Detection of Chlamydia Trachomatis Infection in Infertile and Fertile Females at a Tertiary Care Hospital, Lahore, Pakistan

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Abstract

Objective: To detect anti-chlamydial antibodies in infertile & fertile females of reproductive age.

Methods: A case-control study was conducted in the Department of Microbiology, Medical University, Hospital, Lahore, from January 2021 to August 2022. A total of 154 patients from Hospital, Lahore, satisfying the inclusion criteria (cases) and exclusion criteria (control) were registered in the study. Informed consent was obtained, and demographic data and detailed patient history were recorded with the help of a questionnaire. After aseptic measures, 5 ml of venous blood was drawn for detection of chlamydial antibodies (IgG) using ELISA. Samples were labelled with the patient name and ID. Samples were centrifuged in batches for 15 minutes at 3000 rpm. The serum was then separated and preserved in serum cups at -20 C for further processing with ELISA.

Results: Results showed that infertile women had significantly higher frequency for positive anti-chlamydial antibodies as compared to fertile women. Of the total 77 fertile females, *chlamydia* infection was detected in 8(10.4%) of females while among infertile females, 45(58.4%) females had chlamydia infection with a statistically significant difference between the groups with p-value <0.001. The importance of screening is depicted by the higher prevalence rate of IgG antibodies against *Chlamydia trachomatis* in females of reproductive age.

Conclusion: Results in this study showed a high frequency of *Chlamydia trachomatis* infection among infertile women. i.e. (58.4%) as compared to fertile women. (10.4%). So, women with infertility should be screened for *Chlamydia trachomatis* for early treatment. There should be surveillance programs and interventions to help in screening *Chlamydia trachomatis* among infertile couples.

Keywords: Anti-Chlamydial antibodies, Infertile, Fertile.

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Introduction

Sexually transmitted diseases (STD) are considered a crucial hardship for the health care system and among them is *Chlamydia trachomatis* a common bacterial pathogen in differentials of sexually transmitted diseases worldwide. In past decades the number of cases tends to increase more. About 130 million people are reported globally with chlamydial infections[1]. As per World Health Organization (WHO) statistics over 92 million cases are reported annually and two-thirds of the disease burden is shared by resource-limited countries where there are inadequate diagnostic and treatment modalities available[2]. Infertility is a serious concern of the health system universally and it is defined as "failure to achieve clinical pregnancy after 1 year of unprotected sexual intercourse". 50-80 million females are suffering from infertility due to different etiologies. The rate of infertility is 5%-8% in devel-

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oped countries and 5.8%-44% in developing countries. *Chlamydial* infections are one of the major contributing factors to tubal infertility caused by bacteria. In Pakistan, the prevalence of infertility is about 22 % i.e. approximately one in every five married couples[3]. *Chlamydia* is a gram-negative organism of medical importance. It is an obligate-intracellular non-motile bacteria. They do not produce much energy to survive independently and hence depend on host cells for their energy[4]. *Chlamydia* has a unique life cycle it is present in two morphologically different forms. One is the Elementary body (EB) the infectious form and the other is the reticulate body (RB) the non-infectious form. It has a protein content in the cell wall which is a major outer membrane protein (MOMP). *Chlamydia trachomatis* has more than 15 serotypes from A-L. A-C causes trachoma. D-K causes genital tract infection and occasionally pneumonia and conjunctivitis. L1-L3 causes lymphogranuloma venereum[5].

The prevalence of the disease is found to be 1.7 % in reproductive-age grouped females but 4.7 % in young sexually active females of reproductive age. *Chlamydia* causes lower genital tract infections like cervicitis and ascending infections like salpingitis[6]. Early diagnosis of *Chlamydia* is challenging because of its asymptomatic nature and in chronic patients, its tricky due to low pathogenic load[7]. Up to 80 % of the females remain asymptomatic initially so they have relatively higher chances of being undiagnosed and untreated and having complication sequelae like premature birth, ectopic pregnancy, pelvic inflammatory disease (PID), abortions /miscarriages and tubal factor infertility (TFI)[8]. *Chlamydial* infections are of prime importance medically and socially as they affect females of reproductive age more and also health status of newborn via vertical transmission. TFI in females is caused by adhesive and obliterative inflammation in fallopian tubes. In men, Chlamydia is responsible for impaired spermatogenesis and epididymitis thus being a cause of about 50% male infertility as well[9]. The destruction of the ciliated layer of the fallopian tube and closure of the tube took place as a result of chronic or untreated chlamydial infection. About 20% -40% of untreated and undiagnosed females of the reproductive age group face any or all of these consequences. Due to the high prevalence and severe complications of *Chlamydia* CDC (Centre for Disease Control and Prevention) recommends annual screening of sexually active young adults especially females of reproductive age to avoid long-term health consequences. CDC also recommends 3-month post-treated screening to rule out chronic infection[10].

The study aims to determine the frequency of *Chlamydia trachomatis* infection among infertile and fertile females of reproductive age. By detecting the high prevalence of Chlamydia trachomatis infections among females of reproductive age, fertility-related adverse outcomes can be addressed and prevented by early screening. As there is a very high rate of infertility-related complications, early detection of Chlamydia infection will be a valuable contribution to the field of reproductive pathology.

Methodology

After getting ethical approval from the institutional review board, a case-control study was conducted in the Department of Microbiology, Medical University. Hospital Lahore from January 2021 to August 2022.

The sample size of 154 patients (77 patients in the infertile group and 77 patients in the fertile group) was estimated by using a confidence level of 95%, absolute precision of 10%, expected prevalence of Chlamydia trachomatis among infertile females as 14% and in fertile females of reproductive age as 8.5% [1] by using formula ()

Where**=** Confidence level 95%= 1.96, **d =** Absolute Precision =10%, $\mathbf{P_1}$ **=** Prevalence of chlamydial infection among infertile females=14 % and $\mathbf{P_2}$ **=** Prevalence of chlamydial infection among females of reproductive age =8.5% by using non-probability convenient sampling technique. Married females aged between 15 and 45 years were included. While the females with a history of (1) Uterine diseases like fibroids, uterine anomalies such as the uterine septum, (2) Systemic diseases like renal diseases, chronic liver diseases, thyroid diseases, (3) Antibiotic therapy (especially “Tetracycline e.g.

Doxycycline or Macrolides e.g. Erythromycin/ Azithromycin") taken in last 3 weeks (CDC recommends 1g of oral Azithromycin once daily for 3 days OR 100mg of Doxycycline twice daily for 7 days) (4) Severe factors of the male partner ("sperm count less than 5 million /ml, abnormal forms of sperm more than 90 % and motility less than 10%.") were excluded. After taking informed consent, data was collected and recorded on a predesigned proforma. After aseptic measures, 5 ml of venous blood was drawn for detection of chlamydial antibodies (IgG) using ELISA. Samples were labeled with the patient name and ID. Samples were centrifuged in batches at 3000 rpm for 15 minutes to separate serum and then preserved at -20 C for further processing with ELISA. Data was analyzed by using SPSS version 26. Qualitative variables like marital status, use of contraceptives, histories (like history of vaginal discharge, history of STD), and results of ELISA were presented in terms of frequency and percentages. Quantitative variables like age and duration of marriage were presented as mean and standard deviation. A test of significance was applied. A comparison of two groups of ELISA was done by applying a chi-square test. p-value of less than 0.05 was taken as significant.

Results

Of the total 77 fertile females, *chlamydia* infection was detected in 8(10.4%) of females while among infertile females, 45(58.4%) females had *chlamydia* infection with a statistically significant difference between the groups with p-value <0.001 (Table 1). The mean age of fertile females was 26.27±5.36 years and infertile females was 28.77±5.14 years with no statistically significant difference between the groups found concerning age (p-value 0.945) (Table 1). The mean duration of marriage in fertile females was 3.06±2.87 years while in infertile females was 5.67±3.02years with no statistically significant difference between the groups with p value 0.221 (Table 1). Of the total 77 fertile females, 35(45.5%) were having a history of vaginal discharge while 60(77.9%) were having vaginal discharge among infertile females with a statistically significant difference between the groups with p-va lue <0.001 (Table 1). Of the total 77 fertile female, 6(7.79%) had a history of contraception, while 2(2.59%) had having history of contraception among infertile females with no statistically significant difference between the groups with p value 0.146 (Table 1). The mean pubertal age in fertile females was 12.76±1.13 years while in infertile females was 13.05±1.20years with no statistically significant difference between the groups with p value 0.089 as shown in Table 1. Among 77 fertile females, 24(31.2%) had a history of pain during menstruation while in infertile females, 34(44.2%) had a history of pain during menstruation with no statistically significant difference between the groups with p value 0.096 as shown in Table 1. The mean duration of menstruation was 6.16±1.08 days while in infertile females was 5.76±1.03 days with no statistically significant difference between the groups with p value 0.992 as shown in Table 1. Among 77 fertile females, 19 (24.7%) had a history of discomfort during coitus while 45(58.4%) had a history of discomfort among infertile females with a statistically significant difference between the groups with p-value <0.001 as shown in Table 1. The distribution of fertile and infertile females concerning gravida status, parity status, and miscarriages has been shown in Table 2. By evaluating the primary and secondary infertility among infertile females, it has been found that 49 (63.64%) females were having primary infertility while 28 (36.36%) females were having secondary infertility. The frequency of primary and secondary infertility among infertile females is shown in Figure 1.

Table 1. Cross tabulation of fertile and infertile females concerning Age, Duration of marriage, vaginal discharge, history of contraception, pubertal age, pain during menstruation, duration of menstruation, discomfort during coitus, detection of chlamydia trachomatis (n=154)

Variable	Subgroups	Fertile females(n=77)	Infertile females(n=77)	Chi-square value	*P* value
Age (years)Fertile (26.27±5.36) Infertile (28.77±5.14)	≤30years	42(54.5%)	40(51.9%)	3.12	0.945
	>30years	35(45.4%)	37(48.0%)		
Duration of marriage(years) Fertile (3.06±2.87) Infertile(5.67±3.02)	≤4years	50(64.9%)	41(53.2%)	6.98	0.221
	>4years	27(35.0%)	36(46.7%)		
Vaginal discharge	Yes	35(45.5%)	60(77.9%)	17.17	<0.001
	No	42(54.5%)	17(22.07%)		
History of contraception	Yes	6(7.79%)	2(2.59%)	2.11	0.146
	No	71(92.2%)	75(97.4%)		
Pubertal Age (years)Fertile (12.76±1.13) Infertile (13.05±1.20)	≤13 years	41(53.2%)	35(45.4%)	6.71	0.089
	>13 years	36(46.7%)	42(54.5%)		
Pain during menstruation	Yes	24(31.2%)	34(44.2%)	2.76	0.096
	No	53(68.8%)	43(55.8%)		
Duration of menstruation (days)Fertile 6.16±1.08 Infertile 5.76±1.03	≤ 5 days	32(41.5%)	47(61.0%)	7.28	0.992
	>5days	45(58.4%)	30(38.9%)		
Discomfort during coitus	Yes	19(24.7%)	45(58.4%)	18.07	<0.001
	No	58(75.3%)	32(41.5%)		
Detection of chlamydia trachomatis	Positive	8(10.4%)	45(58.4%)	39.38	<0.001
	Negative	69(89.6%)	32(41.6%)		

Table 2. Distribution of fertile and infertile females concerning Gravida status, Parity status, and Miscarriages (n=154)

Variable	Subgroups	Fertile(n=77)	Infertile(n=77)
Gravida status	1	41 (53.2%)	-
	2	18 (23.4%)	-
	3	8 10.4%)	-
	4	8 (10.4%)	-
	5	1 (1.3%)	-
	6	1 (1.3%)	-
Parity status (No. of alive children)	0	39 (50.6%)	21 (75%)
	1	22 (28.6%)	7 (25%)
	2	11 (14.3%)	0 (0%)
	3	3 (3.9%)	0 (0%)
	4	1 (1.3%)	0 (0%)
	5	1 (1.3%)	0 (0%)
Miscarriages	0	67 (87%)	50 (65.8%)
	1	9 (11.7%)	10 (13.2%)
	2	0 (0%)	8 (10.5%)
	3	1 (1.3%)	7 (9.2%)
	4	0 (0%)	1 (1.3%)

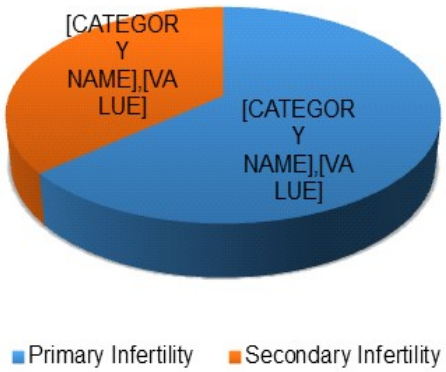

Fig 1. Primary and secondary infertility among infertile females (n=77)

Discussion

The higher prevalence of *C. trachomatis* infection is a global concern due to its potentially detrimental effects on reproductive health. The risk of long-term sequelae increases, especially with recurrent *chlamydial* infections, and in some women, *C. trachomatis* represents a major threat to fertility. Different diagnostic modalities are available for *Chlamydia* like cell culture, immunofluorescence, serological assays, and molecular testing methods. Cell culture is the gold standard but it is resource-intensive and requires special laboratories with proper and advanced equipment. Also, it needs high expertise which makes it impractical for use until under special circumstances. PCR is considered the gold standard in non-culture-based tests and it is highly sensitive and specific. But at the same time, it is relatively expensive and difficult to carry on a routine basis in resource-limited countries for screening purposes[11].

Different studies have been conducted in infertile and fertile individuals to establish the prevalence or frequency of urogenital chlamydial infections. Different and controversial data is reported in some case-control surveys that have investigated the frequency of chlamydia in fertile, infertile symptomatic, and asymptomatic cases[12]. Many studies depict a wide range of occurrences of 9%–55% among infertile patients which shows the prevalence of chlamydial infection in the respective area[13,14]. Mahtab Sattari from Iran figures out the frequency of anti-chlamydial antibodies in infertile females. His findings show that the frequency of anti-C. Trachomatis antibodies were much higher among infertile females. i.e. (“35.88% vs. 18%, p-value =0.004”). 22 Different sociocultural and economical conditions, using different diagnostic techniques or kits used, differences in sample size, a difference of sexual practices and lack of patient follow-up after treatment are some of the factors affecting the frequency in some previous studies.

Based on our study, we have evaluated the primary and secondary infertility among infertile females, it has been found that 49 (63.64%) females were having primary infertility while 28 (36.36%) females were having secondary infertility. Weiming Tang in his study meta-analysis and systematic review reported the alliance of *Chlamydia* with increased risk of many fertility and pregnancy-related inauspicious outcomes like abortions, ectopic pregnancies, infertility etc[15]. As per his results, the odds of *chlamydia* for infertility in several case-control studies were significantly higher among females with infertility comparing with females without infertility (pooled unadjusted OR=2.72)[16]. A study conducted in Ibadan reported females attending infertility clinic had higher prevalence of *Chlamydia trachomatis* infection as 7.33%. Among them 4(36.4%) females have secondary infertility female while 7(63.6%) females have primary infertility [17]. The chief cause of PID and female infertility worldwide is deemed to be *C. trachomatis*. The likelihood of developing PID (pelvic inflammatory disease), extra uterine pregnancy, abortions, and infertility ma-inly because of tubal blockage (TFI), increases by 30% in a female following a single occurrence of genital chlamydial infection, and this risk, especially for pelvic inflammatory disease further increases to 20% more after multiple episodes of disease[18]. Sarah Bagheri described a positive relationship between miscarriages and *chlamydial* disease in his study. According to the result of his study the cases consisting of females having miscarriages the PCR results for *C. trachomatis* are significantly higher than the control group (11.3 vs. 0%, P= 0.007)[19]. Chlamydial infection and its relation to miscarriages has also been reported on molecular study-based evidence by different previous studies[20].

In another study, the prevalence of *C. trachomatis* and spontaneous abortions are found to be interrelated. The endocervical swabs of females having spontaneous abortion showed a prevalence of 22.9% for *chlamydial* infection than 11.9% (p-value=0.031) in females with healthy and normal pregnancy[20]. Females with genital *C. trachomatis* infection may present with symptoms of irregular bleeding, bleeding in between or after a period, foul-smelling vaginal discharge or post-coital discomfort or bleeding[21]. In impoverished countries, it becomes more challenging to deal with infertility due to associated stigma and inadequate resources available for assisted reproductive technologies. Data from previous studies indicate the association of bacterial genital tract infections with infertility especially in resource-limited countries[22]. Effective treatment of the infection can be done by giving appropriate antibiotics provided there is early detection of infection before the development of complications. In resource-limited countries, the prime importance is early diagnosis. Due to inadequate research and surveillance, the current data regarding the prevalence of *chlamydial* infection in Pakistan is insufficient. Therefore, to assess the prevalence of *chlamydial* infection serological assays are used as potential epidemiological tools, especially in young adults who are sexually active to help the public and private health sector build stats and improvise management strategies for the management of this silent killer of the reproductive tract.

Therefore, it emphasizes the need for the integration of antibody testing for *chlamydial* infection as a part of routine screening investigation in the subfertility clinics. This is a mandatory effort needed to be made for early detection and treatment of chlamydial infection hoping to prevent ascend to the upper genital tract or transmission to the sex partners. Our study had certain limitations. It was a single-centered study with a limited sample size. It is recommended to validate the findings of our study by larger prospective studies.

Conclusion

Results of this study depicted a high frequency of *Chlamydia trachomatis* infection among infertile women, i.e. (58.4%) as compared to fertile women (10.4%). There should be surveillance programs and interventions to help in screening *Chlamydia trachomatis* among infertile couples. Females of reproductive age should be screened early for STDs and must be treated accordingly to avoid later complications like infertility. Another conclusive result of our research is that primary infertility (64%) is more frequent than secondary infertility (36%).

Conflict Of Interest: None

Disclaimer: None

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