Care* 2022;11:4255-62.
 10. Vargas S, Calvo G, Oquellon J, Vasquez F, Blondeel K, Ballard R, *et al.* Point-of-care testing for sexually transmitted infections in low-resource settings. *Clin Microbiol Infect* 2022;28:946-51.
 11. Sun YY, Li M, Lenzen M, Malik A, Pomponi F. Tourism, job vulnerability and income inequality during the COVID-19 pandemic: A global perspective. *Ann Tour Res Empir Insights* 2022;3:100046.
 12. Saladino V, Algeri D, Auriemma V. The psychological and social impact of Covid-19: New perspectives of well-being. *Front Psychol* 2020;11:577684.
 13. Kumar B, Sahoo B, Gupta S, Jain R. Rising incidence of genital herpes over two decades in a sexually transmitted disease clinic in North India. *J Dermatol* 2002;29:74-8.
 14. Singh A, Preiksaitis J, Ferenczy A, Romanowski B. The laboratory diagnosis of herpes simplex virus infections. *Can J Infect Dis Med Microbiol* 2005;16:92-8.
 15. Mathew R, Najeem B, Sobhanakumary K, Sunny B, Pinheiro C, Anukumar B. Herpes simplex virus 1 and 2 in herpes genitalis: A polymerase chain reaction-based study from Kerala. *Indian J Dermatol* 2018;63:475-8.
 16. Ray K, Bala M, Gupta SM, Khunger N, Puri P, Muralidhar S, *et al.* Changing trends in sexually transmitted infections at a Regional STD Centre in north India. *Indian J Med Res* 2006;124:559-68.
 17. González-Beiras C, Marks M, Chen CY, Roberts S, Mitjà O. Epidemiology of *Haemophilus ducreyi* infection. *Emerg Infect Dis* 2016;22:1-8.
 18. Muralidhar S, Talwar R, Anil Kumar D, Kumar J, Bala M, Khan N, *et al.* Genital ulcer disease: How worrisome is it today? A status report from New Delhi, India. *J Sex Transm Dis* 2013;2013:203636.
 19. Looker KJ, Johnston C, Welton NJ, James C, Vickerman P, Turner KME, *et al.* The global and regional burden of genital ulcer disease due to herpes simplex virus: A natural history modelling study. *BMJ Glob Health* 2020;5:e001875.
 20. Kaur T, Shergill HK. Seroprevalence of human immunodeficiency virus among reproductive age group females presenting with genital ulcer: Study from a tertiary care Centre in India. *Int J Reprod Contracept Obstet Gynecol* 2020;9:3737-41.
 21. Roett MA. Genital ulcers: Differential diagnosis and management. *Am Fam Physician* 2020;101:355-61.
 22. Tshaka TR, Singh R, Apalata TR, Mbulawa ZZA. Etiology of genital ulcer disease and associated factors among Mthatha public clinic attendees. *S Afr J Infect Dis* 2022;37:a444.
 23. Parra-Sanchez M. Genital ulcers caused by herpes simplex virus. *Enferm Infect Microbiol Clin* 2019;37:260-4.
 24. Corey L, Adams HG, Brown ZA, Holmes KK. Genital herpes simplex virus infections: Clinical manifestations, course, and complications. *Ann Intern Med* 1983;98:958-72.
 25. Rawre J, Rai M, Namdeo D, Das R, Khanna N, Dar L, *et al.* Herpes simplex virus type 2 and cytomegalovirus perigenital ulcer in an HIV infected woman. *Indian J Med Microbiol* 2018;36:441-3.
 26. Maliyar K, Mufti A, Syed M, Selk A, Dutil M, Bunce PE, *et al.* Genital ulcer disease: A review of pathogenesis and clinical features. *J Cutan Med Surg* 2019;23:624-34.
 27. Parasaran SK. Literacy in the context of the Constitution of India. *Vigyan Bhawan Annex*. September 26, 2001. Consultation paper 237-79. Available from: [https://legallaffairs.gov.in/sites/default/files/Literacy in the context of Constitution of India.pdf](https://legallaffairs.gov.in/sites/default/files/Literacy%20in%20the%20context%20of%20Constitution%20of%20India.pdf). [Last accessed on 2023 Jul 01].
 28. Scheidell JD, Beau De Rochars VM, Séraphin MN, Hobbs MM, Morris JG Jr, Célestin JP, *et al.* Socioeconomic vulnerability and sexually transmitted infection among pregnant Haitian women. *Sex Transm Dis* 2018;45:626-31.
 29. Ghebremichael M, Paintsil E. High risk behaviors and sexually transmitted infections among men in Tanzania. *AIDS Behav* 2011;15:1026-32.
 30. Genital ulcer disease syndrome. Guidelines for the Management of Symptomatic Sexually Transmitted Infections. Geneva: World Health Organization; 2021.
 31. Purwoko MIH, Devi M, Nugroho SA, Fitriani F, Pamudji R, Candra NC. Laboratory examination of syphilis. *Biosci Med* 2021;5:726-45.
 32. Tudor ME, Al Aboud AM, Leslie SW, Syphilis GW. In: *Stat Pearls*. Treasure Island (FL): Stat Pearls Publishing; 2023.
 33. Shruthi HN, Kumarguru BN, Ramaswamy AS, Shivakumar V. Role of Tzanck Smear cytology in dermatology: A clinicopathological study. *J Clin Diagn Res* 2022;16:WC13-7.
 34. Morrow R, Friedrich D. Performance of a novel test for IgM and IgG antibodies in subjects with culture-documented genital herpes simplex virus-1 or -2 infection. *Clin Microbiol Infect* 2006;12:463-9.
 35. Nath P, Kabir MA, Doust SK, Ray A. Diagnosis of herpes simplex virus: Laboratory and point-of-care techniques. *Infect Dis Rep* 2021;13:518-39.
 36. Johnston C, Ashley Morrow R, Moreland A, Wald A. Genital

- herpes. In: Morse SA, Ballard RC, Holmes KK, Moreland AA, editors. Atlas of Sexually Transmitted Diseases and AIDS. Philadelphia: Saunders; 2010. p. 169-85.
37. Tada DG, Khandelwal N. Serum HSV-1 and 2 IgM in sexually transmitted diseases-More for screening less for diagnosis: An Evaluation of clinical manifestation. *J Glob Infect Dis* 2012;4:S1-4.
 38. Alfa M. The laboratory diagnosis of *Haemophilus ducreyi*. *Can J Infect Dis Med Microbiol* 2005;16:31-4.
 39. Raffe S, Soni S. Diagnostic tests for sexually transmitted infections. *Medicine* 2018;46:277-82.
 40. O'Farrell N, Hoosen AA, Coetzee K, van den Ende J. A rapid stain for the diagnosis of granuloma inguinale. *Genitourin Med* 1990;66:200-1.