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Prevalence of Chlamydia Trachomatis in First Void Urine Specimens of Women with Symptomatically Cervicitis in Ahvaz, Iran

Mansour Amin[1], Sahar Nasiri[2], Tahereh Navidifar[1]*

[1]Health Research Institute, Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

[2]Department of Microbiology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

*** Corresponding author**

Abstract: C. trachomatis is one of the most common cause of STDs such as urethritis in men and cervicitis in women. Many of the infected adolescents and young adults are reported as asymptomatic reservoirs of C. trachomatis and are unaware of their infections. The untreated cervicitis infections of C. trachomatis cause many reproductive complications including PID, increasing chance of the development of cervical neoplasia, rupture of the membranes in pregnancy and chorioamnionitis. In this study the prevalence of C. trachomatis in FVU specimens of women with cervical infections and their IgM titers were investigated by PCR and serology, respectively. This study was conducted from May to September 2015 at teaching hospital of Razi, Ahvaz, Iran. In this study, from each woman participants one FVU sample and one blood sample was provided and overall 163 FVU and blood samples were collected from sexually active women (15-45 years) who had the symptomatic genital infections. Detection of C. trachomatis DNA was performed in FVU samples by PCR. Also, anti_C. trachomatis IgM antibody in the serum samples were recognized. Out of 163 FVU samples, 41(25.1%) cases were positive based on PCR technique. The anti C. trachomatis IgM was detected in 46 (28.2%) cases. In this study, there was a clear association between C. trachomatis infections with abortion, post coital bleeding and dyspareunia. Also, it seems both PCR and serology methods are appropriate for diagnosis of C. trachomatis infections.

Keywords: Chlamydia trachomatis, PCR, IgM, FVU

Introduction

Chlamydia trachomatis is a sexually transmitted gram-negative bacterium and is one of the most common causes of sexually transmitted diseases (STDs) such as urethritis in men and cervicitis in women [1]. This bacterium is prevalent, especially in the developing countries, where the diagnostic and treatment facilities are seldom [2]. Many of the infected adolescents and young adults are as the asymptomatic reservoirs of C. trachomatis and are unaware of their infections [3]. These individuals establish an ongoing source for the infection transmission to other and also cause a silent, destructive disease in themselves [4]. Subsequently, the untreated cervicitis due to C. trachomatis causes many reproductive complications, including Pelvic inflammatory disease [PID], increasing the chance of the development of cervical neoplasia, the rupture of the membranes during pregnancy and chorioamnionitis. PID ranged from endometriosis, salpingitis, tubo-ovarian abscesses, pelvic peritonitis and peri appendicitis [5]. The annual prevalence of PID is approximately one million cases that in 20 percent cases occur among the adolescents [6]. On the other hand, C. trachomatis increases the risk of HIV transmission and is associated with more severe symptoms in the infected patients

with HIV [7]. In 2012, according to the report of the Centers for Disease Control and Prevention (CDC), 1422976 people were suffering from C. trachomatis infections [8]. Since the treatment of PID and the infertility due to C. trachomatis has high financial costs, the expansion of the screening programs for detecting the asymptomatic women are essential in each community. The main aims of these programs are early detection and the treatment of uncomplicated lower genital tract infections [9]. Currently, there are various diagnostic assays for detection of C. trachomatis, including the isolation in cell culture, direct cytological examination, antigen detection methods based on enzyme immunoassay, direct fluorescent antibody (DFA), serology tests, nucleic acid amplification (NAA) and nucleic acid hybridization (NAH). Out of these methods, NAA is the best diagnostic tool for the screening programs. In women, the excellent clinical specimens for diagnosis of this bacterium by NAA test are first void urine (FVU), vaginal and vulvar swabs [10]. The main advantage of the methods based on NAA is raising the chance of obtaining a positive result in the onset of the illness than serology and lack of a requirement to the viable organisms in the clinical samples. The antibodies have been considered as a marker of the C. trachomatis infections in the disease time. Moreover, serology is a non-invasive screening tool in infertility studies on a large scale [10]. Cell culture of Chlamydia involves delicate processing procedures and well-trained personnel. There is growing recognition that the cell culture has not a sensitive of 100% and, therefore, can not be an acceptable "gold standard" method [11]. To our knowledge, there has not been any adequate research on the detection of C. trachomatis in FVU specimens from women with cervicitis in Ahvaz. For this reason, in this study, the prevalence of C. trachomatis in FVU specimens of symptomatically infected women with and their IgM titers were investigated by PCR and serology respectively.

Materials and Methods

Design of study

This study was conducted from May to September 2015 at Razi Hospital, Ahvaz, Iran. The study was approved by the Research Ethics Committee, Ahvaz Jundishapur University of Medical Sciences, Iran. The written informed consent form was completed by each participant. For sampling, the symptomatically infected women were defined as those presenting with one or more clinical symptoms of genitourinary infections, including abnormal vaginal discharge, dysuria, post coital bleeding, dysmenorrhoea and dyspareunia [12].In this study, from each symptomatically infected woman was provided one FVU sample and one blood sample. An overall 163 FVU and blood samples were collected from the sexually active women (15-45 years). Moreover, the exclusion criteria were pregnancy, a history of cervical cancer and the consumption of any antibiotic in last 10 days [13].

Sample collection and processing

In this study, from each woman 10 ml FVU and two ml blood were taken and transferred to microbiology laboratory of the medical school. The FVU samples were centrifuged at 12000 g for 20 min and their pellets were suspended in 500 microliter PBS. On the other hand, for serology test, the blood samples were centrifuged at 5000 g for 7 min and then their serums were collected in 1.5 ml Microtubes.

Serology test for recognition of C .trachomatis

Anti C. trachomatis IgM antibody in the serum samples were recognized by Anti Chlamydia trachomatis ELISA(IgM) kit (Euroimmune, Germany) according to manufacturer's procedure. The cutoff value established by the manufacturer was used for the interpretation of results of the IgM antibody. Briefly, an IgM titer with the ratio>1.1 was suggested as positive, 0.8 to 1.1 as the borderline range, and under 0.8 as negative.

Detection of C. trachomatis by PCR

A volume of 200 µl of the suspended pellet was taken in a 1.5 ml microtube. DNA was extracted using High Pure PCR Template Preparation Kit (Roche Diagnosis, Mannheim, Germany) according to the manufacturer's procedure. In order to identify C.trachomatis, the amplification of ytfF gene (encoding membrane transport/efflux protein that is conserved in all of C.trachomatis strains) was performed. The used primers for polymerase chain reaction (PCR) were as follows: forward primer: 5'-AGGTAAGAAAGAATCCATCC -3' and reverse primer: 5'-GTTTTCGTGATCCCTAGTAT -3' which amplified a 785bp fragment from the ytfF gene. The volume of PCR reaction was 25 µL and prepared as follows: 12.5µl 2x master mix (Ampliqon – Denmark), 0.4pmol/µl of each primer, 8 µl of the DNA sample and distilled water up to 25 µl. The amplification was carried out in a thermal cycler (Eppendrof-Germany). The cycling program was corresponded to 1 cycle at 94°C for 5 min, 35 cycles at 94°C for 45 seconds, 48°C for 45 seconds and 72°C for 30 seconds and a final extension cycle at 72°C for 5 min. The amplicon of 785bp was visualized on a 1 % agarose gel stained with ethidium bromide.

Statistical analysis

The values of sensitivity, specificity, Negative Predictive Value (NPV) and Positive Predictive Value (PPV) were calculated for the serology compared to PCR method as a gold standard test. Chi-square and Fishers exact test used for comparisons of categorical data. A p value <0.001 was considered statistically significant.

Result

In this study, 163 FVU and serum samples were taken from the sexually active women referred to Razi Hospital, Ahvaz. Their ages ranged from 18 to 39 years old, and their ages mean was 29.65 ± 5.38 years old. The C.trachomatis infections in females were almost in the age range 31 to 35 and followed by age range 21 to 25 years old, respectively. The detection of C. trachomatis was performed by the amplification of ytfF gene and is shown in [**figure 1**]. Out of 163 FVU samples, 41 (25.1%) samples were positive based on PCR technique. The prevalence of C. trachomatis DNA and its risk factors are shown in table1. An acceptable titer of IgM was seen in 46 (28.2%) cases that among them 28 cases had an IgM titer with ratio ≥ 1.1 but in 18 remaining cases, the IgM titer was in the borderline range. The prevalence of anti C. trachomatis IgM and its risk factors are shown in table 2. In our study, the rates of sensitivity, specificity, PPV and NPV for IgM compared to PCR were 97.2%, 93.4%, 82.6% and 97.4% respectively.

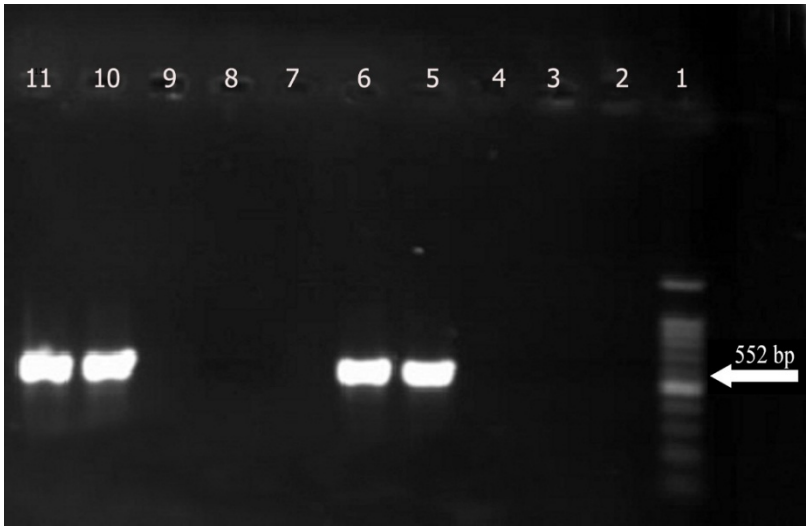

Figure 1: The amplification results of ytf gene (785 bp) in c.trachomatis. Lane of 1: ladder of 100 base pair (cinnagene_Iran), lanes of 2,3,4 and 5: c.trachomatis from clinical samples. lane of 6 : negative control (water).

In our study, 31 women had at least a history of abortion during past five years. Among them, C. trachomatis DNA was detected in FVU samples of 15 women. In addition, 16 women had an acceptable titer of IgM. The prevalence of C. trachomatis DNA in FVU samples of the women with a history of abortion was more than women without a history of abortion (48.4% vs.19.7%). In our study, 36 women complained from post coital bleeding. Moreover, this symptom was seem more among women infected with C. trachomatis than non-infected women (86.1% vs. 7.9%).In addition, there was a meaning association between the post-coital bleeding and the detection of anti C. trachomatis IgM (p= 0.001). Furthermore, this antibody was detected in 80.5% the women with the post-coital bleeding vs. 13.3% women without this symptom. In this study, the prevalence of C. trachomatis DNA and IgM among women with dyspareunia was more than women without this symptom (p<0.001for IgM and p<0.001for DNA). On the other hand, in the present study seems using the condom had an important role in deducing the colonization with C. trachomatis. Furthermore, the prevalence of this bacterium among women who their sex partners had used condom, was lower (13.1%) than the women who had consumed contraceptive drugs (52.4%) or had natural prevention (27.3%).

Discussion

Chlamydia infections are mainly one of the great concerns in women than in men because the manifestations and consequences of this bacterium is more threating for the reproductive organs of women. Since the majority of these infections are asymptomatic in women, they account as a potential source of the infections for their partners and the recurrent and latent infections in themselves [1]. In our study, the prevalence of C. trachomatis based on PCR and serology was 25.1% and 28.2%, respectively. Moreover, based on IgM assay, C. trachomatis DNA had not seen in five cases with an IgM titer in the borderline range. For this reason, for the accurate integration of these five cases, we suggest using a technique with more sensitive such as Real time PCR. In the other hands, the rates of sensitivity, specificity, PPV and NPV for IgM compared to PCR were 97.2%, 93.4%, 82.6% and 97.4% respectively. In similar to our study, Muvunyi et al, reported the high values of the sensitivity, specificity and NPV, respectively (86%, 84% and 99%) [27].However, unlike our study, the value of PPV in Muvunyi et al, study was low (17%).The high values of NPV and PPV give us a high confidence that the negative and positive results of IgM are actually true. For this reason, our study IgM assay as a confident diagnosis method for Chlamydial infections suggests to all laboratories. [27]The prevalence of this bacterium in FVU samples of women in other regions of Iran was reported to be variable, including 12.6% in Tehran [14], 22.44% in Yazd [15], 13.77 % in Sabzevar [16] and 14.99% in Tehran [17]. The

incidence of this bacterium in two regions of USA and New Zealand was 20.6% ,11.6% and 36% , respectively [18, 19, 20]. This variation in prevalence can be explained due to age of the participants, type of population, and using different techniques for detection. Also, the prevalence of this bacterium in our study was more than some studies in other regions of Iran because all these studies were performed at least during past five years or more, in addition population studied in our research were selected only women with symptomatically cervicitis. The abnormal vaginal discharge is a significant cause the morbidity among women because may predispose them to PID, infertility, endometriosis, urethral syndrome, pregnancy loss, and preterm labour The predisposing factors of the symptomatic vaginal discharges are poor hygiene, low socioeconomic status, early sexual activity and multiple partners [21]. In our study, the prevalence of the abnormal vaginal discharge among women with the infection of C. trachomatis was 32.6% but in the study of Beni et al, the frequency of this symptom was 56.2% [12]. Dyspareunia, pelvic pain during sexual activity, is one of the complaint of the cervical infections. In our study, the prevalence of this symptom among women with the infection of C. trachomatis was high. However, in the study of Bourgeois et al, this symptom was reported lower rather than our study (56.8% vs. 32.9%) [22]. The abortion could be induced by spreading a persistent asymptomatic C. trachomatis infection to the fetal tissues or endometrium [23]. In our study, a clear association was found between spontaneous abortion with serologic ($p = 0.003$) and molecular ($p = 0.001$) evidences of the C. trachomatis infection. Similar to our study, Baud et al, also proved this association [23]. Moreover, they documented C. trachomatis in the placenta samples by specific immunohistochemistry. However, several studies have failed to document a clear association between C. trachomatis infection and the spontaneous abortion [24, 25]. According to data of Osser, the frequency of IgM antibody among the women with at least a history of abortion was 137/349 (39.3%) which was not statistically different from that among controls (116/349; 33.2%) [24]. Similarly, Grönroos also did not find any clear association between C. trachomatis infection with abortion [22]. However, these two studies were conducted >10 years ago, i.e., before the recent dramatic increase of C. trachomatis infections and the extension of the diagnostic methods with high sensitivity and specificity for this bacterium. A link between the genital C. trachomatis infections and ectopic pregnancies have mainly been established through sero epidemiologic cross-sectional studies. In these studies, the selected groups of patients with ectopic pregnancy had been compared with women who had normal pregnancies or with non-pregnant controls [26].In our study, there was no a clear association between ectopic pregnancy with serologic ($p = 0.554$) and molecular ($p = 0.669$) evidences of C. trachomatis infections. However, according to data of Bakken et al, a strong relationship was observed between a previous infection of C. trachomatis and ectopic pregnancy in women born 1970 and later, whereas no association was found among women born 1950–1959 and 1960–1969 [26]. It may be due to the high incidence of C. trachomatis infections during years 1970 and later. In conclusion, we detected C. trachomatis DNA in 41 (25.1%) females. Also, an acceptable titer of IgM was found in 46 (28.2%) cases. In this study, the correlation between the IgM and the PCR assay revealed a relatively strong agreement. There was a clear association between C. trachomatis infections with abortion, post coital bleeding and dyspareunia. Also, it seems both PCR and IgM assay can be appropriate for an accurate diagnosis of the C. trachomatis infections.

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Conflicts of interest

All authors declare no conflicts of interest.

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