Polymerase chain reaction *chlamydia trachomatis* examination in nonspecific genital infection patients

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Abstract

Nonspecific genital infection (NSGI) is an inflammation of urethra, rectum, or cervix that caused by nonspecific bacteria. *Chlamydia trachomatis* is known as the most causal organism of NSGI, usually mild (mucopurulent discharge) or asymptomatic, and if untreated it can cause serious complication such as pelvic inflammatory disease that leads to infertility in women. The diagnosis of *Chlamydia trachomatis* needs an advanced method, such as polymerase chain reaction (PCR). PCR has high sensitivity and specificity, and endocervical swab is specimen of choice that also has high sensitivity and specificity to diagnose *Chlamydia trachomatis.* This research aims to evaluate if *Chlamydia trachomatis* is the most causal organism of NSGI by PCR *Chlamydia trachomatis* using 201bp primers. Eighteen NSGI married patients who came to outpatient clinic were evaluated from endocervical swab. The result demonstrated that 16,67% from eighteen NSGI patient positive *Chlamydia trachomatis.* The low incidence of *Chlamydia trachomatis* in low risk population such in this study need further study, the cause of NSGI needs to be known certainly so the exact treatment can be given.

Introduction

Non specific genital infection (NSGI) is an inflammation of urethra, rectum, or cervix that caused by non specific bacteria.[1] The prevalence of NSGI at outpatient clinic Dr. Soetomo General Hospital in 2016 is 47 (1,25%) cases from 3.753 all new dermato and venerology cases while percentage of NSGI is 17,23% from 273 total new cases in sexual transmitted disease division. The diagnose of NSGI is established by detail anamnesis, clinical examination for the sign of urethritis or mucopurulent exudate from cervix, Gram staining from cervical swab which is polymorphonuclear more than 30 in wide microscopic field and all other specific bacteria or fungal have already excluded such as diplococcic Gram negative bacteria (*Neisseria gonorrhea*), *Trichomonas vaginalis*, candidiasis vulvovaginalis and bacterial vaginosis.[1]

Chlamydia trachomatis is known as the most organism causing NSGI is an obligate intracellular bacteria that grow and replicate in eukaryotic host cells. The development cycle is biphasic with morphologically cell types include the infective particles (elementary bodies) and the reproductive particles (reticulate bodies). *Chlamydia trachomatis* infected more than 100 million people every year in worldwide by sexual transmission. Genital *Chlamydia trachomatis* infection affected women mostly asymptomatic that the patients are not aware and do not seek for treatment. The *Chlamydia trachomatis* infection that not treated will cause serious complication in women such as pelvic inflammatory disease which can lead to infertility and ectopic pregnancy.[1-3] To establish the diagnose *Chlamydia trachomatis* infection can not only with conservational microscopy laboratory. The absence of peptidoglycan in *Chlamydia trachomatis* explains why the organism is not seen with standard Gram's staining.

The development of tests based on nucleic acid amplification technology (NAAT) has been the most important advance to diagnose *Chlamydia trachomatis* because nucleic amplification is highly sensitive its capablity of detecting as little as a single gene copy and also highly specific. It does not need invasive sampling and it is a critical advantage to screen infection since the majority of chlamydial infection in women are asymptomatic. DNA amplification tests using noninvasive sampling have been reported to improve screening test that use invasive sampling by at least 30% increase in sensitivity. The most widely known of the DNA amplification technique is polymerase chain reaction (PCR).[4]

Diagnostic methods based on PCR are the most advanced tools for *Chlamydia trachomatis.* Several recent studies demonstrated that PCR has higher sensitivity and specificity superior to the other methods. PCR is based on amplification of a DNA sequence that is highly specific for infectious agent. Such large amount of DNA can easily detected and this is the prime reason why PCR is more sensitive than the other methods, diagnostic sensitivity of PCR based method for *Chlamydia trachomatis* is 90 – 100%. PCR is directly detect the presence of bacterium by identifying specific DNA in the bacterium. PCR amplify and detect a sequence highly specific for the bacterium that make PCR method has high specificity. PCR specificity to diagnose *Chlamydia trachomatis* is 99,6%. PCR is a rapid diagnostic test, it can be processed in 5 – 6 hours and the result is highly reliable. Samples in need for PCR can be from all sources, it may use noninvasive samples as well.[5,6]

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Materials and Methods

This research is descriptive observational study, using cross sectional method to evaluate if *Chlamydia trachomatis* is the most organism causing NSGI. Samples were all NSGI patients that came to dermatovenerology outpatient clinic Dr. Soetomo general hospital Surabaya in sexual transmitted disease division and were taken consecutively in 3 months (June – August 2017). Inclusion criteria include women with IGNS who are married. Women in menstrual period, pregnant and diagnosed with mixed infection are excluded.

Informed consent was obtained from the patients before procedure. All women patients who came to outpatient clinic with mucopurulent discharge were performed an

internal examination, endocervical swab is collected twice at the same time, one sample for the gram staining, wet preparation and KOH examination. The other endocervical swab sample is collected in to tube filled with phosphate buffer saline (PBS). Once the result from gram staining, wet preparation and KOH examination determined if it is found polymorphonuclear leucocyte more than 30 in wide microscopic field and the specific organism such as *Neisseria gonorrhea*, *Trichomonas vaginalis*, candidiasis vulvovaginalis and bacterial vaginosis are already excluded the diagnose of NSGI is established.

The samples in tube filled with PBS are stored in cool place with temperature of refrigerator and the samples were delivered in cooler bag with ice gels inside to keep the samples in cool temperature. The samples then brought to Institute of Tropical Disease (ITD) in order to perform PCR examination, the PCR tool used in this study was MyQ-2 (Bio Rad). The primer used in this study was 201bp targeted in endogenous plasmid CTP1 (*forward strand:* 5'-TAG TAA CTG CCA CTT CAT CA-3') dan CTP2 (*reverse strand:* 5'-TTC CCC TTG TAA TTC GTT GC-3'). Ethical clearance had been approved for this study by Ethical Committee in RSUD Dr. Soetomo general hospital.

Results

This research involved 18 women diagnosed with NSGI in dermatovenerology outpatient clinic - sexually transmitted disease division RSUD Dr. Soetomo general hospital Surabaya. All patients meet the requirement and are willing to participate in this research by signing informed consent and information for consent. The result in this study, *Chlamydia trachomatis* is detected in 3 (16,67%) NSGI patients, the rest of 83,33% were negative. Table 1 and Figure 1 show the percentage of patients with negative and positive PCR *Chlamydia trachomatis*.

Discussion

Based in literature *Chlamydia trachomatis* is the most (30% – 50%) causal organism of NSGI. Genital *Chlamydia trachomatis* infection in women majority is asymptomatic and when there is any symptoms, it is mild but the complication may serious when it is untreated.[1,7] It is important to use sensitive and specific molecular assay like PCR to prevent under diagnosed of genital chlamydial infections.[8]

NAAT has been the most important advancement in chlamydial infection diagnosis since *in vitro* cell culture techniques replaced the yolk sac for culture and isolation of the organism from clinical specimens. This technique detect nucleic acid targets that not depend on either viability or an intact state of the target organism for positive result. NAATs also known as the most sensitive tests for the screening and diagnosis of chlamydial infection of the genital tract.[9] NAATs include PCR and ligase chain reaction (LCR). PCR test utilize two synthetic oligonucleotide primers with sequences that are completing to the flank regions of a specific DNA segment present in the target organism.[4] This study use PCR and the primers used for amplifying a 201 bp fragment of *Chlamydia trachomatis* endogenous plasmid were CTP1 (*forward strand:* 5'-TAG TAA CTG CCA CTT CAT CA-3') dan CTP2 (*reverse strand:* 5'-TTC CCC TTG TAA TTC GTT GC-3'). The specimens used in this study were from endocervical swab which is the appropriate for women undergoing speculum examination which has 100% sensitivity and 98% specificity.[6,10]

The result of this study performed in Dr. Soetomo general hospital Surabaya is 3 (16,67%) of 18 NSGI patients were detected *Chlamydia trachomatis* with PCR using endocervical swab samples. This is consistent with previous studies conducted in Padang, from June 2015 – April 2017 that shows the result of PCR *Chlamydia trachomatis* detected in 10% of 39 NSGI patients.[11]

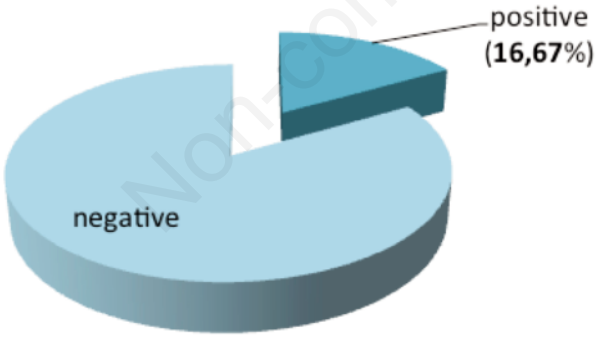

Figure 1. Profile result of Polymerase chain reaction *Chlamydia trachomatis* in Nonspecific genital infection patients.

Table 1. Profile result of Polymerase chain reaction *Chlamydia trachomatis* Nonspecific genital infection.

Profile of polymerase chain reaction *Chlamydia trachomatis*	Patient	Percentage (%)
Positive	3	16,67
Negative	15	83,33
Total	18	100

Conclusions

Chlamydia trachomatis was known to be the most causal organism of NSGI. The study that perform in low risk population such as in this study may result in low detection of *Chlamydia trachomatis.* Detection of *Chlamydia trachomatis* needs an advance technique, to date PCR based method is the most reliable methods for detection of chlamydial infections because its high sensitivity and specificity which are very important characteristic for a laboratory diagnostics. Further study of causal of NSGI in low risk population needs to be done, It is important to know the cause of NSGI certainly so the exact treatment can be given.

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