

Prevalence of urethral pathogens and HIV co-infection among men presenting with urethral discharge

Surafel Fentaw¹, Rajiha Abubeker¹, Negga Asamene¹, Yenew Kebede², Firehiwot Eshetu², Yared Tedla², Afework Mebratu², Ashenafi Haile², Jelaludin Ahmed², Gaston Degmond², Qualls Michael², Almaz Abebe¹, Adugna Woyessa¹, Yibeltal Assefa¹ and Amha Kebede¹

¹Ethiopian Public Health Institute, P.O.Box 1242 or 5456, Addis Ababa, Ethiopia

²CDC-Ethiopia

¹Corresponding author: Surafel Fentaw, sura4f@gmail.com

Abstract

Background: Sexually transmitted infections are major public health challenges currently, due to the high frequency of infections accompanied by a declining of treatment options. Especially in developing nations, the spread of STIs/HIV infection continues to affect millions of young and productive population. This study was aimed to determine the prevalence of STI agents among male patients presenting with urethral discharge syndrome.

Methods: An institutional based cross sectional study was conducted at health centers in Addis Ababa from August 2013-August 2014. Urethral discharge specimens were cultured on Modified Thayer Martin media and suspected gonococcal colonies were confirmed using Oxidase, Superoxol tests followed by API-NH^R. In addition, urine samples were tested against STI agents (*Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis* and *Mycoplasma genitalium*) by Real Time PCR. Serologic Syphilis testing was done using standard tests. HIV test was done by rapid test (KHB^R, Statpack^R and Unigold^R) at the spot.

Results: Five hundred ninety nine urethral discharge specimens were collected and out of these 415 were microbiology culture positive for gonococcus. The Real Time PCR result showed that 321(53.6%), 79(13.2%), 40(6.7%) and 21(3.5%) were found to be positive for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis* and *Mycoplasma genitalium* respectively. Out of the 274 patients who got tested for HIV, 19 of them (7%) were found to be HIV positive. The prevalence of syphilis was 1.5% (9/599).

Conclusion: There is a higher burden of urethral discharge syndrome and HIV co-infection among the young men with risky behavior. Gonococcal infection is the leading urethral discharge syndrome followed by chlamydial infection.

Key words: Sexually transmitted infection, Urethral discharge syndrome.

Introduction

Sexually transmitted infections (STIs) are one of the important public health problems in both developed and developing countries. Sexually transmitted diseases (STDs) are among the first top ten causes of morbidity in young adult males in developing countries and the second in young adult women (Workowski *et al.* 2008). The most frequent curable STI infections are caused by bacteria or protozoa (*Treponema palladium*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and *Trichomoniasis vaginalis*). If STI is left untreated, it has serious adverse health consequences including adverse pregnancy outcomes, reproductive morbidity and mortality, and enhanced HIV transmission (WHO2007). In addition, the high prevalence of these infections accompanied by declining of treatment options are the reasons those considered as public health priorities. The high rate of asymptomatic infections and the consequent under-diagnosis explain the widespread problem of under reporting, difficulty in assessing the true incidence

and prevalence of STI (Mayaud *et al.* 2004). Quantifying the incidence and burden of these infections is important for planning appropriate interventions and advocating for resources, as necessary (WHO 2007). This study focuses on determination of the prevalence of STI agents among male patients presenting with urethral discharge syndrome in Addis Ababa, Ethiopia.

Materials and Methods

The study was conducted in Addis Ababa City Administration. It is geographically located in the central part of the country. The population of Addis Ababa were estimated to be 3,195,000 consisting of 1,515,000 men and 1,680,000 women (CSA 2014). A cross sectional facility based study was conducted in the health centers of Addis Ababa. The selected health facilities were Arada, Teklehaimanot, Addis Ketema, Kirkose, Kotebe, Akaki- Kaliti, Shiromeda and Kassanchis health centers. These health facilities were selected purposively based on assessment made before this study on the flow of STI clients.

There were eight study teams, one nurse and one coordinator. Following training on study protocol, procedures, and research ethics, the study team stayed twelve months at the study site collecting samples.

Specimen Collection: Men presenting to the selected health facilities with urethral discharge syndrome, a sterile cotton-tipped swab were used to obtain a metal swab. Then sterile Dacron swabs tipped applicator were used to collect urethral discharges. The swabs were inoculated on in-house prepared Modified Thayer Martin Agar plates made of Gonococcal agar base supplemented with isovitalex (vitox); vancomycin, colistin, nystatin, and trimethoprim (VCNT); and synthetic hemoglobin (Oxoid and BBL). The inoculated plates were incubated on the site using candle jar and transported to the Ethiopian Public Health Institute Clinical Bacteriology and Mycology Reference Laboratory within the same day of collection. Swab was rolled onto a microscopy slide, labeled, heat fixed, placed in a slide box and sent to EPHI for Gram-stain analysis.

In addition to urethral discharge, first-catch urine specimen (approximately 20ml) were also collected to be tested by a Real-Time, multiplex polymerase chain reaction (RT-PCR) assay to determine the presence of *C. trachomatis*, *N. gonorrhoeae*, *M. genitalum* and *T. vaginalis*. Five ml whole blood was also drawn using plain tube from the same patients for serological test of syphilis.

Laboratory analysis

a. Culture and identification: In the clinical bacteriology laboratory, inoculated plates were incubated at 35°C in carbon dioxide enriched environment (5-8 % CO₂) for 72 hours inspecting every day for the growth of small, translucent and non-pigmented colonies. Isolates with convex, glistening, elevated, mucoid and nonhemolytic colony characteristics were taken as probable gonococcus. In addition, a positive reactions for oxidase and supercool (30% H₂O₂) were considered as presumptive of *N. gonorrhoeae*. Confirmatory test was done using API-NH^R.

b. Multiplex Real-Time PCR: Aliquoted urine samples were extracted manually using Qiagen mini kit. The master mix was prepared mixing ready to use multiplex (5-plex) CDC in-house primers, (containing forward and reverse primers of *Chlamydia trachomatis*, *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, RNP and *Mycoplasma genitalum*), Roche, nucleotides, MgCl₂, Taqman polymerase enzyme were mixed with extracted DNA. Amplification was done by Rotor gene QR machine. To monitor the analysis known positive and negative

controls were used along with each run and the result was interpreted using Cycle Threshold (CT) value less than 35 as positive.

c. Serological tests: From the collected blood samples serum was separated and analyzed using rapid plasma reagin (RPR) assays for screening and *Treponema palladium* passive particle agglutination assay (TPPA) for confirmation of syphilis respectively. HIV screening test was done at the spot using rapid test kits recommended by Federal Ministry of Health (KHB^R) followed by StatPack^R and confirmatory Unigold^R.

Ethical clearance

This study was ethically cleared by Scientific and Ethical Review Office (SERO) of Ethiopian Public Health Institute and the IRB of CDC-Atlanta. At the enrollment visit, all men with urethral discharge (UD) were given written consent diagnosed according to the syndromic treatment guidelines currently approved in Ethiopia. Those who are eligible (> 18 years of age) and willing to participate in the study were asked using structured questionnaire for their demographic and behavioral data. All data were kept confidentially anonymously. Brief counseling on the importance of adherence to STI medications, not having sex while taking medications, HIV/STI prevention, and recommendations to use condoms to reduce STI/HIV acquisition and transmission was given. The newly diagnosed were counseled properly at each facility. The participants were provided condoms and information on use of condoms, partner notification cards. Those clients who had positive syphilis tests, with active syphilis documented by a positive TPPA in the absence of ulcer, were contacted and treated for latent syphilis of unknown duration with Benzathine penicillin.

Results

Socio-demographic characteristics: A total of 599 patients with urethral discharge syndrome were recruited from eight health centers in Addis Ababa from August 2013 to August 2014. About quarter of the patients was reported from Kazanchis health center and 19% were recruited from Arada Health center (Figure 1). Out of 599 participants around 40% patients were in the age group of 25-29 years, and about one third of the patients (33%) were 20-24 years old. A considerable proportion (4.7%) of the patients was 18 or 19 years old (Figure 2).

Laboratory test results: Gram stain was done for 599 collected specimens and 449 (74.8%) were found to be gram negative diplococci. GC culture was done for all collected samples 599 specimens and 415 (69.3%) were culture positive and confirmed by API-NH test.

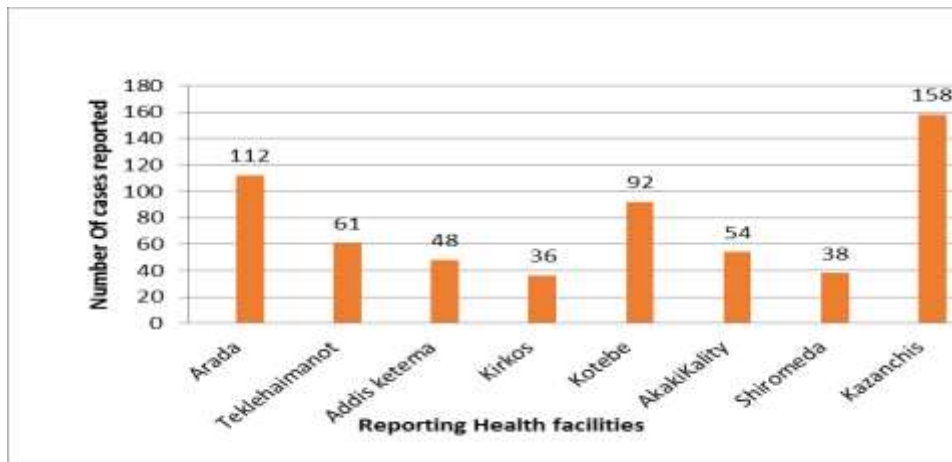


Figure 1: Distribution of study participants with urethral discharge by health facility

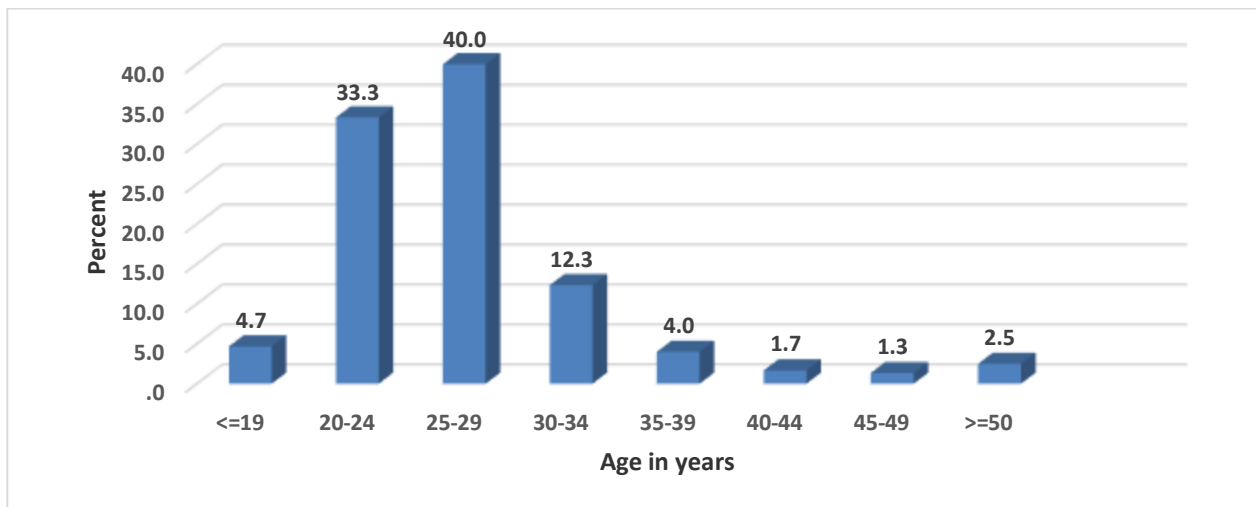


Figure 2: Age distribution of study participants with urethral discharge

Table 1: Gram stains and culture laboratory results

GC confirmation	Method	Result	Frequency	Percent
Gram stain		Positive	449	75%
		Negative	150	25
Culture		Positive	415	69%
		Negative	184	31%
		Total	599	100

HIV status of patients with Urethral discharge and sexual characteristics: Out of the total UD patients enrolled in the study, HIV testing was performed for nearly half (44%) of the patients, 8 patients were known HIV positives and the remaining were not tested since some refused to get tested and others couldn't get the testing at the public health facility due

to critical HIV test kit shortage throughout the country during the study period. Out of the 274 patients who got tested for HIV, 19 of them (7%) were found to be HIV positive, 11 of HIV positives were identified on the survey and 8 patients are known HIV positives (Table 2).

Table 2: HIV and Syphilis status co-infection among men presenting with urethral discharge

HIV testing	Response	Frequency	Percent
Offered for HIV testing	Performed	266	44.3
	Known Positive	8	1.3
	Refused/Not Tested	325	54.2
	Total	599	100
HIV test result	Positive	19	6.9
	Negative	255	93.1
	Total	274	100
Syphilis serology- RPR	Reactive	36	6
	Non-Reactive	563	94
	Total	599	100
Syphilis serology- TPPA	Reactive	9	25
	Non-Reactive	27	75
	Total	36	100

The syphilis screening test was done for all the 599 specimens by RPR test and 36 (6%) of them were found to be reactive. The RPR reactive 36 samples were confirmed using TPPA and 9 (25%) of them were confirmed positive for syphilis infection (Table 2).

Concerning sexual characteristics of patients, majority (65%) claimed that they have only one sex partner, and 13% had 3 or more sexual partners in the last three

months. Majority of the participants (44%) confessed that they have never used condom in the last three months and only 19% responded consistent use of condom. About 71% of the patients did not use condom during the last sex. Last sex was with their regular partner in 42% of the patients, 28% had last sex with a casual partner and about 15% with a sex worker (Table 3).

Table 3: Sexual behavior, prevalence of urethral pathogens and HIV co-infection among men presenting with urethral discharge

Last sexual Partner	Condom use during last sex	Frequency
Spouse/Live in partner	Yes	10
	No	61
Regular Partner	Yes	40
	No	213
Casual Partner	Yes	59
	No	108
Commercial sex worker	Yes	53
	No	31
Unspecified	Yes	3
	No	15

Of the total 599 UD samples subjected to Multiplex PCR analysis 321(53.6%), 79 (13.2%), 40 (6.7%) and 21(3.5%) were found to be positive for

N.gonorrhoeae, *Chlamydia trachomatis*, *Trichomonas vaginalis* and *Mycoplasma genitalum* respectively (Table 4).

Table 4: STI etiologies identified using multiplex PCR from patients treated at health centers in Addis Ababa, August 2013- August 2014

STI etiologies	Multiplex PCR result		
	Positive	Negative	Percent
<i>N. gonorrhoeae</i>	321	278	53.6
<i>C. trachomatis</i>	79	520	13.2
<i>T. vaginalis</i>	40	559	7.0
<i>M. genitalum</i>	21	578	3.5

Discussion

In this study around 40% of urethral discharge patients were in the age group of 25-29 years, and about one third of the patients (33%) were 20-24 years old. Considerable proportions (4.7%) of the patients were 18 or 19 years old. This finding is somehow similar with findings on the EPHI national STI case surveillance report for 2014, where a total of 623 STI cases were reported from eight sentinel surveillance sites located in Amhara, Oromia and Addis Ababa regions, and majority (69%) of the STI patients were in the age group 20-34 and about 7.5% of the patients were below 19 years of age (National STI Case Surveillance Report, 2013- 2014). Moreover, these participants were at age group of 18 up to 29 years and have been exercising unsafe sex with their partners or female commercial sex workers (Table 3). Regarding behavioral characteristics of urethral discharge patients, about 28% of the UD patients had their last sex with a casual partner and about 15% with sex workers. Out of 599 participants in the study around 71% of the patients did not use condom during their last sex. The prevalence of risky sexual behavior in this study is found to be worse than the findings on the national STI surveillance where, 40% of them haven't used condom during the last sexual contact with a casual partner. This difference could be because the current survey is done only in Addis Ababa, which is purely a major urban town. This implies that there is still a prevailing risky behavior of unprotected sexual intercourse among the younger age group despite the existing behavioral change programs being done in the country.

Around 449 urethral discharges had intracellular or extracellular gram negative diplococci with moderate amount of polymorph nuclear cells. Whereas, 415 (69%) urethral discharges were culture positive. The difference number of detection might be due to prior antibiotic treatment that can inhibit culture growth. Out of 599 urine samples, 321 (54%) were positive for *N. gonorrhoeae*, and 79 (13%), 40(7%) and 21 (3.5%) were positive for *Chlamydia trachomatis*,

Trichomonas vaginalis and *Mycoplasma genitalum* respectively on PCR test. The pattern of STI etiologies is in line with the study from South Africa, where urine multiplex PCR showed gonococcus led on 85% of Cape Town and 71% Johannesburg urethral discharge patients (Mhlongo *et al.* 2010).

Chlamydial infection was found the second next to gonococcal infection which accounts 13% of the participants. This result is somehow similar to studies conducted in South Africa, where the KZN study found a 16% rate of infection (Sturm *et al* 2004) and another study from Johannesburg reported 19% (Black *et al.* 2008).

This study shows that the prevalence rate of *Trichomonas vaginalis* was around 40 (7%). This finding is higher than the Korean study which was studied by molecular technique that had a prevalence of 4 % (10) and comparable with a study conducted in South Africa which was 6% (Sturm *et al.* 2004).

Since *M. genitalum* takes a very long time to culture, molecular diagnostic methods are better option to diagnose. The pathogen status of *M. genitalum* in this study was around 3.5% which is lower than a study in South Africa, where it was implicated as the causal agent in 18-46% of men with urethritis and 5% in the overall prevalence of South African study (Sturm. *et al.*2004).

In this study around 75 of participants had multiple infections of gonococcal and or non-gonococcal infections. Of the 599 participants, co-infection with *gonorrhoeae* and *chlamydia* was present only in 53 (8.8%), co-infection with *gonorrhoeae* and *trichomonas* only in 12 (2%), co-infection with *gonorrhoeae* and *mycoplasma* only in 6(1%) and *chlamydia* and *trichomonas* only in 4 (0.7 %). The prevalence of syphilis by RPR test was 36/599 (18.5%) but out of 36 RPR positive only 9 (1.5%) of them were confirmed positive by TPPA which is the more specific test. This result coincides with another study conducted in Gondar (Tessema *et al.* 2010).

Out of the 274 patients who got tested for HIV, 19 of them (7%) were found to be HIV positive, 11 of HIV positives were identified on the survey and 8 patients were known HIV positives. The HIV prevalence in this survey is a bit lower than the one reported on the national STI case surveillance, where HIV prevalence among UD patients was 13% (National STI Case Surveillance Report, 2013- 2014). But the HIV prevalence on this survey may not be accurate enough to show the true picture of HIV status among UD patients, because only 44% of patients with UD were tested due to the critical test kit shortage in the country at the time of the survey. In addition, the low rate of HIV positivity in the presence of confirmed bacterial STI might be explained by the longer seroconversion time of HIV than gonococcal infection (Incidence Assay Critical Path Working Group 2011).

Conclusion

There is a higher burden of urethral discharge syndrome among the young men less than 30 years of age implying a prevailing risky behavior of unprotected sexual intercourse. There is a high prevalence of risky sexual behaviors, multiple sexual partner contact, unprotected casual sex, low utilization of condom, in the community which pause people at risk of acquiring STIs and a significant co-infection of HIV among patients with urethral discharge. *Gonococcal* infection is the leading cause of urethral discharge syndrome followed by *chlamydial* infection.

References

- Black, V., Magooa, P., Radebe, F., Myers, M., Pillay, C. & Lewis, D. (2008). The detection of urethritis pathogens among patients with the male urethritis syndrome, genital ulcer syndrome and HIV VCT clients: Should South Africa's syndromic management approach is revised? *Sexually Transmitted Infectious Diseases*, 84, 254-258.
- Global Strategy for the Prevention and Control of Sexually Transmitted Infections, 2006-2015: Breaking the chain of transmission. WHO Press, Geneva.
- Incidence Assay Critical Path Working Group. (2011). More and better information to tackle HIV epidemics: towards improved HIV incidence assays.
- Mayaud, P. & Mabey, D. (2004). Approaches to the control of sexually transmitted infections in developing countries: old problems and modern challenges. *Sexually Transmitted Infectious*, 80, 174-182.
- Mhlongo, S., Magooa, P., Müller, E., Nel, N., Radebe, F., Wasserman, H. *et al.* (2010). Etiology and STI/HIV co-infections among patients with urethral and vaginal discharge syndromes in South Africa. *Sexually Transmitted Diseases*, 37, 566-70.
- National STI case surveillance report, 2013- 2014. Ethiopian public health Institute.
- Seo, J., Yang, H., Joo, S., Song, S., Lee, Y., Ryu, J. *et al.* (2004). Prevalence of *Trichomonas vaginalis* by PCR in men attending a primary care urology clinic in South Korea. *Korean Journal of Parasitology*, 52, 551-555.
- Sturm, P., Moodley, P. & Khan, N. (2004). Aetiology of male urethritis in patients recruited from a population with a high HIV prevalence. *International Journal of Antimicrobial Agents*, 245, S8-14.
- Tessema, B., Yismaw, G., Kassu, A., Amsalu, A., Mulu, A., Emmrich, F. *et al.* (2010). Seroprevalence of HIV, HBV, HCV and syphilis infections among blood donors at University of Gondar Teaching Hospital, Northwest Ethiopia: declining trends over a period of five years. *BMC Infectious Diseases*, 10, 111.
- Workowski, A., Berman, M., & Douglas, M. (2008). Emerging antimicrobial resistance in *Neisseria gonorrhoeae*: urgent need to strengthen prevention strategies. *Annals of Internal Medicine*, 148, 606-613.
- WHO (2003). Guidelines for the management of sexually transmitted infections, Geneva, pp.1-91.