Chlamydia trachomatis detection in HIV infected patients using polymerase chain reaction

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ABSTRACT

INTRODUCTION: *Chlamydia trachomatis* is a sexually transmitted organism and causes important public health problem in the sexually active age group. Limited studies are found regarding the prevalence of *C. trachomatis* in Nepal.This study attempts to determine the burden of chlamydia on HIV positive patients.

MATERIALS AND METHODS: A total of 117 HIV positive patients visiting a HIV clinic in Kathmandu, were screened for chlamydia infection. For this, urine samples were collected and analyzed using the Polymerase Chain Reaction Technique (PCR).

RESULTS: *C. trachomatis* was detected in 4.2% of the total 117 HIV patients. Out of positive cases 60% were males and 40% were females. Eighty percent of positive cases were asymptomatic.

CONCLUSIONS: Although, the prevalence of chlamydia infection was found less in HIV patients, most of those cases were asymptomatic. Therefore, routine checkup is recommended for all suspected cases for timely management of the disease.

KEY WORDS: HIV, Sexually transmitted infection (STI), Multiplex Polymerase Chain Reaction, Nepal

INTRODUCTION

Chlamydia trachomatis is an intracellular bacterium which causes genital tract infections. Human are its natural host.[1] It causes one of the most common treatable sexually transmitted disease (STD) among sexually active group.[2] Chlamydia can be transmitted by vaginal, oral or anal sex, putting homosexual men at risk as well. Chlamydia infections can also be passed down from an infected mother to her baby during vaginal childbirth.[3]

Chlamydia disease is a "silent disease", with approximately 75% of infected women and 50% of infected men showing no symptoms. If symptoms occur, they usually occur within one to three weeks after exposure.[4] Symptomatic patient may have discharge from penis/vagina or burning and itching around the genital opening and inflammation of the testicles,[5,6] and may lead to sterility and pelvic inflammatory disease.[7]

People with STD are more likely to be infected with HIV as they provide easy passage for the virus to enter the blood streams via the genital ulcerations. STI increases both the infectivity of persons with HIV and the susceptibility of those with STIs to HIV infection.[8] Chlamydia infection is an important biological behavioral marker in HIV infected individuals that may expose others to HIV. Furthermore, chlamydia is associated with increased cervico-vaginal HIV shedding that may increase HIV transmissibility.[9]

Chlamydia infection is an important public health problem in the sexually active age group. Very few studies appear regarding the prevalence of *C. trachomatis* in Nepal. The current study was conducted to determine the burden of chlamydia on HIV positive patients using PCR technique.

MATERIALS AND METHODS

This is a cross-sectional study carried out among HIV positive cases visiting HIV clinic of Sparsha Nepal from 15th January 2011 to 30th March, 2011. Demographic data and other information were collected using data collection sheet and questionnaire through interview. A total of 117 samples were collected and processed for DNA isolation, amplification and detection using agarose gel electrophoresis.[10]

Sample collection and processing: The urine sample was collected and preprocessed using standard protocol. Briefly, from each sample, 10 ml

of urine was taken for centrifugation at 7500 rpm for 10 minutes. The pellet was collected in a clean and sterile test tube, washed with 10-15 ml PBS and re-centrifugation was done at 5000 rpm for 15 minutes. Then, 200 μl PBS was added to the pellet, transferred to 1.5 ml micro-centrifuge tubes and stored at 2-8°C. The processed samples were transported to Everest International Clinic and Research Center (EICRC) for further processing.

DNA isolation, amplification and detection: DNA was extracted as per the standard QIAamp DNA mini kit and handbook protocol (Lot no. 42710823, Cat. no.51306). This DNA sample was used directly for quantification and PCR. Isolated DNA was subjected to amplification using "MPCR kit for sexually transmitted diseases CTR/UU/NG" by Maxim Biotech. Inc., California, USA. In this amplification process the primers were used to amplify 364 bp region of cryptic plasmid gene of *C. trachomatis*. Each 50 μl PCR reaction mixture contained, 25 μl 2X MPCR buffer mixture, 5 μl 10X MPCR primers, 0.5 μl Taq Polymerase (5U/μl), 14.5 μl Water (H_2O) and 5 μl DNA sample. For negative and positive controls, water and positive control provided in the kit were used respectively. The PCR profile used was: 2 cycles of 96°C for 1 min followed by 65°C for 4 min; 35 cycles of 94°C for 1 min followed by 65°C for 2 min; 1 cycles of 70°C for 10 min and at 25°C (for soaking). The PCR product (364 bp) was detected using 1% TAE gel electrophoresis.

Data Analysis: Statistical analysis was done and data was analyzed by using the statistical software SPSS version 13. The association between categorical variable was done using Chi-square test. The p-value less than 0.05 was considered significant.

RESULTS

Among 117 urine samples, 29 (24.79%) were of females and 88 (75.21%) were of males. The age of patients ranged from 20-64 years. The highest number of cases (n=74, 63.5%) belonged to age group 25-40 years.

Prevalence of Chlamydia

Out of the total 117 urine samples collected, only 5 (4.27%) were positive for *C. trachomatis* (Figure) and 112 (95.73%) were negative. Prevalence of *C. trachomatis* in males was 3.4% and that among females was 6.9%. Three positive male were of age 23, 27 and 35 years. On the other hand two positive females were 21 and 22 years of age. No significant difference was observed between sex (χ^2, 6.8; p,

Table 1. Prevalence of chlamydia according to age group and sex

Age Group (years)	Total Patients	Male			Female		
		Total	Positive	Positive %	Total	Positive	Positive %
20-30	42	30	2	6.7	12	2	16.7
31-40	46	37	1	2.7	9	0	0
41-50	23	16	0	0	7	0	0
51-60	5	5	0	0	0	0	0
61-70	1	0	0	0	1	0	0
Total	117	88	3	3.4	29	2	6.9

Association between disease occurrence and sex (χ^2, 6.8; p, 0.44)
Association between disease occurrence and age group (χ^2, 4.6; p, 0.33)

Table 2. Distribution of chlamydia cases according to marital status

Marital status	Number of cases	Positive	Negative	Positive %
Married	98	5	93	5.10
Unmarried	19	0	19	0
Total	117	5	112	4.27

0.44) and also the age group (χ^2, 4.6; P, 0.33).

Chlamydia positive cases according to symptoms and gender

Most cases (n=4) were asymptomatic. One male case was symptomatic with urethral discharge. Statistically, there was no significant difference between the symptomatic cases and disease occurrence (χ^2, 0.42; p, 0.51). Likewise, association between asymptomatic cases and disease occurrence was insignificant (χ^2, 1.68; p, 0.19). Association between marital status and disease occurrence (χ^2, 1.01; p, 0.31) As shown in table 2, all of the positive cases were married. Despite this, there was no statistically significant association between marital status and the occurrence of disease.

Knowledge of STI among Patients

Out of the total 117 cases, only 15 (12.8%) had knowledge of STI where as 102 (87.2%) had no knowledge about the STI. Among the positive cases, two (40%) had knowledge, whereas remaining three (60%) had no knowledge of STI. Statistically, there was no significant association between the knowledge about the disease and the occurrence of the disease (χ^2, 3.35; p, 0.067).

DISCUSSION

Nucleic acid amplification technique like PCR has been used in our study because of its high sensitivity, specificity and rapidity in comparison to conventional culture and serological techniques. There is currently no laboratory in Nepal that offers the chlamydia culture. Neither any antigen detection techniques are more sensitive and specific than
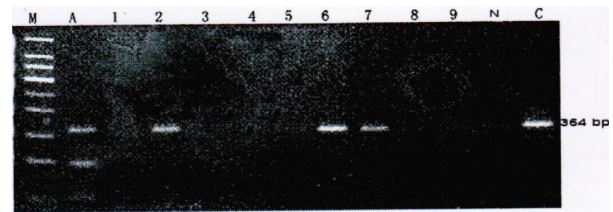

Figure. 1% Gel electrophoresis of amplified PCR products. Lane M, 100bp Molecular weight marker; Lane A and 1 to 9, test samples; Lane N, negative control; and lane C, positive control. Non-specific bands at 100bp and 200bp were noted for sample A.

culture techniques. PCR was found to be 100% sensitive and specific while detecting *C. trachomatis* among female sex workers in Singapore.[11] Even a single organism was said to be detected by PCR with very high sensitivity, specificity and higher predictive values.[12] In the former study, the prevalence was much higher by PCR (4.1%) compared to the one obtained by antigen detection techniques like Enzyme immunoassays (EIA) (1.6%). This prevalence rate as a whole is also in agreement with our study. Less sensitive methods such as EIA result in under-treatment of otherwise undetected cases. PCR based study are of prime importance in such issues. This study, using PCR, clearly showed prevalence of *C. trachomatis* (4.27%) among HIV patients visiting HIV clinics in Nepal. Female population was found to be more susceptible (6.9%) to chlamydial infections than male (3.4%). The Prevalence rate among HIV females was 6 fold greater than in a study carried out among post-partum non-HIV females in rural Nepal.[13] This chlamydia prevalence among HIV individuals in Nepal is found to be greater than that among female sex worker in Singapore (4.1%).[11] These comparisons clearly indicate Nepalese HIV

females are at greater risk of chlamydial infection. No any such studies were carried out previously among male population. However, the sample size may have been inadequate to give out real picture in this study.

Similar prevalence were reported in Amsterdam (4.8%),[14] England and Wales (5.15 %).[15] This prevalence is less than a study conducted in Thailand, where the prevalence rate was 9.7% among 824 HIV seropositive patients.[16] The difference can be due to various reasons, as: the specimens used by them were endo-cervical swabs, and the detection method used was gen-probe. In agreement to this, the overall prevalence rate of *C. trachomatis* in cervical scrapes determined by nested PCR was 10% in 60 Cuban women.[17] Moreover, in a study conducted in Georgia, the seroprevalence of *C. trachomatis* in 234 HIV patients was 23.94%.[18] The strongest predicators in these cases were the history of STI and female gender.

The patients participating in this study ranged from 20 years 64 years. The highest numbers of patients (n=46) were from the age group 31-40. Among the five positive cases, three were within age range 20-25 year, one was twenty seven and the other was thirty-five years of age. Chlamydia infections mainly affect young people, especially young women. In numerous studies, highest incidence was usually reported in age group below 25 years, accounting for more than sixty percentage of all cases.[19,20,21] There are other contradicting studies as well where age group above twenty-five has shown higher prevalence. [22, 23, 24, 25]

Out of total 117 cases, 98 were married and 19 were unmarried. All the positive cases were married. Statistically, no significant association was observed between marital status and occurrence of the disease. Similar insignificant result was observed in a study conducted in Barbados.[21] In contrast, significant result was observed in another study.[26] The relation between the knowledge and occurrence of disease was found to be insignificant in our study, which was in harmony with study by Adams et al., 2008.[21] Of the 117 patients, only 12.8% knew about the STIs and chlamydia whereas the rest 87.2% had no idea of the disease. Out of chlamydia positive cases only 40% knew about STI. This necessitates the increase in effort in disseminating the knowledge of STI to HIV patients as well as to other risk groups.

Asymptomatic cases contributed to 80% of total positive cases. This nature of the disease leaves patients greatly vulnerable to the devastating effect it brings. Along with it, 96.7% of symptomatic individuals were negative indicating the difficulty in relying solely on clinical diagnosis of disease. Chlamydia thus possesses high risk to all infected individuals. The lack of resources, as well as, consequences of the disease has made early screening, diagnosis and treatment process very important.

CONCLUSION

Chlamydia infection is found less common among normal population and most of those cases are asymptomatic therefore go undiagnosed. In contrast, there exists higher prevalence rate among HIV subjects. Asymptomatic nature of chlamydial infection creates difficulty in the timely detection of *C. trachomatis* as well as its clinical sequel, which might be devastating. Therefore, routine checkup is recommended for all risk groups for timely management of the disease. PCR based diagnostic tests for chlamydia need to be introduced as a part of routine checkup for high risk populations.

CONFLICT OF INTEREST: None to declare.

FINANCIAL INTEREST: None to declare.

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