

## Chlamydia Trachomatis Infection in Ectopic Pregnancy: A Case Control Study

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### ABSTRACT

Ectopic pregnancy is a significant cause of maternal morbidity and mortality. It is the leading cause of pregnancy related death in the first trimester and account for 9% of all pregnancy related death. Ectopic pregnancy refers to the implantation of fertilized ovum on any tissue other than the endometrium of the uterus. The main objective of this study is to evaluate the association of Chlamydia trachomatis infection in women with ectopic pregnancy. To do so, a case control study was conducted on 90 patients admitted with ectopic pregnancy in the inpatient department and early normal pregnancy who come in antenatal clinical and OPD of dept. of Obstetrics & Gynaecology of BSMMU, DMCH, SSMC MH, Dhaka. The study duration was almost three years from the start of January 2006 to July 2009. In the present investigation, the results showed that 13.33% & 16.67% had positive IgM and IgG in ectopic pregnancy and only 3.33% had positive IgG in normal pregnancy. Total 9 cases (IgM-4 & IgG-5) were positive in ectopic pregnancy & only 2 cases were positive in normal pregnancy. The difference was statistically significant. Regarding other variables significant difference was present in occupation of husband, past medical & surgical history. Contraceptive history showed that there was significant difference between women of ectopic pregnancy and normal

pregnancy in oral contraceptive pill use. Regarding past obstetric history-repeat ectopic pregnancy was 13.3%. Thusly, chlamydia can cause pelvic inflammatory disease which can result in scarring of the fallopian, with consequent ectopic pregnancies which can be fatal, have reached to morbidity & mortality. Ectopic pregnancy is an important cause of maternal death and still holds a diagnostic challenge.

**Keywords:** Chlamydia Trachomatis Infection, Ectopic Pregnancy, Maternal Morbidity.

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### INTRODUCTION

Ectopic pregnancy is a significant cause of maternal morbidity and mortality. It is the leading cause of pregnancy related death in the first trimester and account for 9% of all pregnancy related death.<sup>1</sup> Ectopic pregnancy refers to the implantation of fertilized ovum on any tissue other than the endometrium of the uterus. More than 95% of extra uterine pregnancy occurs in fallopian tube.<sup>2</sup> A tube that is partly blocked or damaged by scar tissue may cause fertilized egg to get stuck in tube and can cause the tube to rupture or other complication like severe pain, internal bleeding and even death. Exact incidence of this condition is not known in our country. In a multicentric case control study in India the

incidence of ectopic pregnancy was 3.86 per 1000 live birth which is 6 times higher in women who had PID. The incidence varies from place to place even in same country. One of the, determining factor is the frequency of pelvic infection (Chlamydia, Gonococcus, postabortal, tubercular) in that community.<sup>3</sup> The incidence of ectopic pregnancy is found to 1.8% of all women admitted in BB Hospital, Nepal with pregnancy related conditions.<sup>4</sup> In Dhaka Medical College, the incidence of ectopic pregnancy is 4.8% in 2007 & 5.06% in 2008. It has been postulated that PID is a major cause of ectopic pregnancy which is caused by microorganism which can move

upward from vagina or cervix into fallopian tube, ovary and uterus causing infection. Extensive sub epithelial inflammation, epithelial ulceration and scarring are the end results. Many types of organism can cause PID - among them Gonococcus and Chlamydia trachomatis is the most frequent cause. More over studies on the microbial aetiology of PID had shown that C. trachomatis was present in the fallopian tube at the time of salpingitis. So far the mechanism it speculates that persistent infection by C. trachomatis induces tubal damage directly or the indirect result of post inflammatory damage (J. LAN et al). Proposed mechanism for the pathogenesis of chlamydial disease is an immune mediated response.<sup>5</sup>

Current studies indicate that 15% sexually active non-pregnant women have an asymptomatic chlamydial cervical colonization.<sup>5</sup> C. trachomatis infection is a major cause of tubal ectopic pregnancy. Chlamydia plays a major role in tubal infection leading to ectopic pregnancy since tubal disease is nearby always bilateral there is strong tendency for ectopic pregnancy to occur on one side and then at a later date on the other. In a woman, who has already had one tubal pregnancy, the risk of second is 7-13 times greater than the overall risk.<sup>3</sup> In a recent study incidence of repeat ectopic pregnancy was 18%.<sup>4</sup> So to reduce the incidence of ectopic pregnancy exact aetiology should be found out and treated properly. If actual cause is unidentified and if treatment is not ensure adequately -patient of ectopic pregnancy might again be a case of ectopic pregnancy and its adverse consequences. The role of C. trachomatis infection in women who have ectopic pregnancy needs to be explored further.

Chlamydia infections are more insidious in onset. Infection with this agent can be asymptomatic in up to 50% of women which made diagnosis difficult.<sup>6</sup> Identification of Chlamydia directly in specimen material by culture, monoclonal DIFA, ELISA and nucleic acid amplification techniques are the most often used methods for the aid in diagnosis of Chlamydia infections. DIFA testing is highly sensitive, specific & less time consuming. But it requires florescent microscope & expert microscopist. It has been shown that C. trachomatis is rarely isolated from the genital tract and requires invasive procedure- not routinely available in general practice. Serology may be helpful, providing indirect evidence of exposure. So we want to develop a noninvasive screening assay of immunoglobulin for C. trachomatis mediated upper genital tract disease. Chlamydial antibody can be detected by ELISA method.

Because of the severity of the complication and their implication in health economy, several other countries including United Kingdom, France, Holland and Finland have now taken action to reduce the prevalence of this infection and its sequelae like ectopic pregnancy. But we do not know exact situation in our country regarding association of Chlamydia trachomatis infection and ectopic pregnancy. There is no study has been done regarding C. trachomatis infection in ectopic pregnancy in BSMMU. So this study has been designed to find out any association between C. trachomatis infection and ectopic pregnancy.

Ectopic pregnancy is a very important cause of maternal death and diagnosis of cause is not clear until now. Many reports now indicate Chlamydia trachomatis is the major causative agent in the development of PID in women. The major sequelae of PID include ectopic pregnancy. The biggest challenge to control Chlamydial diseases is that as many as 70-80% of women are infected do not

experience symptoms. Hence the diagnostic tests are the only way to detect infection and to treat the disease to prevent complications. Ectopic pregnancy is life threatening condition. Recent advances have been made to diagnose the ectopic pregnancy very early & save the women's like but ectopic pregnancy is still significant cause of mortality and morbidity of women of reproductive age group. Even a small delay one fallopian tube may have to sacrificed in many cases. The issue is great concern for her future fertility.<sup>4</sup>

Unfortunately, we cannot find any report of C. trachomatis infection in ectopic pregnancy in Bangladesh. As a result, we do not give proper antibiotic to be treated. If we do not give proper antibiotic in case of pelvic infection, prevalence of ectopic pregnancy would not come down. 10-50% of ectopic pregnancy has a subsequent ectopic pregnancy and 60-70% has fertility problems.<sup>4</sup>

Considering above facts this study is designed to find out the association of C. trachomatis with ectopic pregnancy by determining anti-chlamydial antibodies in serum of women having ectopic pregnancy. Serology may be one of the methods of identifying chlamydial infection in ectopic pregnancy. so that preventive & curative treatment of Chlamydia can be taken to prevent ectopic pregnancy.

## OBJECTIVES

### Main Objective

The main objective of this study is to evaluate the association of Chlamydia trachomatis infection in women with ectopic pregnancy.

### Specific Objectives

The specific objectives of the study are:

1. To detect Chlamydia trachomatis infection by assessing antichlamydial antibodies (IgM & IgG) level in serum of women with ectopic pregnancy.
2. To detect serum Chlamydia antibodies (IgM & IgG) level in women with normal pregnancy.
3. To compare the Chlamydia antibodies level between two groups.

## METHODS & MATERIALS

### Study Design

Case control study.

### Place of Study

Department of Obstetrics and Gynaecology of BSMMU, Department of Microbiology, BSMMU, Dhaka.

### Study Period

January 2006 to July 2009.

### Study Population

Women admitted with ectopic pregnancy in the inpatient department and early normal pregnancy who come in antenatal clinical and OPD of dept. of obstetrics & Gynaecology of BSMMU, DMCH, SSMC MH, Dhaka

**Cases:** Women having ectopic pregnancy.

**Control:** Women with early normal pregnancy within 12 weeks.

### Sample Size

I have taken 90 samples. Among then 30 was case and 60 was control.

### Sampling Technique

Purposive Convenient sampling

**Inclusion Criteria**

**Cases:** Patients diagnosed as ectopic pregnancy- diagnosed clinically and with the aid of USG.

**Control:** Early normal pregnancy within 12 week- by LMP and USG.

**Exclusion Criteria**

1. Normal pregnancy more than 12 weeks.
2. Women having bleeding disorder.
3. Complicated pregnancy like threatened abortion, multiple pregnancies.
4. Preexisting chronic infective diseases.

**Study Variables**

The socio demographic variables were included in the proposed study are age, socioeconomic status. The obstetric variables like parity, past obstetric history including sub fertility, abortion, M.R were recorded in both the cases and controls. Past medical and surgical history like PID, appendicitis, laparotomy or pelvic surgery due to any cause was included in the study. Clinical and biochemical variables that were recorded and investigated are vaginal discharge, pulse, blood pressure, USG, serum beta HCG& Chlamydia antibodies in both groups.

**Ethical Consideration**

Ethical clearance for the study was taken from the Department of Obstetrics and Gynaecology, BSMMU. A letter of acceptance has given in Appendix III. Permission for the study was taken from concerned department from where I collect out the study subject. All study subjects were thoroughly appraise about the nature, purpose and implication of the study as well as the entire spectrum of benefits and risks of the study. Interest of the study subjects was not compromised to safeguard their right of health

care. All study subjects were assure of adequate treatment of any risk developing associated with the study. Subject also assured about their confidentiality and freedom to withdraw from the study any time they desired. Finally, written consents from the entire subject were taken.

**Procedure**

After selecting the subject written inform consent was obtained from every subject or from authorized person. Sample of 2 ml. venous blood were collected from every subject of the study from anticubital vein using a sterile disposable plastic syringe under all aseptic precaution into aEppendorf tube. Blood or serum was preserved at -2° to -4° C. Test was carried out in the laboratory of Microbiology department of BSMMU. Chlamydial antibodies (IgM &IgG) levels of all samples were tested using ELISA method. Test was carried out according to manufacturer's instructions (DRG Instruments GmbH, Germany).

**Data Collection**

All necessary information and variables of interest were systematically recorded in pre designed data collection sheet. Data were collected by detailed history, clinical examination, necessary investigations and records of patient and hospital. All information was kept confidential.

**Data Analysis**

All relevant data for individual study subjects were collected and recorded in the data collection sheet. The raw data were compiled and analyzed by computer window based SPSS software. Chi-square and students't' tests were done as appropriate to compare differences between the cases and controls. P value <0.05 was considered as minimum level of significance.

**Table 1: Age, parity and gestational age of the study subjects**

Description	Ectopic Pregnancy (n=30)	Percent %	Normal Pregnancy (n=60)	Percent %	P Value
Age in Years	20-25	18	42	70	a 0.390 <sup>NS</sup>
	26-30	08	11	18.3	
	31-35	04	07	11.7	
Mean±SD	24.7	±7.3	22.5	±3.4	
Parity	Primi	17	41	68.3	a 0.390 <sup>NS</sup>
	Multi	13	19	31.7	
Gestational Age (wk)	Mean±SD	9.3±2.0	10.0±1.7		a 0.114 <sup>NS</sup>
	Range	(6-12)	(6-12)		

NS= not significant; a =P value reached from unpaired 't' test; b= P value reached from chi-square test

**Table 2: Occupational status of husband the study subjects**

Occupational Status	Ectopic Pregnancy (n=30)	%	Normal Pregnancy (n=60)	%	P Value
Service	4	13.3	36	60.0	0.024 <sup>S</sup>
Garments Worker	9	30.0	9	15.0	
Daily Labour	9	30.0	9	15.0	
Deiver	8	26.7	6	10.0	

S= significant; P value reached from chi-square test

**Table 3: Contraceptive history of study subjects**

Contraceptive Used	Ectopic Pregnancy (n=30)	%	Normal Pregnancy (n=60)	%	P Value
OCP	10	33.3	9	15	0.044 <sup>S</sup>
Condom	4	13.3	17	28.3	0.112 <sup>NS</sup>
Nothing	12	40.0	26	43.3	0.762 <sup>NS</sup>
Deiver	4	13.3	08	13.3	0.618 <sup>NS</sup>

S= significant, NS= not significant; P value reached from chi-square test

Table 4: Past obstetric history of study subject

Past Obstetric History	Ectopic Pregnancy (n=30)	%	Normal Pregnancy (n=60)	%	P Value
Ectopic	4	13.3	0	0.0	0.010 <sup>S</sup>
MR	14	46.7	5	8.3	0.001 <sup>S</sup>
Abortion	6	20.0	5	8.3	0.001 <sup>S</sup>
Still birth/IUD	4	13.3	1	1.7	0.041 <sup>S</sup>
Pureperal Sepsis	1	3.3	0	0.0	0.333 <sup>NS</sup>
Nothing	1	3.3	54	90.0	0.001 <sup>S</sup>

S= significant, NS= not significant; P value reached from chi-square test

Table 5: Past medical history

Past Medical History	Ectopic Pregnancy (n=30)	%	Normal Pregnancy (n=60)	%	P Value
PID	12	40.0	2	3.3	0.001 <sup>S</sup>
Salpingitis	6	20.0	1	1.7	0.005 <sup>S</sup>
Appendicitis	15	50.0	1	1.7	0.001 <sup>S</sup>
UTI	03	10.0	5	8.3	0.536 <sup>NS</sup>
Nothing	0	0.0	51	85.0	0.001 <sup>S</sup>

S= significant, NS= not significant; P value reached from chi-square test

Table 6: Past surgical history

Past Surgical History	Ectopic Pregnancy (n=30)	%	Normal Pregnancy (n=60)	%	P Value
Laparotomy	5	16.7	0	0	0.003 <sup>S</sup>
LSCS	10	33.3	10	16.7	0.072 <sup>NS</sup>
Appendicetomy	15	50.0	1	1.7	0.001 <sup>S</sup>
UTI	3	10.0	5	8.3	0.536 <sup>NS</sup>

S= significant, NS= not significant; P value reached from chi-square test

Table 7: Presenting complaints of the ectopic pregnancy patients

Ectopic Pregnancy (n=30)		Number of Patient	%
Presenting complaints	P/V bleeding	25	83.3
	Pain in Lower Abdomen	05	16.7
Abdominal Examination	Tenderness	25	83.5
	Rigidity	05	16.7
Pre vaginal Examination	Positive Cervical excitation test	25	83.3
	Full post fornix	20	66.6
	Bulky uterus	09	30.0

Table 8: Chlamydia antibodies (IgM &amp; IgG) in case &amp; control

Test	Case (Ectopic pregnancy) (n=30)	Control (Normal pregnancy) (n=60)	P Value
Chlamydia positive	9(30%)	2 (3.3%)	0.001 <sup>S</sup>
Chlamydia negative	21 (70%)	58 (96.67%)	

S= significant Data were analyzed using chi-square test

Table 9: Mean distribution of IgM and IgG between two groups

Description	Ectopic Pregnancy (Mean±SD)	Range	Normal Pregnancy (Mean±SD)	Range	P Value
IgM	10.37±2.0	(3-56)	6.07±0.21	(3-9)	0.008 <sup>S</sup>
IgG	10.47±1.0	(4-34)	6.55±0.40	(3-24)	0.005 <sup>S</sup>

Data were analyzed using student't' test

## RESULTS

Majority of the patients were 20-25 years in both group, which were 60.0% and 70.0% in ectopic and normal pregnancy respectively. The mean±SD age was 24.73±7.3 years in ectopic pregnancy and 22.48±3.38 years in normal pregnancy. Regarding the parity it was observed that primi were predominant in both groups. Gestational age was varied from 6 to 12 weeks of gestation in both groups. No statistical significant (P>0.05)

difference were found regarding age, parity and gestational age in two groups.

Table 2 shows that occupational status of husband 13.33% were service, 30% were garments worker, 30% daily labour and 26.67% were driver in ectopic pregnancy. In normal pregnancy 60% were service, 15% were garments worker, 15% daily labour and 10% driver. The difference was statistically significant (P<0.05) between two groups.

Table 3 shows that history of contraception 33.3% were used OCP, 13.3% were used condom, 40% were used nothing and 13.3% were used withdrawal method in ectopic pregnancy. In normal pregnancy 15% were used OCP, 28.3% were used condom, 43.3% were used nothing and 13.3% were used withdrawal method. Oral contraceptive pill was statistically significant ( $P<0.05$ ) between two groups, but others methods were not significant ( $P>0.05$ ) between two groups.

Table 4 shows that past obstetric history 13.3% had ectopic, 46.7% MR, 20% abortion, 13.3% still birth/IUD, 3.3% pureperial sepsis in ectopic pregnancy. In normal pregnancy 1.7% still birth /IUD and only 8.3% MR. Regarding past obstetric history ectopic, MR, abortion, still birth IUD was statistically significant ( $P<0.05$ ) between two groups.

Table 5 shows that past surgical history, 40.0% was PID, 20.0% was salpingitis, 50.0% was appendicitis and 10.0% was UTI in ectopic pregnancy. In normal pregnancy majority (85.0%) had no relevant medical history, 3.3% was PID, 1.7% was salpingitis, 1.7% was appendicitis and 8.3% UTI. PID, salpingitis, and appendicitis were statistically significant ( $P<0.05$ ) between two groups.

Table 6 shows that past surgical history 16.7% were laparotomy, 33.3% were LSCS, 50.0% appendectomy in ectopic pregnancy. In normal pregnancy 16.7% LSCS. Laparotomy and appendectomy were statistically significant ( $P<0.05$ ) between two groups.

Table 7 shows that presenting complaints, majority (83.3%) had PN bleeding, tenderness and positive cervical excitation test in ectopic pregnancy.

Table 8 shows that positive serum IgM & IgG was 9 which was in control 2. There was significant difference between case & control.

Table 9 shows that the mean $\pm$ SE IgM was 10.332.5 with range from 3 to 56 and 6.07 $\pm$ 0.21 with range from 3 to 9 in ectopic and normal pregnancy respectively. On the other hand the mean $\pm$ SE IgG was 10.46 $\pm$ 9.04 with range from 4 to 34 in ectopic pregnancy and 6.90 $\pm$ 3.34 with range from 4 to 24 in normal pregnancy. The difference was statistically significant ( $P<0.05$ ) in IgM and IgG between two groups.

## DISCUSSION

This case control study was carried out with an objective to evaluate the association of Chlamydia infection in women with ectopic pregnancy by comparing the antichlamydial antibodies (IgM & IgG) in serum of ectopic pregnancy and normal pregnancy. Chlamydia trachomatis is one of the most commonly diagnosed sexually transmitted infections while the majority of women are asymptomatic. This study have shown that serum IgM & IgG against Chlamydia trachomatis was positive in 4(13.33%) & 5(16.7%) cases among 30 cases of ectopic pregnancy. IgM was not found and IgG was positive in 2(3.3%) cases in normal pregnancy. The difference was statistically significant between two groups. So it is indentified that Chlamydia trachomatis infection associated with ectopic pregnancy.

Hill et al. found 25% of the ectopic pregnancies in the population related to Chlamydia antibodies.<sup>7</sup> Another study by Robertson et al (1988) had shown that 76% antibody to C. Tachomatis was positive in case compared with 38% in control.<sup>8</sup>

In current study it has found that mean IgM and IgG antichlamydial antibodies more than in the normal pregnancy

group (mean $\pm$ SE 10.33 $\pm$ 2.5 vs 6.15 $\pm$ 0.21 and 10.46 $\pm$ 1.7 vs 6.55 $\pm$ 0.40). In this study difference of positive antiChlamydial antibodies between cases controls was statistically significant. The result of this study might suggest that women having ectopic pregnancy had once been infected with Chlamydia trachomatis who are positive by ELISA.

In this study there was no statistical significant difference were found regarding age, parity and gestational age between two group. Husbands occupation, use of oral contraceptive pill, past medical and surgical history were significant difference between two groups. A study by Tuomivaara (1990) reported that gynaecological infections are more prevalent among factors predisposing to ectopic pregnancy Regarding past obstetric history repeat ectopic pregnancy was 13.33% in this study.<sup>9</sup> A study of Nepal recently has been shown 18% of repeat ectopic pregnancy.<sup>4</sup> These findings are more alike to my study.

Thus by this it is proved that Chlamydia infection is a risk factor for ectopic pregnancy. The incidence of pelvic inflammatory diseases and its sequelae has fallen markedly in countries is like Sweden and parts USA where there are screening programme for Chlamydia. The rate of ectopic pregnancy has been reduced in the 1st year after introduction of chlamydia screening.

This case control study to compare the Chlamydial antibodies level between women with ectopic pregnancy and women with normal pregnancy was carried out to determine any association between ectopic pregnancy and Chlamydia trachomatis infection in the department of Obstetrics and Gynaecology of BSMMU and Department of Microbiology, BSMMU, Dhaka during the period of January 2006 to July 2009. I have enrolled 30 women of ectopic pregnancy as case & control subject were 60 women of normal pregnancy.

This study showed 13.33% & 16.67% had positive IgM and IgG in ectopic pregnancy and only 3.33% had positive IgG in normal pregnancy. Total 9 cases (IgM-4& IgG-5) were positive in ectopic pregnancy & only 2 cases were positive in normal pregnancy. The difference was statistically significant. Regarding other variables significant difference was present in occupation of husband, past medical & surgical history. Contraceptive history showed that there was significant difference between women of ectopic pregnancy and normal pregnancy in oral contraceptive pill use. Regarding past obstetric history-repeat ectopic pregnancy was 13.3%.

## CONCLUSION & RECOMMENDATIONS

Women who have had Chlamydia trachomatis, a sexually transmitted disease, are more than as likely as other women to have an ectopic pregnancy, Chlamydia can cause pelvic inflammatory disease which can result in scarring of the fallopian, with consequent ectopic pregnancies which can be fatal, have reached to morbidity & mortality.

Ectopic pregnancy is an important cause of maternal death and still holds a diagnostic challenge. MDG-5 of Bangladesh is to reduce MMR 3/4 by the year 2015. To reduce ectopic pregnancy & its consequences causative agent should be identified. So this study has been tried to found out whether there is any association of ectopic pregnancy and Chlamydia trachomatis infection and it has been proved by this study that there is an association. Though this small study has some limitation- still it may create some awareness regarding preventive & therapeutic measure of

Chlamydia trachomatis infection to reduce incidence & consequences of ectopic pregnancy in long run. Thusly, the recommendations are:

- Chlamydial serology might be use in women suffering from PID.
- Treatment of both partner with clamydial IgM positive cases may reduce the incidence of PID and ectopic pregnancy.
- Antichlamydial therapy should be given to all cases with pelvic Infection and pelvic surgery
- Further large scale study is needed to determine the Chlamydia Trachomatis as a risk factor for ectopic pregnancy
- C. trachomatis antibody testing is simple, less expensive and cause minimal inconvenience to the patient.
- C. trachomatis antibody testing deserves to be an integral component of future prevalent study of PID to reduce the incidence of ectopic pregnancy.

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