Molecular Detection of Chlamydia Trachomatis in Patients with Pelvic Inflammatory Disease Visiting a Tertiary Care Hospital in Pakistan

Afnan Naeem, Hafsa Waseem, Sakhawat Ali, Javaid Usman, Mehreen Gilani, Amna Javed

Army Medical College/National University of Medical Sciences (NUMS), Rawalpindi Pakistan

ABSTRACT

Objective: To detect the frequency of Chlamydia trachomatis in urine samples of women with Pelvic Inflammatory Disease visiting a tertiary care hospital using a Polymerase Chain Reaction assay and find an association between different risk factors.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Microbiology, Army Medical College/National University of Medical Sciences Rawalpindi Pakistan, from Mar 2018 to Jan 2019.

Methodology: Over eleven months, 60 diagnosed urine samples of married females with pelvic inflammatory disease between the ages of 14-49 years were included in the study. DNA of Chlamydia trachomatis from urine samples was extracted manually using the commercially available kit. It was detected by performing a real-time Polymerase Chain Reaction assay using a forward primer (5′-CATGAAAACTCGTTCCGAAATAGAA-3′) and a reverse primer (5′-TCAGAGCTTTACC-TAACAACGCATA-3′) of sequence mentioned above for amplification of target sequences, of the Chlamydia trachomatis.

Results: Out of sixty cases included in this study, Chlamydia trachomatis DNA was detected in 12 cases (20%), and 48 cases (80%) were negative. So, the frequency of Chlamydia trachomatis in our study population was estimated at 20%. In addition, age, socioeconomic status, education and no of sexual partners were all risk factors were evaluated for their role in acquiring infection.

Conclusion: Females having Chlamydia trachomatis infection and other sexually transmitted infections are at an increased risk of developing Pelvic Inflammatory disease due to the presence of risk factors and the asymptomatic nature of the disease.

Keywords: Chlamydia trachomatis, Pelvic inflammatory disease, Polymerase chain reaction, Sexually transmitted infections.

INTRODUCTION

Sexually transmitted infections (STIs) are a silent epidemic with substantial health and economic consequences. STIs carry not only a risk of morbidity but also considerable mortality. According to World Health Organization (WHO), 350 million new STIs occur per annum, out of which 131 million cases are of Chlamydia trachomatis. It is the most common sexually transmitted bacterial infection worldwide.[1]

Around 75 percent of total cases of Chlamydia trachomatis take place in the developing world, making the situation more worrisome as these countries lack diagnostic and treatment facilities.[2] Centers for Disease Control and Prevention (CDC) reported 1.7 million infections in the United States in 2017.[3]

Chlamydia trachomatis infection is mostly asymptomatic. In females, it causes endometritis, cervicitis, ectopic pregnancy, Pelvic Inflammatory Disease (PID), infertility and sometimes death due to ruptured ectopic pregnancy.[4]

One of the main challenges of Chlamydia trachomatis infection is its asymptomatic nature. It occurs more frequently in young women, putting them at risk of acquiring and transmitting infection, especially in our setup where most cases go undetected due to lack of knowledge, screening programs, testing facilities and cultural barriers.[5]

In our study, we used a urine sample over an endocervical swab as the sensitivity of PCR of both swab and urine is equal, but urine specimen is a non-invasive sample collection technique.[6]

Pelvic Inflammatory Disease is ascending inflammation of the endometrium, fallopian tubes, ovaries or pelvic peritoneum. Clinically it is diagnosed as pelvic pain combined with inflammation of the lower genital tract. Infection with Chlamydia trachomatis is the foremost cause of it in females.[7]

There is no gold standard diagnostic test for detecting Chlamydia trachomatis DNA. Traditionally tissue culture was used for its diagnosis, but it has limitations. The sensitivity of culture is between 70-90 percent, and specificity is around 99 percent.[8] Other methods used for diagnosis are serology and antigen

Correspondence: Dr Afnan Naeem, Department of Microbiology, Army Medical College, Rawalpindi Pakistan

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detection. The sensitivity of serology is 50-60 percent, and specificity is 90-95 percent.[9] However, PCR is not only specific but also sensitive. The specificity of PCR is more than 99 percent, and sensitivity is above 90 percent.[10]

Females are among the highest risk groups that can acquire Chlamydia trachomatis infection. Our study uses PCR, which is a superior method to other diagnostic modalities as it is rapid, cost-effective, accurate, and requires a non-invasive sample collection technique.

METHODOLOGY

After obtaining approval from the Institutional Review Board of the institute, we carried out a cross-sectional study at the Department of Microbiology; Army Medical College (National University of Medical Sciences) Rawalpinfi Pakistan from March 2018 to January 2019.

We included 60 newly diagnosed cases of Pelvic Inflammatory Disease in our study. The sample size (n=60) was determined using the WHO calculator while keeping the confidence interval at 95% and taking the anticipated population with Chlamydia trachomatis as 4 %with an absolute precision of 5%.[11] Non-probability convenience sampling technique was used for obtaining samples.

Inclusion Criteria: All the sexually active females, 14 - 49 years of age, presenting with PID were included in the study.

Exclusion Criteria: All the females who had already received treatment for PID were excluded from the study.

PID was diagnosed based on clinical manifestations, including a history of severe lower abdominal pain, dysuria, dyspareunia, high fever, intermenstrual bleeding and prolonged menstrual cycle or based on transvaginal ultrasound or MRI demonstrating tubo-ovarian abscess or thickened tubes with or without free pelvic fluid.

Urine samples were obtained from patients under aseptic conditions and were transported to the laboratory within one hour of collection without any added transport medium. Upon receipt,10 ml of urine was centrifuged for 10 minutes at 1400 g. The supernatant was discarded, and the pellet was stored at-20℃. A real-time PCR assay detected Chlamydia trachomatis DNA in urine samples. DNA was extracted manually using a commercially available kit (Pure Link Micro-biome DNA purification kit Cat A29790 M/s Invitrogen). The instrument used was a Smart cycler by Cepheid PCR system. Two sets of primers, i.e., a forward primer and a reverse primer, were used for amplification of target sequences of the Chlamydia trachomatis cryptic plasmid gene. Sequences from a cryptic plasmid were used for designing (GenBank accession nos M19487, Y00505, J03321, X06707 and X07547) of five different Chlamydia trachomatis strains (serotypes A, B, D, L1 and L2, respectively), and designed using Primer Express software (Applied Biosystems). The primers were prepared by e-oligos by Gene Link.

Forward primer (5'-CATGAAAACTCGTTCCGA-AATAGAA3'), reverse primer (5'-TCAGAGCTTTA-CCTAACAACGCATA-3'), having the sequences as mentioned above, were used for amplification of target sequences of the Chlamydia trachomatis gene.

After DNA extraction, the microbial pellet was suspended in 800 µ1 of S1 lysis buffer and was transferred into a bead tube. After that, 100 µ1 S2 lysis enhancer was added and briefly vortexed. The sample was then incubated for ten minutes at 65 ^{0}C. It was homogenized in by beating for ten minutes. After that was centrifuged at 14000 x g for two minutes, and the supernatant was shifted to a new Eppendorf tube. After that, 900µl of binding buffer was added to the Eppendorf tube containing supernatant and was vortexed. Next, 700µl sample mixture was transferred to spin column, centrifuged and flow through was cast away. The spin column was placed in a collection tube, and 500µ1 of wash buffer was added. Centrifugation was done for one minute and then thirty seconds at 14000 x g. After that, 50µl of elution buffer was added to obtain purified DNA. The purified DNA was stored at -20℃. DNA was extracted from 200µl thawed or 200µl aliquot of pellet from 10 ml of centrifuged urine.

The DNA sample was defrosted, centrifuged and diluted with Nuclease-free water. The final volume of 20µl consisted of a forward and reversed primer of 0.25 µM and 5 µl. DNA, 10µl. Thermo Scientific Maxima SYBR Green/ROX qPCR Master Mix (2X). The SYBR Green dye, instead of a probe-specific sequence, was used for DNA recognition and analysis.

Cycling conditions of PCR were kept at 50℃ for 2 minutes, followed by 10 minutes at 95°C. After that, 45 cycles of 15 seconds each at 95°C were given. The last cycle was 1 minute at 60℃, leading to amplification and PCR product detection. Negative and positive control was used for each run of amplification.

A positive result was taken as threshold cycle (Ct), i.e., the cycle number at which dye emission was

above the background noise and was taken as a negative result if the fluorescent signal did not rise within 45 cycles.

The statistical package for social sciences (SPSS 23) was used for data analysis. For qualitative variables, frequency and percentages were calculated, and mean and standard Deviation (SD) were calculated for quantitative variables. The *p*-value of ≤0.05 was considered statistically significant, calculated using Pearson chi-square.

RESULTS

A total of 60 diagnosed females suffering from Pelvic Inflammatory Disease were included in our study. Our study participants were of varying ages. The mean age of study participants was 24.38±4.85 years ranging from 16 – 40 years.

Out of sixty participants included in this study, Chlamydia trachomatis DNA was detected in 12 (20%) samples, and 48 (80%) samples were negative. Therefore, the frequency of Chlamydia trachomatis in our study population was 12 (20%), as shown in Table-I.

Table-I: Presence of Chlamydia Trachomatis in Urine Samples of Females Having Pelvic Inflammatory Disease (n=60)

Samples	n (%)
Positive	12 (20)
Negative	48 (80)

Different factors play a role in acquiring Chlamydia trachomatis infection. Several sexual partners play a role in acquiring Chlamydia trachomatis infection and affect its prevalence; therefore, we divided our study participants into three groups. The first group included individuals with one sexual partner, individuals in the second group had two sexual partners and the third group comprised individuals with more than two sexual partners. A total of 54 (90%) of study subjects had one sexual partner, 6 (10%) had two sexual partners, and none of the participants had more than two sexual partners. Out of the total participants, 11 (91.7%) positive cases had only one partner, and 1 (8.3%) had two partners, as shown in Table-II.

Table-II: Presence of Chlamydia Trachomatis according to Number of Sexual Partners

Number of Sexual Partners	Chlamydia Trachomatis n (%) n=12	Pelvic Inflammatory Disease n (%) n=60
One partner	11 (91.7)	54 (90)
Two partners	1 (8.3)	6 (10)

We calculated the correlation between no of sexual partners and frequency of Chlamydia trachomatis infection (*p*=0.046), showing that the two variables are statistically significant, as shown in Table-III.

Table-III: Presence of Chlamydia Trachomatis according to number of Sexual Partners (n=60)

Number of Sexual Partners	Presence of Chlamydia Trachomatis n (%)		*p*-value
	Positive (n=11)	Negative (n=55)	
One partner	11 (20.4)	43 (79.6)	0.046
Two partners	1 (16.6)	5 (83.3)	

A positive association was found between Chlamydia trachomatis and the number of sexual partners. The study subjects were also grouped according to their socioeconomic status. Study subjects having an income of less than Rs-30,000 per month were categorized in the low socioeconomic class, and those having income between Rs-30,000 and Rs-60,000 per month were considered in the middle class, while those participants having an income of more than Rs-60,000 per month were considered in the higher middle class. Most of our participants belonged to a lower socioeconomic class.

There were 54 (90%) sub-jects with low socioeconomic status, while 5 (8.3%) subjects belonged to middle socioeconomic status, and one subject were cate-gorized as having upper middle socioeconomic status. Out of 12 positive subjects, 10 (83.3 %) belonged to low socioeconomic status, 1 (8.3 %) belonged to middle socioeconomic status, and 1 (8.3%) belonged to higher middle socioeconomic status, as shown in Table-IV.

Table-IV : Presence of chlamydia trachomatis in study subjects according to level of socioeconomic status(n=60)

Socioeconomic Status	Presence of Chlamydia Trachomatis	
	Positive (n=12)	Negative (n=48)
Low income status	10 (83.4)	44 (91.6%)
Middle income status	1 (8.3)	4 (8.4%)
Higher income status	1 (18.3.)	0 (0)

The frequency of Chlamydia trachomatis infection according to education level was also determined. The study subjects were grouped according to their level of education. 13 (21.7%) were educated up to grade five, 17 (28.3%) had education up to grade eight level, 14 (23.3%) were educated up to grade ten level, 11 (18.3%) had education up to grade twelve level, 5 (8.3%) were

educated more than grade twelve level. Out of positive cases, 41.7% had completed education till grade five, 16.7 % till grade eight, 33.3% till grade ten, and 8.3% till grade twelve. No positive case has completed education more than grade twelve level. No positive association was found between the presence of Chlamydia trachomatis and level of education.

DISCUSSION

The prevalence of STIs is increasing globally, especially Chlamydia trachomatis. It is the most frequent curable source of STIs worldwide. According to WHO, Approximately 131 million cases of Chlamydia trachomatis occur per annum globally, making it a global public health problem.[12]

The prevalence of Chlamydia trachomatis and its associated complications is on the rise worldwide, especially in the developing world. A substantial research effort is taking place worldwide to provide prompt diagnosis, timely management and prevent Chlamydia trachomatis infection. Much published data is not available from Pakistan that deals with Chlamydia trachomatis prevalence and its detection through PCR in women having PID, so we compared our study results with studies done in neighbouring countries as they have the same socio-demographic conditions. Our study results showed the percentage to be around 23% which is comparable with the results of other studies conducted in this region, especially in India.[13,14] A similar study on Iranian women showed the prevalence of Chlamydia trachomatis to be on the lower side, i.e. 10.5 percent.[15] However, our frequency was higher than reported in Sri Lanka, 17.1% respectively.[16] We could not find any similar study or published data from our country to compare results.

The mean age of females having PID infected with Chlamydia trachomatis was 24.38 years, according to our study, which was more or less the same as studies conducted by Kreisel *et al*.[17] In this study, most cases were seen in 20-25 years old individuals.[5] Chlamydia trachomatis infection is asymptomatic. Therefore, there is an urgent need for its timely diagnosis before complications develop. Screening should be mandatory for all females under 25 years of age to prevent Chlamydia trachomatis infection.[18]

Chlamydia trachomatis occurs more in persons with a homosexual or bisexual orientation or those with a greater number of sexual partners, especially in males, but our study population comprises heterosexual females.[19,20] Increased number of sexual partners is a risk factor for acquiring Chlamydia tracho-

matis infection.[5] However, in our study, only 8.3 percent of Chlamydia-positive females had more than one sexual partner. This difference from international studies could be mainly due to cultural and social barriers to disclosing or having more than one sexual partner and different sexual orientations.

In our study, socioeconomic status and education did not affect the PID and Chlamydia trachomatis infection among females. However, a systemic review and meta-analysis conducted on disadvantaged youth of Europe, North America and Australia showed that infection prevalence was higher in people of lower socioeconomic status.[21] In addition, low socioeconomic status is associated with complications resulting from Chlamydial infection, as demonstrated by various studies.[22,23] However, in our study, there is no significant difference due to socioeconomic status mainly because the majority of our study population belonged to a disadvantaged background as our hospital mainly caters for people belonging to lower socioeconomic status, so a significant comparison cannot be made.

CONCLUSION

The findings of our study confirm the widely held belief that females with Chlamydia trachomatis infection are at an increased risk of developing complications like Pelvic Inflammatory Disease due to the asymptomatic nature of the disease. In addition, our study employed the modern technique PCR, which eliminates certain limitations posed by conventional tests and is a rapid and accurate diagnostic tool.

Conflict of Interest: None.

Author's Contribution:

AN: Principle investigator, HN: Data collection, SA: Agree to be responsible for all aspects of research, JU: Final approval of documents, MG: Critical analysis, AJ: Data analysis.

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