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Molecular Detection of Chlamydia Trachomatis and Neisseria Gonorrhea Prevalence in Pregnant Women

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ABSTRACT:

Background: Chlamydia trachomatis *and Neisseria gonorrhea are the most public health concern in developing countries. Screening for sexually transmitted infection such as Neisseria gonorrhea and Chlamydia trachomatis was suggested by CDC at first visit and also last trimester of pregnancy because early infection can asymptomatic and also may complicated by severe sequela.*

Objective: *This paper has aimed at estimating the prevalence of infections by Chlamydia trachomatis and by Neisseria gonorrhea in pregnant women. This study was carried out to determine prevalence of* C. trachomatis and *N. gonorrhea among pregnant women in Tehran, Iran'*

Methods: *In this study, 196 urine specimens were collected from pregnant women referred to Rasuol-e- Akram hospital. Detection of organisms was done using duplex PCR method with specific primers for each organisms.*

Results: *Overall, 6.1% and 4.1% of the specimens were positive for* C. trachomatis and *N. gonorrhea respectively using duplex PCR assay. Co-infection was found in 4.1% of the patients.*

Conclusion: *In comparison to other studies, a moderate and high prevalence of chlamydial and gonococcal infections were seen in pregnant women. According to potentially dangerous complications of chlamydial and gonococcal infections, the results endorse that pregnant women should be screened routinely for detecting the Chlamydia and gonococcus infections.*

Keywords: *Neisseria gonorrhea,* chlamydia trachomatis, *prevalence, pregnant women*

I. INTRODUCTION

Genitourinary tract infections are a major public health concern globally. C. trachomatis and N. gonorrhea are the most common causes of lower genital tract infection. The Co-infection of C. trachomatis with other bacterial vaginosis has been found in 12.7% cases[1]. C. trachomatis is one of the prevalent bacteria found in genital tract infections worldwide and manifest in a variety of syndromes and sever complications including mucopurulent urethritis, epididymitis, cervicitis, acute salpingitis, pelvic inflammatory diseases (PIDs) and infertility[1-3].

According to the World Health Organization report , 101.5 million chlamydial infections are reported annually worldwide[2]. The prevalence of infection caused by C. trachomatis in pregnant women ranges from 2-35%[4]. Gonorrhea is a sexually transmitted infection usually manifested by urethritis, cervicitis and salpingitis[5]. Approximately, 62 million new infections with N. gonorrhea occur annually worldwide [6]. Gonorrhea rates in women are slightly higher than in men. Similar to C. trachomatis infection, N. gonorrhea is an important cause of PID and consequently can lead to infertility or ectopic pregnancies[7].

Early diagnosis and treatment of infected individuals is needed for preventing spread of the disease and also severe complications. Traditionally, tissue culture was considered as the gold standard for diagnosis of the disease. This method has several limitations including low sensitivity, long testing time and high cost. However, the diagnosis has become fast and easy using newer diagnostic techniques especially molecular methods which are not only highly sensitive and specific but also cost-effective [1].

Recently molecular amplification assays like Polymerase Chain Reaction (PCR) have been found to be highly sensitive and specific methods for detection of N. gonorrhea and C. trachomatis in urethral cervical and urine specimens [8]. Asymptomatic nature of the disease and broad spectrum of infections caused by C. trachomatis and N. gonorrhea highlight using of the sensitive and reliable diagnostic laboratory methods. In present study, the species specificity duplex PCR assay was used to detection of N. gonorrhea and C. trachomatis in infected pregnant women, simultaneously. This assay is very specific and may allow the proper diagnosis of infections with lower false-positive results [9].

Effective public health of genitourinary tract in pregnant women is important to avoid the maternal and neonatal complications. It can achieve with accurate estimation of prevalence and incidence of infections based on highly sensitive and specific approach. Thus, this epidemiological study was carried out to determine prevalence of C. trachomatis and N. gonorrhea among pregnant women.

II. MATERIAL AND METHODS

Subjects

All subjects were requested for filling a questionnaire to record demographic information, history of abortion, and symptoms. We excluded women who had taken antibiotic in the past 14 days. The 30-50 ml urine sample was collected from 196 pregnant females, referred to Rasoul-e-Akram hospital in Tehran, Iran, from May 2014 to October 2014.

All specimens for possible Duplex PCR testing (one urine container per patient) were kept at 4°C for less than 18 hours until transported to the laboratory, and 10 ml aliquots of unspun urine were pipetted into polyethylene tubes and also frozen -70°C until Duplex PCR testing. Demographic characteristics, history and symptoms of patients were obtained and pregnant females who used antibiotics were excluded.

DNA Extraction

On the day of Duplex PCR, urine samples were thawed and vortexed and one ml aliquot was transferred to a labelled micro centrifuge tube. The samples were centrifuged for 30 min at 6,000 rpm. The urine supernatant was then removed by pipet, and 1 ml of urine Duplex PCR resuspension buffer was added to each tube. The micro centrifuge tubes were sealed with lid locks and vortexed to resuspend the pellet. DNA extraction was done using DNA extraction Kit (Roche Diagnostics GmbH, Mannheir, Germany) according to the manufacturer's protocol.

Duplex PCR assay

The Duplex PCR reaction mixture included 1 µl of extracted DNA, 20 µl of Taq DNA Polymerase Master Mix RED (Ampliqon), 0.5 µl of each specific oligonucleotide primers (10) in a total volume of 40 µl.
The Duplex PCR cycling parameters were 95°C for 10 min, 35 cycles of denaturation for 40 sec at 95°C, annealing for 40 sec at 54°C, an extension for 40 sec at 72°C, with a final extension of 8 min at 72°C. The PCR products were visualized by 1.5% agarose gel electrophoresis in TAE buffer.

III. RESULTS

The age of participants in this study ranged from 17 to 41 (28.73±5.52). Screening for sexually transmitted diseases was regularly done in 32.7% of women. Abortion, sexually transmitted disease and preterm delivery were records in 21.4%, 10.2% and 8.2% of the studied patients, respectively. Overall, 6.1% and 4.1% of the specimens were positive for C. trachomatis and N. gonorrhea by the duplex PCR assay (Fig 1). Totally, co-infection was found in 4.1% of patients. N. gonorrhea and C. trachomatis were detected in 75% of patients suffering from asymptomatic infections using duplex PCR. No significant association was seen between age, screening, awareness and infection in this study (P-value $\geq$ 0.05).

IV. DISCUSSION

Although there isn't screening program for chlamydial and gonococcal infections during pregnancy, some evidence proves screening for C. trachomatis and N. gonorrhea in high risk women during pregnancy can reduce the adverse outcomes of labor[11].

Chlamydial infection may be transmitted to infant during delivery [12]. Two previous studies indicated treatment of chlamydial infections can improve unexpected outcomes of pregnancy such as low birth weight and neonatal death[13,14]. In previous reports from Iran, the variable rates of chlamydial infections among Iranian women are showed (2.75-22%)[15-17]. Therefore, screening of C. trachomatis infection is one of the most important hygiene concern during pregnancy.

In present study, prevalence of C. trachomatis and N. gonorrhea were reported 6.1% and 4.1%, respectively. These findings were nearly similar to previous studies were done in Tehran[18-20]. In Badami and Khazardoost et al. studies, C. trachomatis infection on pregnant women was reported 2.75% and 2.9% using Direct Immuno flourescent and ELISA assays, respectively[21,22].

Reports from other province in south and south-west of Iran, Hormozgan and Khozestan, the prevalence of C. trachomatis infection on pregnant women were estimated 5.2% and 10%, respectively[23,24]. High prevalence of C. trachomatis infection(15.81%) was reported from Sabzevar city(northeast era of Iran) [22]. Taken together, prevalence of C. trachomatis differs in different regions because of treatment regimen in these areas, use of several diagnostic methods, differences in sampling, health status, geographic area and the awareness rates of individuals.

In general, the prevalence of N. gonorrhea in differfent regions of Iran is variable. In studies performed in Yasuj, Kermanshah and Kashan for simultaneously detection of C. trachomatis and N. gonorrhea using Multiplex PCR, high prevalence of N. gonorrhea were reported [25-27].

In this study, the rate of N. gonorrhea infection (4.1%) in participants was higher than the infection rate reported by Hassanzadeh et al (1.18%)[26]. In another research was performed on 328 pregnant and non-pregnant women in Zanjan, Iran, the prevalence of N. gonorrhea was reported 0.9%[28]. In a study performed in Sabzevar, Iran, the reported prevalence of gonococcal infection using Triplex PCR technique was 1.25% [29]. The results obtained from study in Tonekabon were showed the rate of N. gonorrhea infection among pregnant female about 4.54% [30]. The variable degree of gonorrhea rates among pregnant female are not well understand. It may be due to differences in access and use of health services, geographic clustering of populations, social and economic factors.

The variable prevalence of C. trachomatis and N. gonorrhea was found in Brazilian pregnant women, as 9.8% to 11.1% for C. trachomatis and 0% to 1.5% for N. gonorrhea[31]. According to recent reports from USA, the high prevalence of C. trachomatis and N. gonorrhea (49%) were reported in pregnant individuals[32,33]. Several studies on European pregnant women for detection of C. trachomatis have revealed the prevalence rate of infection varies from 0 to 37%[33-37].

The prevalence of chlamydial and gonococcal infections in pregnant women is nearly consistent with the studies from other Islamic communities. The 8.7% incidence of C. trachomatis infection in pregnant women was reported in Saudi Arabia[38]. While, N. gonorrhea among pregnant women was 0.0% in Saudi Arabia[39]. C. trachomatis infection is an important causative agent of abortions in pregnant women in Iraq[40]. The prevalence of gonorrhea and chlamydial infections reported in developing countries range from 2–7% and 3–29%, respectively [3] .

The low prevalence rate of infections in Muslim community may be due to religious belief in these populations. While, it has been shown that demographic factors such as youth, non-white race, multiple sexual partners and the use of oral contraceptives in women increase the risk of chlamydial infection in USA and Europe. In conclusion, the results suggest that the Duplex PCR assay is a useful, rapid, cost effective and satisfactory diagnostic tool for the detection of N. gonorrhea and C. trachomatis in clinical urine samples, simultaneously. Since many chlamydial infections are asymptomatic, it seems that periodic testing of individuals at risk must be done for effective control of infection. According to potentially dangerous complications of chlamydial and gonococcal infections, the results endorse that pregnant women should be screened routinely for detecting of Chlamydia and gonococcus bacteria. Finally, more studies on the epidemiology of C. trachomatis and N. gonorrhea must be carried out to determine the true prevalence of this organism in all regions of Iran.

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Competing Interests: None

Ethics approval:

This study was approved by the institutional review board (IRB) of the Iran University of Medical Sciences, Rasoul-e- Akram Hospital (IRB No. IR.IUMS.REC.1392.24182) and also each participating hospital. The requirement for informed consent of subjects was waived by the board.

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