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Chlamydia Trachomatis–A Cause of Infertility at Tertiary Care Hospital, Pakistan

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ABSTRACT

Objective: To determine the association of Chlamydial infection with infertility in patients reporting to tertiary care set up for evaluation.

Study Design: Cross-sectional study.

Place and Duration of Study: Armed Forces Institute of Pathology, from Jun 2015 to Jun 2016.

Methodology: A total of 100 women with primary or secondary infertility were included. Endo cervical swabs were taken. PAP smear was performed. One drop of anti-chlamydial monoclonal antibody was added to each slide, incubated at 37oC for 10 minutes, and examined for Chlamydia trachomatis under the fluorescent microscope.

Results: The mean age was 29.87±3.48 years. 89.0% of the patients had primary infertility, and 11.0% had secondary infertility. The mean age of primary infertile patients was 29.98±3.51 years, while the same was 29.00±3.28 years among patients with secondary infertility. Chlamydial infection was detected positive by direct immunofluorescence (Elementary Bodies) in 6 patients with mean age of 27.17±1.72 years and positive in 13 patients by polymerase chain reaction with mean age of 28.38±1.85 years, having a significant *p*-value (<0.05).

Conclusion: There was an association between Chlamydia trachomatis infection and female infertility.

Keywords: Chlamydial infection, Cause of infertility, Tertiary care hospital, Pakistan.

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INTRODUCTION

Chlamydia trachomatis is one of the causes of infertility.[1] It involves various sites of the urogenital system in females, mostly the cervix and urethra and in males, only the urethra. Most infections are asymptomatic, but ascending infections in females can cause pelvic inflammatory diseases, ectopic pregnancy and infertility.[2]

The prevalence of Chlamydia trachomatis in infertile women is 15%.[3] Tubal factor is the main cause in 56.8%, and tubal occlusion is found in 67.8% of infected cases.[4] The prevalence of Chlamydial infections is 15.3% in infertile women when direct immunofluorescence is used as a diagnostic modality and 32% by PCR.[5]

Chlamydial infection is the cause of primary infertility in 67.9 % of infertile women.[6] Because of the asymptomatic nature of the disease and to decrease the risk of complications, early detection of the organism should be done using sensitive and reliable tests such as PCR and Direct immunofluorescence.[7] These two tests were preferred over gold standard tissue culture, which was later invasive, costly, expertise dependent and time-consuming.[8]

The objective of our study was to identify this treatable cause of infertility in our setup and help formulate a strategy for essential screening of Chlamydial infection in infertile women.

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METHODOLOGY

This study was carried out at the Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan, along with the collaboration of the Outpatient Department of Gynecology of our tertiary care hospital for one year after the approval of the Ethical Committee. Sample size was calculated by WHO sample size calculator, taking population pro-portion: 0.153, confidence interval: 95%, absolute precision: 0.05 and sample size (n)=100. Non-probability consecutive sampling was used.

Inclusion Criteria: Women of age between 20-45 years with primary or secondary infertility were included in the study.

Exclusion Criteria: Women with other diagnosed causes of infertility like PID were excluded.

All patients fulfilling inclusion criteria were elaborately explained about the study to get informed consent. Infertile patients who fulfilled the inclusion criteria were selected. History and physical examination were carried out before taking an endocervical swab. An experienced gynaecologist performed a PAP smear. Further processing of all samples was carried out at AFIP by direct Immunofluorescence and PCR.

For direct testing, endocervical swabs were transferred on clean slides and incubated at room temperature for drying and fixation. Next, one drop of antichlamydial monoclonal antibody was added to each slide and incubated at 37 oC for 10 minutes. Next, slides were washed with phosphatebuffered saline followed by distilled water for 10 and 5 minutes, respectively. Finally, the slides were examined for Chlamydia trachomatis under a fluorescent microscope.

For PCR, Gene Proof Chlamydial trachomatis kit was used. DNA was extracted from the specimen using a kit Bacterial Xpress DNA extraction reagent (Millipore). 30ul of master mix and 10ul of the DNA extract or 10µl of the positive control were collected in a tube, centrifuged and inserted into the device and am-plified for a total of 45 cycles.

Statistical Package for Social Sciences (SPSS) version 22.0 was used for the data analysis. Frequent and percentage were calculated for qualitative variables like Chlamydial infection and infertile women. For quantitative variables like age, mean and standard deviation were calculated. The chi-square was applied, and *p*-value ≤0.05 was considered significant.

RESULTS

A total of 100 women aged 20-45 years with primary or secondary infertility were included in the study to determine the frequency of Chlamydial infection in patients presenting to our tertiary care setup. It was observed that 89.0% of the patients had primary infertility while 11.0% had secondary infertility. The chlamydial infection was positive in 6 patients (6%) by direct immunofluorescence and in 13 patients (13%) by polymerase chain reaction. Association of types of infertility with Chlamydial infection detected by direct immunofluorescence and polymerase chain reaction were presented in Table-I. The results showed that the mean age was 29.87±3.48 years.

The frequency and association of types of infertility with age groups WERE given in Table-II. The mean age of patients who observed positive Chlamydial infection detected by direct.

Table-I: Association of Infertility Caused by Chlamydial Infec-Tion as Detected by DIF and PCR (n=100)

Type of Infertility	Direct Immunoflouresence			*p*-value
	Positive	Negative	Total	
Primary	5	84	89	0.647
Secondary	1	10	11	
Type of Infertility	**Polymerase Chain Reaction**			***p*-value**
	Positive	**Negative**	**Total**	
Primary	11	78	89	0.588
Secondary	2	9	11	

Table–II: Association of Infertility according to Age Groups (n=100)

Age Groups	Type of Infertility			*p*-value
	Primary	Secondary	Total	
≤30 years	53	9	62	0.151
>30 years	36	2	38	

Immunofluorescence (Elementary Bodies) was 27.17±1.72 years, and it was 28.38±1.85 years when detected by polymerase chain reaction. Frequency and association of age with Chlamydial infection detected by a polymerase chain reaction and direct immunofluorescence are given in Table-III. The stratification according to age and types of infertility was done. Post-stratification association of Chlamydial infection detected by the two methods was observed, with these modifiers using (*p*-value <0.05).

Table-III : Association of Age Groups According to Chlamydial Infection as Detected by Polymerase Chain Reaction (PCR) and Direct Immunoflouresence (DIF) (n=100)

Age Groups	Polymerase Chain Reaction			*p*-value
	Positive	Negative	Total	
≤30 years	12	50	62	0.016
>30 years	1	37	38	
Age Groups	**Direct Immunoflouresence**			***p*-value**
	Positive	**Negative**	**Total**	
≤30 years	6	56	62	0.048
>30 years	0	38	38	

DISCUSSION

Chlamydia trachomatis is the most prevalent cause of sexually transmitted diseases caused by bacterial aetiology. It has different prevalence rates in different countries and populations. It is generally more detrimental to female reproductive health than males.[5]

The exact burden of disease caused by Chlamydia trachomatis remains underestimated because of the asymptomatic nature of the course, and so neither diagnosed nor reported.[9] In a study, the prevalence of

asymptomatic Chlamydia trachomatis was 20.5% which was higher than studies done in the UK and other western countries, which was 10.4%.[10,11]

Marashi *et al.*[5] reported that the average age amongst infertile women was 24.3 years. Direct immunofluorescence was positive in 15.3% and PCR in 32% of the patients. Malik *et al.*[12] showed that C.Trachomatis was detected in 31 (28.1%) of 110 infertile women; C.Trachomatis antigen was found in 16.37%, and cell culture showed positivity in 22.72% of patients.Chlamydia was found positive in 27% of women with primary infertility and 30.6% with secondary infertility.

The overall prevalence of Chlamydia trachomatis in infertile women was found 15% in a study done in Saudia Arabia.[13] Abdella *et al.*[14] revealed that the prevalence of Chlamydial genital infection was 6% when detected by PCR during screening for C. Trachomatis in Egyptian women with unexplained infertility.

Another study in Ghana,[15] showed a relatively low prevalence rate of C.Trachomatis at 1.6% with PCR. Nevertheless, it was done on a less sensitive urine sample. In comparison to the above, our study had 6.0% of patients with Chlamydia trachomatis detected by direct immunofluorescence (elementary bodies) and 13.0% by polymerase chain reaction. Furthermore, in our study, 89.0% were diagnosed with primary infertility while 11.0% with secondary infertility, compared to Rashidi *et al.*[16] who revealed primary infertility in 67.9% of cases.

In our study, the infertile women with positive Chlamydial infection had an average of 27.17±1.72 years diagnosed with direct immunofluorescence (elementary bodies) and 28.38±1.85 years for who were diagnosed with polymerase chain reaction when compared to Marashi *et al.*[5] which revealed 24.3 years for case group. This mean that age difference may be due to late marriage or presentation to tertiary care setup.

Post-stratification association of Chlamydial infection detected by the two methods in our study was observed with these modifiers using the Chi-square test, with a significant *p*-value of <0.05.

To the best of our knowledge, there is no national data available on this disease burden, so much study has been carried out.

We carried out this study to identify a treatable cause of infertility in our setup to minimize the use of costly invasive intervention and help formulate a strategy for essential screening of Chlamydial infection in all infertile women presenting to hospitals in developing countries. We further suggest PCR (being more sensitive than cell culture,[17,18] and/or ELISA / direct immunofluorescence to be used for early detection of Chlamydial infection as they are reliable and sensitive tests.[19,20]

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LIMITATIONS OF STUDY

The sample size was small, and the study was confined to a single centre and targeted only local subjects. In addition, the study was conducted in an urban environment.

CONCLUSION

Our results suggested an association between C. trachomatis infection and infertility. Therefore, C. trachomatis can be one of the main etiological factors for female infertility. To identify C. trachomatis, PCR results are more reliable than immune fluorescence tests. Due to the unknown prevalence of Chlamydia infection in our setup, screening is suggested in all infertile women presenting to the hospital for workup.

Conflict of Interest: None.

Authors' Contribution

SY: Direct contribution, AI: Supervision, SI:, SSH: Data analysis, SN: Editor, SSA: Intellectual contribution.

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