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Detection of gonorrhoea among HIV infected patients by polymerase chain reaction

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ABSTRACT

Objective: To determine the burden of gonorrhoea among HIV patients using PCR technique.**Methods:** A cross sectional study was carried out in a HIV clinic of Sparsha Nepal from January to March, 2011. Standard microbiological procedures were followed during collection and processing of urine samples. DNA amplification, isolation and detection were carried out. Gonorrhoea diagnosis was done by multiplex polymerase chain reaction.**Results:** Among the 119 HIV positive patients, 12 (10.08%) were positive for *N. gonorrhoeae*. The cases were more among the males than in females. The age of patients ranged from 20-45 years and the highest prevalence was among the age group 20-35 years. The distribution of gonorrhoea infected patients according to marital status revealed that 8 cases were among the married patients.**Conclusions:** This study revealed that the incidence of gonorrhoea was higher in males and in married people. So, the control measures should be targeted to these groups of people.

1. Introduction

Sexually transmitted infections (STIs) present a major public health concern in both industrialized and developing countries. Gonorrhoea is caused by a bacterium *Neisseria gonorrhoeae* (*N. gonorrhoeae*), which is the second commonest bacterial STI in the world[1,2]. It is a fastidious gram-negative coccus that requires nutrient supplementation to grow in laboratory cultures. It facultatively intracellular and typically appears in pairs[3].

Gonorrhoea is a common adult disease that primarily infects the urogenital tract, giving rise to intense local inflammation and a range of clinical manifestations[4]. Infection can be uncomplicated, complicated and may lead to disseminated gonococcal infections[5]. Infected men usually have symptoms and seek treatment spontaneously but women frequently have minor symptoms and are more asymptomatic. *N. gonorrhoeae* is successful human colonizer pathogen because it is able to evade the immune mechanism by constantly varying its surface structures by mimicry of host molecules. It is not found outside the human host niche[6]. The infection is transmitted through

vaginal, oral, or anal sexual relations, though transmission occurs rarely with safe sex practices. Gonorrhoea, if left untreated, can result in infertility, pelvic inflammatory disease, ectopic pregnancy and scrotal swelling and its disseminated infections can result in endocarditis, meningitis or gonococcal dermatitis-arthritis syndrome[7]. Gonorrhoea also increases the likelihood of acquiring and transmitting HIV infection. People who are infected with another STI also tend to be at increased risk of HIV infection and vice-versa. However, beyond this correlation resulting from common risk behaviors, STIs and HIV may facilitate each other's transmission. HIV infected people with gonorrhoea can transmit HIV more easily and detection of gonorrhoea especially in HIV infected patients is more necessary[8].

Among STIs, gonorrhoea remains a significant disease globally. While it is more frequent in poorer countries, disease rates remain unacceptably high in developed countries, and appear to be increasing at present, at least in some population subgroups. Consequences of a high disease rate include a high incidence of complications and long-term morbidity, as well as increased HIV transmission[9]. It is a severe public health problem worldwide with more than 87 million new infections each year[10]. It has high prevalence among teens and young adults[4]. The problem is further compounded by the emergence of resistance to antimicrobial agents that are commonly used against *N. gonorrhoeae*, making the treatment expensive and prolonged[11]. Very few studies have been conducted regarding the prevalence of

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N. gonorrhoeae in Nepal. This study was conducted to determine the burden of gonorrhoea among HIV patients using PCR technique.

2. 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 119 samples were collected and processed for DNA isolation, amplification and detection using agarose gel electrophoresis.

2.1. 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 r/min for 10 min. The pellet was collected in a clean and sterile test tube, washed with 10-15 mL phosphate buffer solution and re-centrifugation was done at 5000 r/min for 15 min. Then, 200 μ L phosphate buffer solution was added to the pellet, transferred to 1.5 mL micro-centrifuge tubes and stored at 2-8 $^{\circ}$ C. The processed samples were transported to Everest International Clinic and Research Center for further processing.

2.2. 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 "multiplex polymerase chain reaction (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 298 bp region of the *cppB* gene of *N. gonorrhoeae*. Each 50 μ L PCR reaction mixture contained 25 μ L 2X MPCR buffer mixtures, 5 μ L 10X MPCR primers, 0.5 μ L *Taq* polymerase (5 IU/ μ L), 14.5 μ L water (H_2O) and 5 μ L 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 $^{\circ}$ C for 1 min followed by 65 $^{\circ}$ C for 4 min; 35 cycles of 94 $^{\circ}$ C for 1 min followed by 65 $^{\circ}$ C for 2 min; 1 cycles of 70 $^{\circ}$ C for 10 min and at 25 $^{\circ}$ C (for soaking). The PCR product (364 bp) was detected using 1% Tris-acetate-ethylene diamine tetraacetic acid (TAE) gel electrophoresis.

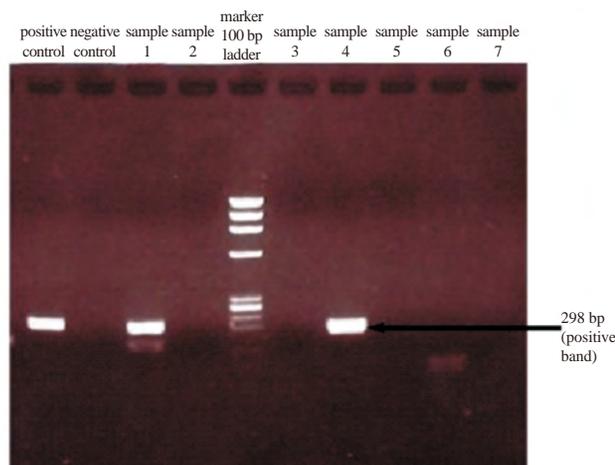
2.3. Data analysis

Statistical analysis was done and data was analyzed by using the statistical software SPSS version 17. The association between categorical variable was done using *Chi*-square test. The *P*-value less than 0.05 was considered significant.

3. Results

A total of 119 HIV positive patients (90 males and 29 females) were involved in the study. Among them, 12 (10.08%) were positive for *N. gonorrhoeae* (Figure 1). The cases were more among the males (7) than in females (5). The age of patients ranged from 20-45 years and the highest prevalence was among the age group 20-35 years, followed by 40-45 years (Table 1). Similarly, the prevalence was higher among the laborers (50%), followed by job holders (33.33%)

and housewife (16.67%). But there was no prevalence of gonorrhoea among farmer, businessman, and unemployed people.



Note: The faint bands seen are non specific bands.

Figure 1. Gel electrophoresis of amplified PCR products.

Table 1

Age wise distribution of the cases.

| Age group | No. of observed patients | Male | | | Female | | |
|-----------|--------------------------|----------------|----------------|--------------|----------------|----------------|--------------|
| | | Observed cases | Positive cases | Positive (%) | Observed cases | Positive cases | Positive (%) |
| 20-25 | 16 | 10 | 1 | 10.0 | 6 | 2 | 33.3 |
| 25-30 | 28 | 22 | 1 | 4.5 | 6 | 2 | 33.3 |
| 30-35 | 23 | 20 | 3 | 15.0 | 3 | 0 | 0.0 |
| 35-40 | 23 | 17 | 1 | 5.9 | 6 | 0 | 0.0 |
| 40-45 | 15 | 10 | 1 | 10.0 | 5 | 1 | 20.0 |
| 45-50 | 11 | 8 | 0 | 0.0 | 3 | 0 | 0.0 |
| 50-55 | 3 | 3 | 0 | 0.0 | 0 | 0 | 0.0 |
| Total | 119 | 90 | 7 | 7.8 | 29 | 5 | 17.2 |

Most of the patients (88) had no symptoms. Among the gonorrhoea infected patients, 6 males and 2 females had symptoms relating to gonorrhoea (Table 2). The distribution of symptoms between the male and female patients is shown in Table 3. There was significant association between symptoms and gonorrhoeal infection ($P = 0.001$). Significant association was also seen between symptoms and gender ($P = 0.044$). The distribution of gonorrhoea infected patients according to marital status revealed that 8 (66.66%) cases were among the married patients and 4 (33.33%) cases were among the unmarried ones (Table 4).

Table 2

Symptoms wise status of patients according to the gender.

| Symptoms | Male | | | Female | | |
|--------------------|-----------------------|----------------|--------------|-----------------------|----------------|--------------|
| | No. of observed cases | Positive cases | Positive (%) | No. of observed cases | Positive cases | Positive (%) |
| Asymptomatic cases | 66 | 1 | 14.28 | 22 | 3 | 60 |
| Symptomatic cases | 24 | 6 | 85.71 | 29 | 2 | 40 |
| Total | 90 | 7 | 100.00 | 29 | 5 | 100 |

Table 3

Distribution of symptoms among the patients.

| Symptoms | Male | | | Female | | |
|-------------------------------------|----------------------|----------------|--------------|----------------------|----------------|--------------|
| | Total observed cases | Positive cases | Positive (%) | Total observed cases | Positive cases | Positive (%) |
| No symptoms | 66 | 1 | 14.28 | 22 | 3 | 60 |
| Urethral discharge | 11 | 3 | 42.85 | 2 | 1 | 20 |
| Mucopurulent discharge | 9 | 1 | 14.28 | 2 | 0 | 0 |
| Dysuria | 0 | 0 | 0.00 | 1 | 1 | 20 |
| Abdominal pain | 0 | 0 | 0.00 | 2 | 0 | 0 |
| Urethral and mucopurulent discharge | 4 | 2 | 28.57 | 0 | 0 | 0 |
| Total | 90 | 7 | 100.00 | 29 | 5 | 100 |

Table 4

Distribution of cases according to marital status.

| Marital status | Number of cases | Positive | Negative | Positive (%) |
|----------------|-----------------|----------|----------|--------------|
| Married | 99 | 8 | 91 | 8.08 |
| Unmarried | 20 | 4 | 16 | 20.00 |
| Total | 119 | 12 | 107 | 10.08 |

4. Discussion

The presence of gonorrhoea in HIV patients has been a serious problem in developing countries which has made the cases further complicated. It is of prime importance to screen the cases infected with gonorrhoea in proper time to reduce the morbidity of the disease. In this study, multiplex PCR was used for determining the presence of gonorrhoea. This technique is used to detect *N. gonorrhoeae*, generally in the genitourinary specimens like urine. The prevalence of gonorrhoea among HIV patients in this study was 10.08% which is in accordance to the results obtained from a previous study [12] in which the prevalence was 9.5%. The presence of gonorrhoea in HIV patients indicates the lack of awareness of STIs and HIV infection.

Gonorrhoea is a curable and preventable disease but the disease has been more prevalent in the productive ages. Similar to these results are those in a previous study from Nepal [13] where the age group 20-30 years is found to be the most infected followed by age groups 30-40 years and 40-50 years. The sex wise distribution of the cases revealed that males (58%) had higher prevalence of gonorrhoea than females (42%). Similar results were obtained in a study in Thailand [14], where higher prevalence (74%) was seen in male than (26%) in female.

From symptom wise status, more asymptomatic patients were seen in both males and females. Majority of the male patients were asymptomatic and most of the female patients were asymptomatic. The greater number of HIV cases were asymptomatic but in case of gonorrhoea, most of the cases were symptomatic. Within infected patients, urethral discharge, mucopurulent discharge, abdominal pain and dysuria were the most complained symptoms. In this study, most of the females were asymptomatic and also they were not having urethral or vaginal discharge, due to which they did not have cases of gonorrhoea and infection rate was quite low among them. Based on this finding, it is important to screen prevalence of gonorrhoea in all HIV infected people. Although those patients had already caught the HIV infection, they should be made aware of STIs and have knowledge of impact of gonorrhoea on HIV infection and vice-versa. In this study, even though 89.92% had no prevalence of gonorrhoea, they should also be advised for safe sex behavior.

The results of this study have a great significance for the health authorities and medical practitioners related to STIs, but still there are some limitations. The major limitation includes the short study period due to which only a few cases were obtained. Similarly, the site of sample collections only confined in Sparsha (National Government Organization) and does not reveal the total picture of the whole country. So surveillance and treatment should be initiated in every geographical part of Nepal.

This study revealed that the incidence of gonorrhoea was higher in males and in married people. So, the results of this study provide essential information for formulation and implementation of control measures which should be targeted to these groups of people.

Conflict of interest statement

We declare that we have no conflict of interest.

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