Gonorrhoea Infection Prevalence in Human Immunodeficiency Virus Positive Patients Based on Polymerase Chain Reaction Examination

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Abstract

Objective: To determine the prevalence of gonorrhoea infection in male and female HIV positive patients based on polymerase chain reaction (PCR) examination at the Teratai Clinic, Dr. Hasan Sadikin General Hospital Bandung.

Methods: This study was conducted in July, 2012 at the Teratai Clinic, while the PCR examination was performed at the Molecular Biology Laboratory of Microbiology Department, Faculty of Medicine, Universitas Padjadjaran, Bandung. This was a cross-sectional observational study. The subjects were 81 HIV positive patients who were taken in consecutive admission. They underwent history taking and physical examination. Samples were taken from urethral swab in males and cervical swab in females for PCR examination.

Results: The PCR examination result was positive for gonorrhea in 36% subjects. From all male subjects participating in the study, 37% were positive while 33% of the female subjects were also positive for gonorrhea.

Conclusions: The prevalence of gonorrhoea infection in male and female HIV positive patients at the Teratai Clinic, Dr. Hasan Sadikin General Hospital Bandung is quite high, i.e 37% and 33%, respectively.

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Introduction

Gonorrhea is the second most common sexually transmitted infection (STI) in the United States and it infects more than 700,000 people per year with an equal ratio between male and female.[1] It was stated by the United Nations Joint Program on Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (UNAIDS) that the gonorrhea prevalence in the general population in Indonesia was 20 to 31% in 2004.[2] Meanwhile, data from 2007 Integrated Biological and Behavioral Surveillance (IBBS) on the prevalence of STIs in female sexual workers (FSWs) in Indonesia show that the prevalence of gonorrhea ranged between 16% and 44%, with the highest incidence found in West Java.[3]

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Established diagnosis of gonorrhea infection is made through *Neisseria gonorrhoeae* (*N. gonorrhoeae*) identification in the genital tract, rectal, pharyngeal, and ocular secretion, which can be done using several methods, such as microscopic examination, culture, and molecular techniques.[1,4–6] Polymerase chain reaction (PCR) examination is one of the molecular techniques with a higher sensitivity, from 90–100%, than culture technique which has 68% sensitivity.[4–7] This technique has been stated to replace the culture technique, including for asymptomatic patients.[7]

Human immunodeficiency virus (or referred to as HIV) infection causes an immunosuppressed condition that could increase the number of STIs and lead to greater co-diagnosis condition.[8,9] People with active STIs are more prone to HIV infection, and vice versa. HIV positive patients who are exposed to STIs also have a greater risk to transmit HIV.[9] Some epidemiological data have suggested that gonorrhea contributes to

increased HIV transmission.[10] The frequency of mucosal HIV increases by 32–41% in gonorrhea patients and its transmission risk increases to approximately 2–5 times higher.[11–14]

Several factors that contribute to the increase of HIV infection are HIV-infected cell withdrawal in the genital tract as a part of the immune response and cluster of differentiation, in this case the CD4, lymphocyte stimulation that will eventually lead to increased replication of HIV.[12]

From the immunology perspective, the presence of gonorrhea increases HIV shedding on the genital tract which attracts CD4 T lymphocytes as a part of the inflammatory response against patogen.[10,11,15] In addition to the viral sheddings, the fact that some people with HIV and AIDS are still engaged in some high risk sexual behaviors becomes the key factor in STI co-infections, including gonorrhea, with HIV and AIDS.[16–20]

Sexually transmitted infections in people with HIV suggest the possibility that these people may still be engaged in risky sexual behaviors. These infections could be a risk factor for HIV transmission to uninfected population.[16] Other studies also support this.[9,17] Manning *et al*.[18] revealed that high-risk sexual practices still occur among some segments of HIV positive population. To decrease associated morbidity and prevent the secondary spread of HIV and STIs, prevention efforts should focus on HIV positive patients who are still engaged in high-risk sexual practices.

Thus, data regarding gonorrhea infection in people with HIV are critical for the prevention, screening, and treatment of HIV to reduce HIV transmission.[16,19] However, until recently, data on STI prevalence, including data on asymptomatic gonorrhea in individuals with HIV positive, are still limited.[3,10,21]

Until now, there are still limited numbers of publication available on the prevalence of gonorrhea infection among HIV positive patients in Indonesia; therefore this study aimed to determine the prevalence of gonorrhea infection in male urethra and female cervix of HIV positive patients based on PCR examination results in Teratai Clinic, Dr. Hasan Sadikin General Hospital, Bandung.

Methods

This study is a cross-sectional observational study performed to 81 HIV positive patients who visited Teratai Clinic, Dr. Hasan Sadikin General Hospital Bandung, Indonesia. The subjects were taken in consecutive admission in July 2012. The HIV serostatus of the study population had already been determined and confirmed based on the procedures stated in the National Guideline for HIV before the study. Sexually active HIV positive patients attending Teratai Clinic were offered to participate in this study. Fourty eight males and 33 females were enrolled after signing written informed consent. Women having her menstrual period were excluded from the study.

The subjects underwent history taking and physical examination. Sample was collected from urethral swab in male and cervical swab in female for PCR examination. Comprehensive data on patient's characteristics and sexual history were obtained through history taking and medical records. The history taking, clinical examination, and specimens collections were performed in Teratai Clinic while the specimen processing was conducted at the Biology Molecular Laboratory, Microbiology Department, Faculty of Medicine, Universitas Padjadjaran, Bandung.

The protocol of this study was reviewed and approved by the Ethical Committee of the Faculty of Medicine of Universitas Padjadjaran-Dr. Hasan Sadikin General Hospital, Bandung.

Specimen collection kits (Roche) was used for urethral swab for male patients and endocervical swab for female patients. Transport medium tubes (Roche) were used for transporting the specimens to the laboratory on the day of the collection. Immediately after arrival at the laboratory, the urethral and endocervical swab specimens were prepared for the PCR assay as instructed by the manufacturer, kept frozen at -20 °C, and analyzed within 14 days of sampling. Transport medium tubes were thoroughly mixed and 100 mL was withdrawn and diluted to 1 mL with sucrose-phosphate medium. After that, 1 mL of swab specimen diluent (F. Hoffmann-La Roche Ltd., Basel, Switzerland), was added and the prepared specimens were frozen at -20 °C for PCR.

The PCR assay was accomplished using Roche CT/NG Amplicor PCR kit and the amplification process was conducted by using a thermocycler machine. Roche CT/NG Amplicor PCR kit for *N. gonorrhoeae* (Roche Moecular System, Inc., Brancburb, USA) is a qualitative in vitro test for the detection of *N. gonorrhoeae* DNA in urine from symptomatic and asymptomatic males and in endocervical swab specimen from symptomatic and asymptomatic females as evidence of *N. gonorrhoeae* infection. *N. gonorrhoeae* DNA is detected by PCR amplification of target DNA and by hybridization capture of amplified target. After the amplification, the amplified nucleotide sequences were detected using an enzyme immunoassay and the A450s were then

measured with a spectrophotometer (Multiskan; Labsystems Ltd., Findland).

As stated in the Operator's Manual for the Amplicor Analyzer, the results were seen in the print out for the flags. Interpretation of the results is as follow: positive result for *N. gonorrhoeae* is confirmed if the value of the examination materials >3.5 and a negative result is confirmed if the value of the examination materials <0.2. The results were calculated by statistic test SPSS 17.0 and presented descriptively by percentage.

Results

Subjects of the study were 81 patients averaging 32.8 years old with the youngest age was 23 years old and the oldest was 62 years old (data not shown). Table 1 shows the characteristics of subjects by sex, education level, occupation, and marital status. The most frequently found characteristics of the subject were male gender (59%), high school education (51%), self-employed (43%), and married (57%). We can see that of the 81 subjects, 29 people (36%) gave positive results and 52 subjects (64%) were negative (Table 2).

The average age of subjects with PCR positive result for *N. gonorrhoeae* in this study was 32 years old with the youngest being 23 years old and the oldest being 62 years. Meanwhile, for the subjects with negative PCR results, the average age was 33 years old with the youngest was 23 years old and the oldest was 44 years old (data not shown). Based on sex, 18 males (37%) and 11 females (33%) had positive PCR result for *N. gonorrhoeae* while 30 males (62%) and 22 females (67%) were negative (Table 3).

Fifteen subjects with positive *N. gonorrhoeae* PCR results were self-employed. A total of nine subjects were merchants, two of the subjects were the owner of a beauty parlour, and the others were owner of a bike shop, a tattoo shop, an EO, and a catering company. Of the seven subjects with positive PCR results who worked as private employees, two subjects were hotel employees, two were salesmen, one was a social worker, and the others were private employees of unknown companies.

As many as 30% unemployed subjects showed positive results and 70% were negative. Subjects who worked as commercial sexual workers and students gave negative PCR results.

Most of the subjects were married. Among subjects with positive PCR result, 14 of them were married, 9 were single, and 6 were widows or widowers.

Data show that the subjects with positive PCR result for *N. gonorrhoeae* had their first sexual intercourse at an average age of 19.5 years, while the average age of the subjects with a negative PCR result were 21.3 years. Most of the subjects in this study were heterosexual.

The majority of subjects with homosexual orientation (60%) showed gonorrhea infection. Of the 29 subjects with positive PCR results, as many as 23 subjects were heterosexual, 3 subjects were homosexual, and 3 subjects were bisexual (Table 4).

Table 1 The Baseline Characteristics of Participants

Variabel	n	%
Sex		
Male	48	59
Female	33	41
Education		
No formal education	0	0
Primary school	4	5
Junior high school	4	5
Senior high school	41	51
College	32	49
Occupation		
Self-employed	35	43
Employee	21	26
Commercial sexual worker	1	1
Student/college student	1	1
Unemployed	23	28
Status		
Single	18	22
Married	45	57
Widow	18	22

Eleven subjects (52%) who had more than 6 lifetime sexual partners showed positive PCR test results, and 10 (48%) were negative. Most of the subjects in both groups stated that they always use condoms during sexual intercourse. Fourteen subjects wih positive PCR results stated that they constantly used condoms, 6 expressed that they sometimes used condoms, and 9 declared that they had never used a condom during sexual intercourse. In patients with HIV

infection in this study, the history of previous STI was not associated with the incidence of gonorrhea. Most of subjects with positive PCR result stated that they did not have a previous history of STIs.

Table 2 The Results of PCR *N. gonorrhoeae* Examination

The Results of PCR *N. gonorrhoeae* Examination	n	%
Positive	29	36
Negative	52	64
Total	**81**	**100%**

Discussion

Belongia *et al*.[21] in Minessota found that the prevalence of gonorrhea infection in patients with HIV infection and AIDS was more common in the age group of 25–34 years. Bastos *et al*.[22] in Rio de Janeiro found that gonorrhea in patients with HIV infection related to the young age of approximately in the early 30s. In 2001, Stolte *et al*.[23] in Amsterdam found that gonorrhea infection in people with HIV and AIDS were more common at age less than 31 years. The average age of subjects with *N. gonorrhoeae* positive PCR result in this study was 32.1 years old.

Gonorrhea is more commonly found in young people, probably because at young age there is a tendency of high risk sexual intercourse, such as having sex with multiple sexual partners or without using condom.[21–24] In this study, gonorrhea was more commonly found in male

Table 3 *N. gonorrhoeae* PCR Result Based on Characteristic of Participants

Variabel	PCR *N. gonorrhoeae* Result		Total
	Positive	Negative	
Sex			
Male	18	30	48
Female	11	22	33
Education			
No formal education	0	0	0
Primary school	0	4	4
Junior high school	2	2	4
Senior high school	15	26	41
College	12	20	32
Occupation			
Self-employed	15	20	35
Employee	7	14	21
Commercial sexual worker	0	1	1
Student/college student	0	1	1
Unemployed	7	16	23
Status			
Single	9	9	18
Married	14	31	45
Widow	6	12	18

subjects than in females, i.e. 37% vs 33%. Hamlyn *et al*.[25] found that gonorrhea infection in people with HIV were more commonly found in males than in females, i.e. 53% vs 17%. Studies by Manning *et al*.[18] in New York and Gore-Felton *et al*.[13] also found the incidences of gonorrhea infection were higher in males than in females, 84% vs 16% and 59% vs 41%, respectively.

Norms lead to differences in sexual behavior between men and women. Women are more constrained by the norms; thus less exposed to STIs due to less frequent sexual relations.[26]

Most of the subjects with positive PCR results in this study were senior high school graduates. Individuals with low levels of education tend to have risky sexual behaviors. Lu *et al*.[27] found a significant association between the level of education and gonorrhea infection in people with HIV. In this study, most subjects who were infected with gonorrhea had lower educational

Table 4 ***N. gonorrhoeae*** **PCR Examination Result Based on Sexual History of Participants**

Variable	PCR *N. gonorrhoeae* Result		Total
	Positive	Negative	
Average age of sexual debut	19	21	
Sexual orientation			
Heterosexual	23	49	72
Homosexual	3	2	5
Bisexual	3	1	4
Number of sexual partners in the last month			
0 person	11	16	27
1 person	15	33	48
≥2 persons	3	3	6
Number of sexual partners in the last 3 months			
0 person	8	12	20
1 person	16	30	46
≥2 persons	5	10	15
Number of lifetime sexual partners			
1 person	9	12	21
2–5 persons	9	30	39
≥6 persons	11	10	21
Condom use			
Never	9	11	20
Seldom	6	15	21
Always	14	26	40
STI history			
Positive	12	31	43
Negative	17	21	38

levels which was senior high school level or lower. Most of the subjects with positive PCR results were self-employed and employees. A study conducted by Castor *et al*.[28] in India found a significant relationship between occupation and the incidence of gonorrhea. In the study, subjects who worked as an employee or self-employed with an income between $1,000–5,000 per year had 3-fold higher risk for gonorrhea as it was possible for them to have sex with multiple partners.

Research conducted by Lu *et al*.[27] in China in 2005 found that gonorrhea in patients with HIV infection were more frequent in unmarried, widow, and widower subjects. Similarly, the study conducted by Castor *et al*.[28] in India found a significant association between the incidence of gonorrhea and the unmarried status. This might be because the subjects who are not married, widow, or widower, are likely to have no steady sexual partner.[27]

Studies regarding sexual behaviors often give inaccurate results due to social desirability bias and recall.[25,29,30] Social desirability bias might increase in direct anamnesis data retrieval if it is compared to audio computer assisted self-interview because subjects tend not to disclose the actual data.[25,30]

The subjects with PCR positive result for *N. gonorrhoeae* in this study had their first sexual intercourse at the average age of 19.5 years. The majority of women with HIV infection who were exposed to gonorrhea in Venkatesh *et al*.[31] study had their first sexual intercourse between the ages of 16–20 years old. First sexual intercourse at young age was closely related to the number of sexual partners, delayed marriage, as well as high-risk sexual behaviors affected by STIs.[27,29]

Most of subjects with a positive PCR result in this have heterosexual orientation. Studies by Young *et al*.[32] and Castor *et al*.[28] found a high incidence of gonorrhea in heterosexual subjects. It was suspected that it is related to the number of multiple sexual partners and a history of previous gonorrhea.[28,32]

A total of 11/21 subjects who had more than six lifetime sexual partners had positive PCR results. Significant correlation between the incidence of gonorrhea infection and the number of lifetime sexual partners was found in the study conducted by Venkatesh *et al*.,[31] namely in women with 2–3 and more than 4 multiple sexual partners. A study regarding female sexual workers by Jin *et al*.[33] found that multiple sexual partners was a risk factor for gonorrhea infection. Reitmeijer *et al*.[24] in his study found that the number of multiple sexual partners in the last 4 months was one of the risk factors for incidence of gonorrhea and other STDs in men who have sex with men (MSM) patients with HIV infection.

Based on the research by Ghanem *et al*.[30] it is stated that data on the sexual behaviors of a sensitive nature are more at risk for bias. Hamlyn *et al*.[25] suggested the possibility of sexual relations or sexual partners reported by the subjects affect research results.

In this study, most subjects with positive PCR results stated that they always use condom when having sex. About 90% of subjects who were infected with gonorrhea in the study by Jin *et al*.[33] declared that they always use condom when having sex. In this study, there was no significant difference between condom use and gonorrhea infection. This result was suspected due to a bias in reporting condom use by the subjects. In addition, incorrect condom use is also suspected as a risk factor associated with the transmission of gonorrhea.[33]

Study by Hamlyn *et al*.[25] toward HIV infection patients found that most subjects exposed to gonorrhea stated inconsistent condom use. Only less than 50% of subjects who stated that they always use condom when having sex but more than 25% of the subjects showed the positive PCR results for *N. gonorrhoeae*. This may be due to inaccurate research data from history taken.[25]

In this study, data on condom use history only include the frequency of condom use without information about how the subjects use it. Lee *et al*.[34] in his research in Thailand found that 68.3% of patients with HIV infection stated that they always use condom when having sexual intercourse but 8.9% of them were exposed to gonorrhea and other STIs. This result was considered as the result of the fact that the questionnaire used in the study did not include any question about correct condom use.

Several other studies found that condom use is associated with gonorrhea infection, although some did not reach statistical significance.[22,24,27,35] In research by Lu *et al*.[27] in people living with HIV, most of the subjects who were infected by gonorrhea claimed that they rarely or never use condoms. Similarly, the research by Bastos *et al*.[22] in Rio de Janeiro stated that an increase in the incidence of gonorrhea is associated with less consistent condom use.

History of previous STIs was not associated with the incidence of gonorrhea in patients with HIV infection in this study. Majority of subjects with positive PCR result stated that they do not have a previous history of sexually transmitted infections. These results are in accordance with the results of Rieg *et al*.[20] on MSM patients with HIV infection in Los Angeles in 2006 which found

that a history of previous STIs was not associated with the incidence of gonorrhea. It is presumably because most STIs are asymptomatic so they are not recognized by the patients.[20]

In conclusion, the prevalence of gonorrhea infection in male urethra and female cervix of HIV positive patients based on PCR examination in Teratai Clinic of Dr. Hasan Sadikin General Hospital Bandung were quite high, 37% and 33% respectively. Identifying gonorrhea infection based on PCR examination should be considered for routine screening test in HIV positive patients.

Positive *N. gonorrhoeae* PCR results were found in subjects with an average age of first intercourse at 19.5 years old. In addition, positive results were more common in heterosexual subjects and those with more than six lifetime sexual partners.

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