

Prevalence of *Neisseria Gonorrhoea* Infection by PCR Method Using Urine Samples From HIV Sero-Positive Women



Microbiology

KEYWORDS : *Neisseria gonorrhoeae*, PCR, HIV, Primers, urine.

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ABSTRACT

Neisseria gonorrhoeae infection was diagnosed in human immunodeficiency virus (HIV) sero-positive women in Ebonyi state, Nigeria. The samples were screened for HIV at the laboratory of Applied Microbiology, Ebonyi State using diagnostic dipstick, while PCR study was carried out at university of Ibadan. Species specific primers were designed for the specific amplification of a 260bp region of the *N. gonorrhoeae* porA pseudo gene based on the numbering from strain MS11. All strains of *N.gonorrhoeae* tested were successfully detected by the designed PCR primer giving a single predicted product of 260bp amplicon in gel electrophoresis. The primer could not amplify DNA extracted from other *Neisseria* species. HIV status was established using rapid immunochromatographic assays as per national standards. Out of the total number of 105 urine samples, *N. gonorrhoeae* was detected in 16 (15.2%). There was significant association between *N. gonorrhoeae* infection and age but infection was not related to occupation and marital status. Analysis showed that *N. gonorrhoeae* infection is significantly associated with HIV status (15.2% vs 100%). *N. gonorrhoeae* is known to facilitate HIV transmission, hence there is the need for routine testing and treatment of all positive cases of infection to control HIV.

INTRODUCTION

N. gonorrhoeae is a major sexually transmitted infection prevalent in many countries (Conte *et al.*, 2006). In men, *Neisseria* infects the urethra causing dysuria and acute urethritis with a purulent discharge. The most common site of gonococcal infection in women is the endocervix (80% - 90%) followed by urethra (40%), and pharynx (10% -20%) (CDC, 2006). Symptoms often manifest within 10 days of infection and majorly include vaginal discharge, dysuria, intermenstrual bleeding, dyspareunia, and mild lower abdominal pain (Holder, 2008). In men, the infection may spread to prostate, bladder, and epididymes, causing inflammation and swollen epididymes and may lead to sterility (Brian, 2009).

A number of techniques have been developed to detect genital infections caused by *N. gonorrhoeae*. The current "gold standard" for diagnosis of infection is by culture on selective media. However, even under optimal laboratory conditions, the sensitivity of gonococcal cultures ranges from 85 to 95% for acute infection (Schmale *et al.*, 1969) and falls to approximately 50% for females with chronic infections (Bassiri *et al.*, 1997). This is largely due to poor specimen collection, transport, and storage (Geraats-Peters *et al.*, 2005). Nucleic acid amplification-based techniques, including the ligase chain reaction, strand displacement amplification assay, nucleic acid sequence-based amplification, and PCR, have been shown to have both high sensitivity and specificity for the detection of *N. gonorrhoeae* (Palladino *et al.*, 1999; Farrell, 1999; Martin *et al.*, 2000; Mahony *et al.*, 2001; Akduman *et al.*, 2002; Koenig *et al.*, 2004 and Tabrizi *et al.*, 2004) and a number of commercial assays are available (Geraats-Peters *et al.*, 2005).

Gonorrhoea has been found to be a cofactor in HIV transmission. Most sexually transmitted infections (STI) that cause inflammation increases the concentration of HIV in the urethra, semen, and cervical fluid (Wu *et al.*, 2003). *Neisseria gonorrhoeae* and other STI are known to be associated in several ways with HIV. This is true because STI and HIV are spread in like manner through sexual activities. Person who contracts

STI may also have put themselves at risk for HIV and should be tested (Nwele *et al.*, 2013). Hence this study is aimed to ascertain the prevalence of *Neisseria gonorrhoea* infection among HIV seropositive women using urine samples via PCR technique.

MATERIALS AND METHODS

Study Area

The samples used for the study were collected from the Federal Teaching Hospital (FETHA I) Abakaliki.

Sample Collection and Analysis

Urine and blood samples were collected from a total of 105 HIV-positive women within the age range of 15-49 years attending Federal Teaching Hospital Abakaliki I (FETHA I). Wide mouthed sterile containers were given to the participants with instruction for urine collection which was stored at 4°C for PCR studies. Sociodemographic parameters (age, occupation and marital status) were obtained from participants by use of structured questionnaire. Blood samples were collected aseptically and introduced into sterile tubes for serum extraction. Enzyme Link Immunosorbent Assay (ELISA) was used to test for HIV following the principle of antigen-antibody reactions.

DNA Extraction

DNA components of *N. gonorrhoeae* were extracted from urine sample using the ZR Genomic DNA TM -Tissue Miniprep kit. According to the manufacturers direction, the sample was digested with the applied proteinase K. Genomic Lysis Buffer was added and mixture was vortexed and transferred to the supplied zymo-spin TM column to elute the purified DNA. Eluted DNA was stored at 4°C for PCR studies (Lawing *et al.*, 2008).

PCR Primer

The primers based on *N. gonorrhoea* repeated DNA target for PCR identification were used to amplify a 60bp piece of genome. The sequences of primers (por A pseudo gene) were as

follows:

5'-CAAGAAGACCTCGGCAA-3' forward.

5' -CCGACAACCACTTGGT-3' reverse (Guillermo *et al.*, 1998).

PCR Protocol

The specificity of the primers was determined by previously using specific laboratory strains from stock culture. It was established that our primers could not amplify DNA extracted from related and unrelated microorganisms. However, the primers were able to amplify all the specific isolates tested. Southern blot analysis with the primer probes confirmed the specificity of the amplicons. PCR reaction was performed with an automated thermal cycle (Eppend of Mastercycle gradient) with the total volume of PCR reactions set at 25 µl in 0.2ml micro tubes. Negative control contained all components except template DNA and the procedure was as follows:

- Initial denaturation step at 94°C for 5 minutes
- 40 cycle s repetitions of 1minute at 92°C (denaturation), 45 seconds at 62°C annealing), and 2 minutes at 92°C (extension).
- Final incubation at 72°C for 7 minutes to ensure polymerization of any remaining PCR products.

Statistical Analysis

Data were analyzed using percentages and chi-square. Results were accepted to be significant at p>0.05.

RESULTS

Samples containing 260bp in gel electrophoresis were considered positive for *N. gonorrhoea*. Out of the 105 urine samples screened, 16 (15.2%) were positive for *N. gonorrhoea*. The highest prevalence was observed among the HIV positive women within the age range of 22- 28 years as well as the unemployed housewives and the unmarried respondents. Analysis indicated significant association between *N. gonorrhoea* and age (p<0.05), but infection was independent of occupation and marital status (p<0.05).

All the screened samples were HIV positive. Hence, infection is significantly associated with HIV (100% vs 15.2%).

Table 1: Distribution of N. gonorrhoeae among HIV Positive Women as evaluated by PCR studies

PARAMETER INVESTIGATED	NUMBER EXAMINED	NUMBER INFECTED BY PCR	PERCENTAGE INFECTED
Age			
15 - 21	16	2	12.5
22 - 28	38	8	21.1
29 - 35	14	2	14.3
36 - 42	25	3	12
43 - 49	12	1	8.3
Total	105	16	15.2
(p < 0.05)			
Occupation			
House wife	17	3	17.6
Business	46	6	13.0
Student	12	2	16.7
Civil servant	30	5	16.7
Total	105	16	15.2
(p > 0.05)			
Marital status			
Single	28	5	17.9
Married			
Monogamous	57	8	14.0
Polygamous	20	3	15.0
Total	105	16	15.2
(p > 0.05)			

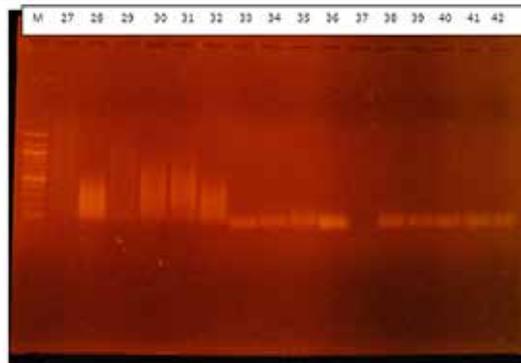


Figure 1: DNA bands of Neisseria gonorrhoea
 Line M: Size marker DNA Ladder (100 bp)
 Line 33, 34, 35, 36, 38, 39, 40, 41, 42: Specimens positive for *N. gonorrhoeae* (260 bp PCR bands)
 Line 27, 28, 29, 30, 31, 32: Specimens negative for *N. gonorrhoeae* (no banding pattern)
 Line 37: Negative control

DISCUSSION

Early diagnosis of *Neisseria gonorrhoeae* infection is important with regard to patients' health and infectivity (Geraats-Peters *et al.*, 2005). Result from this study showed that *N. gonorrhoea* is a significant co-infection of HIV and this is in agreement with the findings of Coombs *et al.* (2003) which stated that STD that cause ulcers such as *N. gonorrhoea*, can directly increase the shedding of HIV in the genital tract. Wright, (2001) reported that in patients with urethritis, gonorrhoea seems to have a great effect on the viral load in the genital tract due to the associated inflammation. Also, majority of the women were of child bearing age suggesting that HIV and gonorrhoea infection could be as a result of promiscuity. This agrees with the report of Weinstock *et al.* (2004) that sexually active persons can contract sexually transmitted infections.

In this study, women aged between 22-28 years as well as the unmarried unemployed respondents were mostly infected. Al-suleiman *et al.* (1983) reported that demographic risk factors for gonorrhoea transmission include age, race, low socioeconomic level, unmarried marital status, urban-residence, early onset of sexual activity and history of STI. Thus preventive measures should be targeted at all women of child bearing age especially the low income and single groups. In women, symptomatic gonococcal infection may lead to tubal scarring and infertility. The incidence of ectopic pregnancy in infected women is increased from 7 to 10 fold, increasing both fetal and maternal morbidity, including tube varian abscess, endometritis or Fitz-Hugh-Curtis syndrome (perihepatitis) (Holder, 2008). Vaginal delivery from women infected of gonorrhoea can lead to bilateral conjunctivitis (opthalmia neonatorum) as well as systemic illnesses, including septicemia and arthritis in neonates (Alexander, 1988 and PHC, 2004). Neonates may also acquire pharyngeal, respiratory, rectal infection or Disseminated Gonococcal Infection (DGI) (Alexander, 1988).

The use of PCR technique in this study is of obvious advantage to enhance sensitivity and specificity in detection of infectious agents. PCR method offers high degree of selectivity and the ability to amplify targets sequence. Extracting DNA components of *N. gonorrhoeae* directly from urine sample was simple, inexpensive and easy to perform and contamination as well as inhibition due to manipulation of specimens was prevented by the use of chelating resin (chelex 100).

In our study, the urine samples which were self-collected by

participants were rapid, easy and practical. Invasive specimen collection procedures in women often results in reduced patient compliance with testing and has been a problem in research procedures. In men, discharge from the meatus or an intra-urethral swab collected from patients who have not voided for at least 2 hours have been recommended as a preferred specimen for the isolation of *N. gonorrhoea* (Janda and Knapp, 2003). However, Horii *et al.* (2009) maintained that urine specimen is suitable in nucleic acid methods for detecting sexually transmitted pathogens in clinical sample. Thus in this study, urine specimen was adopted for PCR studies.

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