ORIGINAL ARTICLE

Chlamydia trachomatis Infection in Tubal Ectopic Pregnancy

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Abstract

Introduction: Ectopic pregnancy (EP) is a pregnancy complication where the fertilized egg is implanted in an area other than the uterus. In 98% of EP cases, the implantation of the egg occurs within the fallopian tube. It can happen due to many factors related to the patient's history and sexual and reproductive history. This research is aimed to study the association between EP and *Chlamydia trachomatis* infection and also other risk factors.

Methodology: The study was conducted in the Department of Obstetrics and Gynecology, Chattogram Medical College Hospital, Bangladesh. Women with ruptured EP were selected as cases; women undergoing tubal ligation were taken as control. The biological test was conducted using PCR to detect the presence of the *Chlamydia* bacteria in the specimens collected from both the cases and controls. The collected data were analyzed using IBM SPSS version 20.0.

Results: The average age of the cases was 27.55 ± 5.32 years, and the average age of the control was 31.61 ± 6.89 years; the age difference was not statistically significant ($p > 0.1$). Medical history of individuals with sexual–reproductive health issues, such as parity, PID, abdominal pain, and vaginal bleeding, showed a significant connection ($p = 0.1$) with EP. In women, infection with *C. trachomatis* was strongly linked to tubal EP (15 positive cases out of 22). The presence of *C. trachomatis* infection, parity, abdominal discomfort, vaginal bleeding, and PID results was significant at a 90% confidence interval ($p < 0.1$) using binary logistic regression for all variables to predict which factors affect the dependent variable. Tubal EP is 5.7 times more common in women infected with *C. trachomatis*.

Conclusion: This study proved a strong association between the presence of *C. trachomatis* infection and EP. For future studies, using a greater sample size and investigating other microbes are suggested for better results.

Keywords: *Chlamydia trachomatis*, Ectopic pregnancy, Polymerase chain reaction.

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Introduction

When the fertilized egg is implanted outside a woman's uterus, it is said to be an EP and is a pregnancy complication. It is a crucial cause of pregnancy-related death in early pregnancy.[1] About 98% of EP occurs in the fallopian tube.[2] There are many risk factors associated with EP like PID, smoking, age, genital tract infection, tubal surgeries, and history of previous EP.[3,4]

For women who are suffering from sexually transmitted bacterial diseases, *Chlamydia trachomatis* infection is most common.[5] The estimated prevalence of *Chlamydia* infection in females is approximately 2.5% in the United States.[6] In developing countries, including China and India, high incidence and prevalence of *Chlamydia* infection are observed.[7]

It may cause a clinically evident or asymptomatic PID where it is difficult to diagnose.[8] The failure to diagnose *C. trachomatis* infection might cause fibrosis in the fallopian tube and many other complications. Due to aggressive inflammation, chronic scarring of the tube, occlusion, and hydrosalpinx might occur.[9] Ectopic pregnancy is one of those complications.[10]

A case-control study conducted in women with EP and women with tubal ligation identified many factors associated with EP like smoking, age, history of PID, and a previous infection of *C. trachomatis*, but failed to prove the association between the presence of *C. trachomatis* antibodies and EP[11–13] and between previous tubal surgeries and EP, which were established by other studies.[14]

C. trachomatis is isolated by culture from the upper genital tract of women with infertility and resected fallopian tubes of women with EPs, in some cases. *C. trachomatis* DNA in the fallopian tube tissues was detected using the polymerase chain reaction (PCR) tool.[15] They have used a specific primer to set the PCR reaction mixture to bind to the DNA of *C. trachomatis*. Then, DNA fragments were separated on agarose gel electrophoresis and investigated by the length of restriction fragments.

This research is aimed to identify *C. trachomatis* PCR in the fallopian tube in women who had a ruptured EP and therefore to contribute to the overall understanding of how *C. trachomatis* causes EP in women in Bangladesh.

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Methodology

Study Population

A case-control observational study was conducted in the Obstetrics and Gynaecology Department, Chattogram Medical College Hospital from October 2019 to March 2020. Women admitted with ruptured EP were taken as cases, and women who had fallopian tube ligation during a cesarean section were selected as control. In total, 40 women were enlisted for the study; the ratio of cases to controls was 1:1, resulting in 18 controls and 22 cases. The primary dependent variable in this study was *C. trachomatis* in the resected fallopian tube tissue samples of cases and controls.

Specimen Collection

After taking consent, data were collected using a structured questionnaire which includes sociodemographic characteristics and medical data. During the surgery, resected fallopian tubes were collected as a sample from both cases and controls. The specimens were stored in a sterile sample bottle after surgery and then immediately frozen at –20°C.

DNA Extraction Process

After measuring the required amounts of tissues, *C. trachomatis* DNA was detected using a PureLink Genomic DNA Kit according to the manufacturer's instructions. The primers used in the first PCR test were 5′-CTG CAG CCT CCG TAG AGG TCT GGG CAG TGT C-3′ and 5′-AAG CTT TTC TTA ACA ATG CAA ATG AGA TAG-3′.[10]

Five microlitres of extracted DNA was added after the master mix was introduced to the PCR (0.2 mL) tube, and the total volume was adjusted to 50 µL using distilled water. The PCR program was set as initial denaturation for 3 minutes at 95°C, anneal primers for 1 minute at 55°C, extended DNA for 1 minute at 72°C. PCR cycle was repeated 25–30 times, and the final extension was done for 5 minutes at 72°C. When the reaction was completed, results were analyzed on 2% agarose gel electrophoresis.

PCR Assay

Positive results were amplified again using a thermal cycler, and the same master mix was prepared for the second test, but the primer was replaced with a different primer called *omp2* gene; the sequence of this primer set was 5′-ATG TCC AAA CTC ATC AGA CGA G-3′ and 5′-CCT TCT TTA AGA GGT TTTACCC-A-3′. The PCR program for this test was different; it started with 94°C for 4 minutes as initiation, followed by 35 cycles of 94°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute, and 72°C for 10 minutes.[10]

Results were analyzed on 1.5% of agarose gel. After this, 10 µL of the PCR products were mixed with one unit of enzyme AluI, 2 µL of 10× buffer, and 7 µL of water. The mixture was incubated for 1 hour at 37°C. After the incubation period, products were analyzed by 4% of agarose gel electrophoresis.

Data Management

Data were coded and analyzed using IBM SPSS software. The prevalence of *C. trachomatis* infection was calculated and adjusted, and an unadjusted odds ratio with a 90% confidence level was to determine the association between EP and other risk factors rather than the usual 95% as the sample size was minimal (case of 22 and control of 18).

Results

Of the 40 participants recruited in this study, 22 women with ruptured tubal pregnancy were enrolled into the case group and 18 women underwent tubal ligation after being enrolled in the control group. The age of the participants ranged from 15 to 45 years, the average age for controls was 31.61 ± 6.89 years and 27.55 ± 5.32 years for cases, and the age difference was not statistically significant ($p > 0.1$).

Ninety percent of the case and control group women fall in the age category of 20–40 years; no cases were recorded with women over 40 years, whereas only two control subjects were over 40 years (Table 1).

Table 1: Age and other selected characteristics of the study participants

Variables	*Cases N = 22 (%)*	*Controls N = 18 (%)*	*p-value*[*]
Age (Mean ± SD)	27.55 ± 5.32	31.61 ± 6.89	0.186 (two samples *t*-test)
Residence			
Urban	14 (63%)	8 (44%)	0.225
Rural	8 (27%)	10 (56%)	
Age			
<20 years	2 (9.09%)	16 (88.8%)	0.613
20–40 years	20 (90.9%)	2 (11.1%)	
Occupation			
Housewife	19 (86.3 %)	17 (94.4%)	1.000
Other occupations	3 (13.6%)	1 (5.5%)	
Socioeconomic status			
Upper	0	1 (5.5%)	0.642
Upper middle	4 (18.1%)	5 (27.7%)	
Upper lower	7 (31.8%)	4 (22.2%)	
Lower	11 (50 %)	8 (44.4%)	
Educational level			
Illiterate	2 (9.09)	4 (22.2%)	0.403
Primary	10 (45.4%)	8 (44.4%)	
SSC	5 (22.7%)	1 (5.5%)	
HSC	3 (13.6 %)	1 (5.5%)	
Graduate	1 (4.54)	3 (16.6%)	
Postgraduate	1 (4.54)	1 (5.5%)	

[*] *p*-value refers to the results of the Chi-square test

This study navigated many possible risk factors associated with EP. Medical history of patients related to sexual–reproductive health reported a significant association (p-value <0.1) with EP like the history of parity, PID, abdominal pain, and vaginal bleeding (Table 2).

As stated in different literature sources, other risk factors can be associated with the occurrence of EP like the use of combined oral contraceptive pill (COCP), smoking, sexual activity with various partners, antenatal care (ANC), history of STD, and menstrual cycle pattern. These variables were also subjected to the Chi-square test; however, the association between these variables and EP was not statistically significant (Table 3).

The primary variable of this study was the presence of *C. trachomatis* infection in women with tubal EP, which was found to be positive in 15 and 6 among cases and controls, respectively (Fig. 1). The Chi-square test resulted in a significant association between *C. trachomatis* infection and the occurrence of EP (Table 3).

The crude (unadjusted) odds ratio was calculated using binary logistic regression for all the variables to predict which factors affect

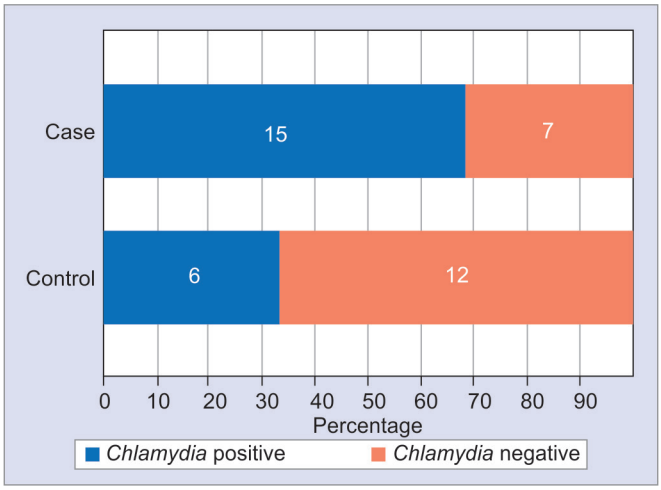

Fig. 1: Presence of *Chlamydia trachomatis* infection in the study

Table 2: Association between risk factors and ectopic pregnancy

Variables	*Case N = 22 (%)*	*Control N = 18 (%)*	*Chi-square value*	*p-value*
Abortion				
Yes	10 (45%)	6 (33%)	0.606	0.436
No	12 (55%)	12 (67%)		
Parity				
Yes	15 (68.1%)	17 (94.4%)	4.268	0.039
No	7 (31.2%)	1(6.6%)		
PID				
Yes	8 (36.6%)	1 (5.5%)	5.389	0.027
No	14 (63.4)	17 (94.5 %)		
Dysuria				
Yes	1 (4.5%)	2 (11.1%)	0.615	0.579
No	21 (95.5%)	16 (88.9%)		
History of infertility				
Yes	3 (13.6%)	1 (5.5%)	0.718	0.613
No	19 (16.4%)	17 (94.5%)		

p-value of Fisher's exact test was used for variables with count numbers less than 5

Table 3: Association between ectopic pregnancy and risk factors from patients' medical history

	Cases (N = 22) (%)	*Control (N = 18) (%)*	*Chi-square value*	*p-value*
Presence of *C. trachomatis*				
Yes	15 (68.18%)	6 (33.3%)	4.821	0.021
No	7 (32.82%)	12 (66.7 %)		
No. of sexual partners				
One partner	19 (86.4%)	18 (100%)	1.807	0.490
More than one	2 (13.6%)	0 (0%)		
Abdominal pain				
Yes	20 (90.9%)	8 (44.4%)	10.178	0.002
No	2 (9.1%)	10 (55.6%)		
Menstrual cycle				
Regular	18 (81.8%)	18 (100%)	3.636	0.114
Irregular	4 (18.2%)	0 (0%)		
COCP				
Yes	7 (31.8%)	8 (44.4%)	0.673	0.412
No	15 (68.1%)	(55.6%)		

p-value of Fisher's exact test was used for variables with count numbers less than 5

Table 4: Factors affecting ectopic pregnancy

		Binary logistic regression			*Multiple logistic regression*		
Variable name	*Category*	*Unadjusted odds ratio*	*p value*	*Confidence interval*	*Adjusted odds ratio*	*95% CI*	*p-value*
Presence of *C. trachomatis*	Present Absent	4.28 Ref	0.032	3.95–5.1 Ref	5.77 Ref	4.95–6.85 Ref	0.084
Parity	Yes No	7.93 Ref	0.066	1.2–50.5 Ref	0.04 Ref	0.09–23.5 Ref	0.115
Abdominal pain	Yes No	12.50 Ref	0.004	1.02–34.97 Ref	3.67 Ref	1.65–7.26 Ref	0.050
Vaginal bleeding	Yes No	3.50 Ref	0.077	2.68–6.84 Ref	5.16 Ref	5.01–7.39 Ref	0.050
PID	Yes No	9.71 Ref	0.042	6.45–13.75 Ref	2.17 Ref	1.65–8.94 Ref	0.038
COCP	Yes No	0.58 Ref	0.414	0.58–5.07 Ref	0.90 Ref	0.899 Ref	0.899
Previous abortion	Yes No	1.66 Ref	0.438	0.20–1.77 Ref	0.81 Ref	0.11–7.29 Ref	0.769

the dependent variable. As demonstrated in Table 4, the presence of *C. trachomatis* infection, parity, abdominal pain, vaginal bleeding, and PID findings was significant at a 90% confidence interval (*p*-value <0.1).

The adjusted odds ratio was calculated to find the effect of one variable on causing EP while adjusting other variables. Women infected with *C. trachomatis* are 5.7 times more likely to develop tubal EP. Women who have abdominal pain, vaginal bleeding, or PID are more likely to have an EP than women who do not have any of these symptoms (Table 4).

Discussion

In this study, the adjusted odds ratio of *C. trachomatis* infection in EP was 5.77 times higher (95 % CI 4.95–6.85) among the women with EP (case of 68.18%) than the women who had a tubal ligation during a cesarean section (control of 33.3%). In a prior study in Bangladesh, the prevalence of latent *C. trachomatis* infection among women with EP using both ProbeTec ET and real-time PCR was 0% (0/16).[16] Many studies in Europe showed a high prevalence of this infection in EP subjects; a survey in Netherland revealed (29.5%) prevalence of *C. trachomatis* infection.[17–19] A study was conducted in Vietnam on the association between present/previous infections of *C. trachomatis* with the occurrence of EP. Their investigation resulted in 24.9% of prevalence of infection in the study subjects.[20] The reason behind the difference in the prevalence between this study and other studies worldwide is that this study was conducted in a public hospital. Over 75% of study subjects belonged to lower/upper-lower socioeconomic class (50%) and low education levels (54%) (Table 2), and they had less access to reproductive health care (ANC <6%). *C. trachomatis* infection prevalence increased in women with low socioeconomic status and women occupation, specifically homeworkers and housewives. Applying this to our study, we find that the increased rate of *C. trachomatis* infection is associated with the low socioeconomic status.

Women with previous PID are at 9.7 times higher risk of developing EP than women with no previous PID. Adjusting other risk factors increased the risk of developing EP. Results showed that these women are more prone to develop EP by 33.517 times than women with no PID. Hillis et al. showed that the presence of any PID increases the risk of EP by 2.8 times (95% CI, 1.3–6.1).[18] This study cannot prove any association between STD and EP. In contrast, Anorlu et al. discussed that STD increases the risk of EP by 14 times.[19] This may indicate that Bangladeshi women have different characteristics or it may be related to the relatively limited sample size and loss of information, as many subjects may not know their medical history.

Also, the present study could not prove any association between EP and previous tubal surgeries, abdominal surgeries, or induced abortion which was not the case in other studies.

Limitation

Limited time and budget failed to complete the calculated sample size.

Conclusion

As the results stated, there is a strong correlation between the occurrence of tubal EP and the presence of *C. trachomatis* infection. As it is an asymptomatic infection, increasing the regular scanning for the infection and treating it will dramatically decrease the prevalence of tubal EP. The small sample size was a limiting factor in this study; therefore, continuing the research and including more subjects will give more accurate results and conclusions. Further research needs to be done to study the association between EP and other microorganisms using a greater sample size.

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