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Research article

Chlamydia trachomatis among HIV infected patients using PCR technique

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ABSTRACT

Background and Objectives: *Chlamydia trachomatis* is a sexually transmitted organism and an important public health problem in the sexually active age group. Limited studies are found regarding the prevalence of *Chlamydia trachomatis* in Nepal. Moreover, no study in Nepal reports the association of Chlamydia and HIV infection. The current study attempts to determine the burden of Chlamydia on HIV positive patients.

Material 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 Multiplex polymerase chain reaction technique (MPCR) and Agarose gel electrophoresis. DNA isolation was performed using QIAamp DNA and Blood mini kit handbook protocol.

Results: *C. trachomatis* was detected in 4.27% of the total 117 HIV patients. Out of positive cases 60% were males and 40% were females. However, Chlamydia is found more prevalent among females (6.89%) than in males (3.4%). Eighty percent of positive cases were asymptomatic.

Conclusion: Chlamydia infection was found less commonly among studied patients and most of those cases were asymptomatic. So there is difficulty in timely detection of *C. trachomatis* and track the clinical sequel, which might be devastating. Hence, routine checkup is recommended for all suspected cases for timely management of the disease.

Keywords: *Chlamydia trachomatis*, HIV, Sexually transmitted infection (STI), MPCR

INTRODUCTION

Chlamydia trachomatis is a bacteria which causes ocular and genital tract infections. It is one of the most common sexually transmitted disease (STD) of bacteriological etiology found worldwide. It is prevalent primarily in areas of poverty and overcrowding with millions of new cases occurring yearly. Humans are its natural host [1]. The organism is an obligate intracellular parasite, and has an extremely unique life-cycle alternating between non-replicating infectious "elementary body" and replicating non-infectious "reticulate body".

Prevalence of Chlamydia varies enormously across the world. It makes for about 20-25% of sexual infections worldwide. In 1999, 101.5 million Chlamydia cases were estimated worldwide, affecting 53.9 million women and 47.4 million males in the 15-49 year old population; 6.6 million cases among them occurring in south and Southeast Asia [2]. It is one of the most common treatable bacterial STD [3] among sexually active group. The greater the number of sex partners, the greater the risk of infection. Chlamydia can be transmitted by vaginal, oral or anal sex, putting homosexual men at risk as well. Chlamydia infections are also reported be associated with miscarriage, stillbirth or down infant birth rate. It can also be passed down from an infected mother to her baby during vaginal childbirth [4].

Chlamydia disease is as 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 [5]. Symptomatic patient may have discharge from penis/vagina or dysuria; burning and itching around the genital opening and inflammation of the testicles [6, 7]. In a single Chlamydia infection there is a 25% chance of sterility, 50 % with a second infection and almost guarantees sterility in third due to pelvic inflammatory disease (PID) [8].

People with STD have greater chances 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 [9]. 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 [10].

No doubt, Chlamydia infection is an important public health problem in the sexually active age group. Very few studies appear regarding the prevalence of *Chlamydia trachomatis* in Nepal and not a single study is repoted among individuals suffering from Human Immunodeficiency virus (HIV) has been done. The current study was conducted to determine the burden of Chlamydia on HIV positive patients using PCR technique.

MATERIAL AND METHODS

This is a cross-sectional study carried out among HIV positive cases visiting HIV clinic of Sparsha Nepal from 15thJanuary 2011 to 30th March, 2011. Demographic data and other information were collected using data collection sheet and questionnaire through interview. After that urine samples were collected and processed. A total of 117 samples were processed for DNA isolation, amplification and identified using Agarose gel electrophoresis. Nucleic acid amplification based methods are reported to be of prime imoportance for chlamydial disgnosis. The main advantage of these tests is that such methods combine unsurpassed sensitivity with good specificity [11]. The detail of the process is mentioned below.

Sample collection and processing: The urine sample was collected and preprocessing was done at the spot using standard protocol. 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 microcentrifuge 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: 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. The materials to be discarded were discarded in a container containing freshly prepared 0.5% sodium hypochlorite solution.

DNA amplification: Isolated DNA was subjected to amplification by MPCR technique using commercially available "MPCR kit for sexually transmitted diseases CTR/UU/NG". In this amplification process the primers were used to amplify 364 bp region of cryptic plasmid "cppB" gene of *C. trachomatis*. Whereas the DNA marker contained linear double stranded DNA band of 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 base pairs. Master Reaction Mixture (MRM) was prepared according to the protocol for each sample. For this, 6.25 µl 2 X MPCR buffer mixture, 1.25 µl 10 X MPCR primers , 0.125 µl Taq Polymerase(5U/µl), and 3.625 µl Water (H2O) were mixed together to make a total volume of 11.25 µl, followed by brief vortexing and brief centrifugation. The prepared MRM was dispensed into each labeled PCR tubes and brief centrifugation was done. Thereafter, 1.25 µl of DNA samples were added into respective PCR tubes and mixed well. For negative and positive controls, water and positive control provided in the kit was used. The PCR tubes were then run in the thermocycler as programmed below. Four steps included:

- 96˚C 1 min followed by 65˚C 2 min., 2 cycles
- 94˚C 1 min, followed by 65˚C 2 min., 35 cycles
- 70˚C 10 min., 1 cycle
- 25˚C (for soaking)

The PCR tubes were then taken out and used for gel electrophoresis.

Agarose Gel Electrophoresis: Firstly, 1X TAE Buffer was prepared [i.e., 40 ml of stock solution (25X) was added to 960 ml of distilled water to make final volume 1 liter with 1X strength]. Then 2 % agarose gel was prepared. Amplified sample was pipette out in 10 µl and mixed with 2 µl of the loading dye. The mixed sample was then loaded in previously prepared agarose gel. The electrophoresis was run at 100 V for 30-35 minutes. After completion of the process, the gel was viewed using UV illuminator. Different bands were observed on the gel in which the band observed on 354 bp marker was considered as positive for *C.trachomatis*, and the positivity was compared with band of positive control. If unclear bands were observed then these were considered as non specific band or might be due to protein contamination **Photograph 1**.

Data Analysis: Statistical analysis was done and data was analyzed by using the statistical software SPSS version 13.

RESULTS

Urine sample were collected from HIV patients visiting HIV clinic of Sparsha Nepal and the samples were processed at EICRC.

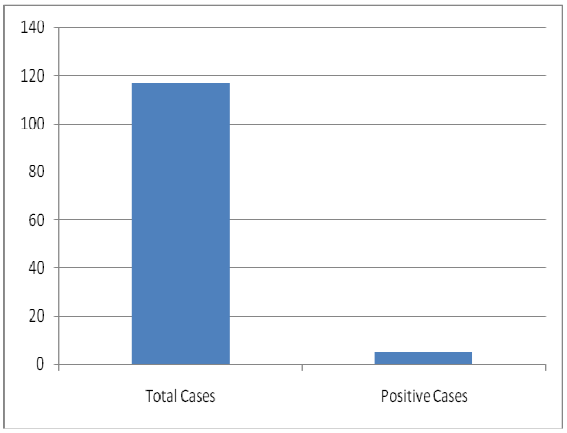
Table 1: prevalence of Chlamydia according to age group and sex

Age Group	Total Patients	Male			Female		
		Total	Positive	Positive %	Total	Positive	Positive %
20-25	14	8	1	12.5	6	2	33.33
25-30	28	22	1	4.55	6	0	0
30-35	23	20	0	0	3	0	0
35-40	23	17	1	5.88	6	0	0
40-45	13	10	0	0	3	0	0
45-50	10	6	0	0	4	0	0
50-55	3	3	0	0	0	0	0
55-60	2	2	0	0	0	0	0
60-65	1	0	0	0	1	0	0
Total	117	88	3	62.76	29	2	33.33

Association between Disease Occurrence and Sex (χ^2=0.648, p=0.421)
Association between Disease Occurrence and Age group (χ^2=312.427, P=0.133)

Prevalence of Chlamydia: Out of the total 117 urine samples collected, 5 (4.27%) showed positive result from PCR, whereas 112 (95.73%) showed negative result (fig. 1).

Fig 1: Distribution of Chlamydia in HIV patients



Distribution of chlamydia infection among different age groups and sex is shown clearly in Table 1. Among 117 samples, 29 (24.79%) were of females and 88 (75.21%) were of males. The patients under investigation were the age range 20-64 years. The highest number of cases, 74 (63.52%), were from age group 25-40 years.

Among the positive samples three were male (60%). They were of age 23, 27 and 35. There were two positive females (40%) of age 21 and 22 years. Within male group 3.40% are found to be Chlamydia positive while among females prevalence rate observed was 6.89%. No significant difference was observed between sex (χ^2=0.648, p=0.421) and also the

age group (χ^2=312.427, P=0.133) statistically at 5% level of significance.

As shown in table 2, four out of five cases were asymptomatic whereas only one case was symptomatic, that of a male with urethral

discharge. Statistically, there was no significant difference between the symptomatic or asymptomatic cases and disease occurrence at 5% level of significance.

Table 2: Distribution of Chlamydia positive cases according to symptoms and gender

Gender	Chlamydia Positive cases		
	Symptomatic	Asymptomatic	Total
Male	1	2	**3**
Female	0	2	**2**
Total	1	4	**5**

Association between Asymptomatic cases and Disease occurrence (χ^2=1.681, p=0.195)
Association between Symptomatic cases and Disease occurrence (χ^2=0.423, p=0.516)

As shown in table 3, all of the positive cases were married. No unmarried cases were found to be Chlamydia positive. However, there was no statistical significant association between the marital status and the occurrence of disease.

Table 3: Distribution of Chlamydia cases according to marital status

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

Association between Marital status and Disease Occurrence (χ^2=1.013, p=0.314)

Figure 2 and 3 shows the knowledge status of the study sample. As shown in figure 2, out of the total 117 cases, only 15 (12.82%) had knowledge of STI where as 102 (87.18%) had no idea about the STI. Among the positive cases (figure 2), two (40%) had knowledge, whereas, three (60%) had no ideas of STI.

Fig 2: Knowledge of STI among total Patients

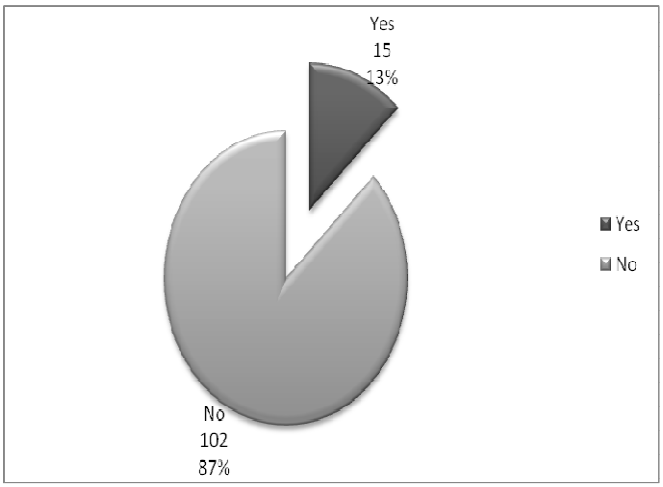
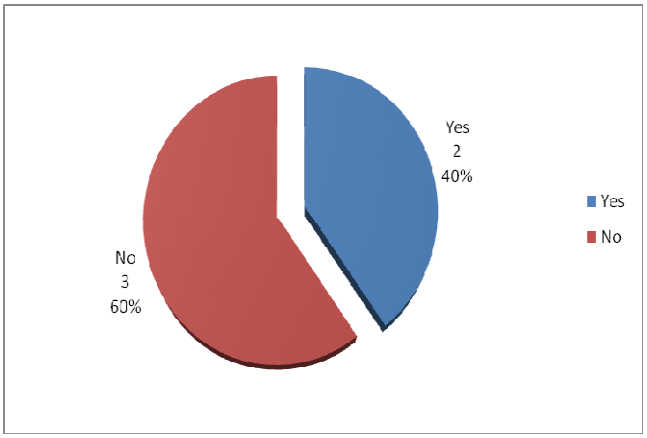


Fig 3: Knowledge of STI among Chlamydia Positive cases



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 tests. Neither any antigen detection

techniques are more sensitive and specific than culture techniques. PCR was found to be 100% sensitive and specific while detecting Chlamydia among female sex workers in Singapore [12]. Even a single organism is said to be detected by PCR with very high sensitivity, specificity and higher predictive values [13]. In the former study, the prevalence percentage was much higher by PCR (4.1%) compared to the one obtained by antigen detection techniques like 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 MPCR, clearly showed prevalence of *Chlamydia trachomatis* (4.27%) among participants. This prevalence rate implies to HIV patients visiting HIV clinics in Nepal. Female population was found to be more susceptible (6.89%) to Chlamydial infections than male (3.4%). The Prevalence rate among HIV females is 6 fold greater than in a study carried out among post-partum non-HIV females in rural Nepal [14]. This Chlamydial prevalence among HIV individuals in Nepal is found to be greater than that among female sex worker in Singapore (4.1%) [12]. 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%) [15], England and Wales (5.15 %) [16]. This prevalence findings is less than a study conducted in Thailand, where the prevalence rate was found to be 9.7% among 824 HIV seropositive patients [17]. 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 *Chlamydia trachomatis* in cervical scrapes determined by nested PCR was 10 % in 60 Cuban women [18]. Moreover, in a study conducted in Georgia, the seroprevalence of *C. trachomatis* in 234 HIV patients was 23.94% [19]. 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 (28) were from the age group 25-30. Among the five positive cases, three were from age group 20-25, one was twenty seven and the other was thirty-five years of age. Chlamydia infections are diffused widely in general population and unlike other STIs, appear not to be restricted to a particular risk group, mainly affecting young people, especially young women. In numerous studies, highest incidence is usually reported in age group below 25 years, accounting for more than sixty percentage of all cases [20],[21],[22].

There are other contradicting studies as well where age group above twenty-five has shown higher prevalence [23],[24],[25],[26].

Out of total 117 cases, 98 were married whereas 19 were unmarried. All the positive cases were of the married status. 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

[22]. In contrast, significant result was observed in another study [27].

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. Of the 117 patients, only 12.82% knew about the STIs and Chlamydia whereas the rest 87.17% 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 and most of those cases are asymptomatic therefore go undiagnosed. However we cannot ignore higher prevalence rate among HIV subjects than the normal population. Besides, knowledge regarding STI including Chlamydia is rare among the studied subjects. Due to this, it creates difficulty in the timely detection of *C. trachomatis* as well as clinical sequel, which might be devastating. Hence, routine checkup is recommended for all suspected cases for timely management of the disease.

Diagnostic tests for Chlamydia need to be introduced. It can be inferred on the basis of available literatures and present study that Nucleic acid amplification tests such as PCR are sensitive and specific for diagnosis. The main goal of Chlamydia identification is to prevent overt and silent Chlamydia PID and its sequel. Preventions efforts should be increased especially among sexually active adolescents. Timely diagnosis and treatment should be performed for the identification and management of Chlamydia.

Therefore, documentation of *C. trachomatis* infections in high risk population can assist in designing HIV risk reduction strategies as well. Thus, early diagnosis and treatment of Chlamydial infection is important to prevent high risk and devastating clinical consequences.

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