DOI: http://dx.doi.org/10.18203/2320-1770.ijrcog20184162

Original Research Article

Chlamydia infection as a risk factor in ectopic pregnancy: a case control study

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Received: 09 August 2018 Accepted: 04 September 2018

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ABSTRACT

Background: Chlamydia trachomatis has been linked to 30-50% of all ectopic pregnancies, due to irreversible tissue damage. Hence it is pertinent to explore the risk factors pertaining to Chlamydia infection and ectopic pregnancies. The aim is to study whether Chlamydia infection is a risk factor for ectopic pregnancy in comparison with early intrauterine pregnancy, and study other risk factors in ectopic pregnancy

Methods: Case-control study conducted at Sree Avittom Thirunal Hospital, Thiruvananthapuram over a period of six months taking 43 subjects in each group, i.e., cases and controls. An interview followed by collection of venous blood sample was done, which was subjected to Enzyme linked immunoassay test to detect Immunoglobulin G antibodies to Chlamydia. Comparison of qualitative variables such as age, socioeconomic status, history of infertility, history of pelvic inflammatory disease, previous history of ectopic pregnancy, use of IUCD, history of IVF, use of OCP or progesterone only pills and presence of Chlamydia IgG antibodies - between two groups was analyzed by chi-square test and the strength of association expressed in terms of Odds Ratio. A p-value <0.05 was considered statistically significant.

Results: Out of 86 samples 54 were positive for Chlamydia infection. Subjects with Chlamydia infection had an increased risk of developing ectopic pregnancy. Among the other risk factors history of pelvic inflammatory disease and history of infertility were the most significant with Odds of 3.46 and 3.98 respectively. History of oral contraceptive use also had a significant risk associated with developing ectopic pregnancy. Other factors with significant association included, age more than 25 years, Upper Socioeconomic Class, previous ectopic pregnancy and history of IUCD use for more than 5 years.

Conclusions: The increased number of Chlamydia infection and its increased risk for developing irreversible sequels such as ectopic pregnancy, it is pertinent to vigilantly diagnose, treat and prevent vaginal infection and pelvic inflammatory disease.

Keywords: Chlamydia infection, Ectopic pregnancy, Risk factors

INTRODUCTION

Ectopic pregnancy is a life-threatening condition and one of the most important causes for maternal mortality worldwide and in our country, with incidence in developed countries as 1-2% and higher incidence in developing countries. Chlamydia trachomatis is the most common bacterial sexually transmitted infections worldwide.¹ Disproportionately, it is women who suffer bulk of the complications. These complications include infertility, pelvic inflammatory disease (PID), and ectopic pregnancy. The disease is on a decline in developed countries owing to the increased awareness. Unfortunately, 1.2 billion people still live in endemic areas. Recent studies from India have revealed the prevalence of Chlamydia trachomatis infection to be 23% in gynaecology outpatient department and 19.9% in STD patients.^{2,3} It has been recovered from 30-60% cases of salphingitis and pelvic inflammatory disease patients in India. 20% of women who develop PID become infertile, 18% develop chronic pelvic pain, and 9% have a tubal pregnancy. Chlamydia trachomatis infection is the most preventable cause of pelvic inflammatory disease in young women.

It has also been linked to 30-50% of all ectopic pregnancies.⁴ Ectopic pregnancy is a direct result of damage to the fallopian tubes. Ectopic pregnancy is one of the most serious sequels to acute salpingitis. Chlamydia trachomatis seems to be the most common etiologic agent of acute salpingitis.

Chlamydia trachomatis is a strong immunogen, which stimulates both humoral and cell mediated immune responses. In addition to the immunogenic antigens, the outcome of Chlamydia infection depends on interaction and balance of cytokines secreted by the activated lymphocytes.

Chlamydia trachomatis infection may be primary or a chronic re-infection. Primary infection is a serial infection of the mucosal cells. The damaged and infected epithelial cells secrete numerous proinflammatory chemokines and cytokines, causing vasodilatation, increased endothelial permeability, activation and influx of neutrophils, monocytes and T-lymphocytes, and elevated expression of adhesion molecules. Chronic infection is associated with persistence of Chlamydia in the host cells, which is more dangerous. It leads to tissue damage, fibrosis and cicatrisation within the affected organs. Irreversible consequences like PID leading to mechanical infertility, ectopic pregnancy, chronic pelvic pains and chronic urethritis may occur.

Hence it is pertinent to explore the risk factors pertaining to Chlamydia infection and ectopic pregnancies. The aim of the study was to determine whether chlamydia infection is a risk factor for ectopic pregnancy, in comparison with early intrauterine pregnancy admitted in SAT Hospital, tertiary care centre in Thiruvananthapuram. And also study other risk factors in ectopic pregnancy.

METHODS

This was a case-control study conducted on patients admitted as a case of ectopic pregnancy and comparing them with the controls, which were taken as patients with early intrauterine pregnancy, in the Sree Avittom Thirunal (SAT) Hospital, the maternity hospital of the Government Medical College, Thiruvananthapuram, Kerala. The data pertaining to this study was collected between July and December 2016. Cases were selected by confirming ectopic pregnancy via trans abdominal and transvaginal sonography, urine pregnancy test positive and high beta human chorionic gonadotrophin hormone levels, more than 1500mIU/ml. while controls were taken as those with urine pregnancy test positive and trans abdominal and transvaginal sonography showing early intrauterine gestation, up to 8 weeks gestation. Among controls those with previous history of ectopic gestation or fallopian tube surgery were not included; along with those who did not give consent to partake in the study.

The total number of patients taken was 86, 43 in each group. All were subjected to an interview to collect baseline data, following which a venous blood sample was drawn from each patient to detect antibodies to Chlamydia trachomatis Immnuoglobulin G using Enzyme Linked Immunosorbent Assay readymade kit. The study variables used was age, socioeconomic status, regularity of menstrual cycles, parity, history of more than one sexual partner, history of infertility, history suggestive of pelvic inflammatory disease (chronic pelvic pain, dyspareunia, discharge per vaginum, dysuria), previous history of ectopic pregnancy, use of IUCD, history of tubal surgery/ any other abdominal surgery, history of taking combined pills or progesterone only pills, presence of Chlamydia IgG antibodies.

RESULTS

A total of 86 patients were examined, 43 cases and 43 in the control group. As per this study, it was evident that females above 25 years of age were susceptible to developing an ectopic pregnancy with Odds ratio of 2.13, giving a two-fold increase risk in women over 25 years, Table 1.

Table 1: Comparison of the variable, age, between the
cases and controls.

		Case		Cor	ntrol	Total		
		Ν	%	Ν	%	Ν	%	
Age	>25	27	62.8	19	44.2	46	53.5	
	≤25	16	37.2	24	55.8	40	46.5	

Looking at the socioeconomic status, 76.7% of the cases were from the upper socioeconomic status thus it was found that subjects from the Upper socioeconomic class, shown in Figure 1, were more predisposed to develop ectopic pregnancy than the lower socioeconomic class with Odds of 2.16, Table 2.



Figure 1. Comparison between cases and controls with respect to socioeconomic status

In the case of number of sexual partners, the highest number of partners observed was 2. Among 4 in the ectopic group had two sexual partners each, 9.3% and in the control group 2, 4.7%. The observed p value in this category is 0.397 which is not significant when compared to the group of controls, Table 3. History of infertility was found in total 9 patients out of the total 86.

Table 2: Comparison between the cases and controls with respect to socioeconomic status.

		Case		Cor	ntrol	Tot	OR	
		Ν	%	Ν	%	Ν	%	
SES	Upper	33	76.7	26	60.5	59	68.6	2.16
	Lower	10	23.3	17	39.5	27	31.4	

Table 3: Comparison between cases and controls with respect to number sexual partners.

Sexual partners	Case		Control	Control		Total		df	р
	Ν	%	Ν	%	Ν	%			
One	39	90.7	41	95.3	80	93	0.717	1	0.397
Two	4	9.3	2	4.7	6	7			
Total	43	100	43	100	86	100			

Table 4: Comparison between cases and controls with respect to history of pelvic inflammatory disease.

		Case		Control		Т	Total		n	OD	95% (CI for OR
		Ν	%	Ν	%	N	N	%	P	UK		
Pelvic Inflammatory disease	Yes	22	51.2	10	23.3	3	32	37.2	0.007	3.46	1.37	8.73
	No	21	48.8	33	76.7	5	54	62.8				

Out of which 7 were among the cases and 2 were in the control group, as shown in Figure 2. The Odds ratio calculated was 3.98. Out of 86 subjects, 32 gave a history of pelvic inflammatory disease, i.e. 37.2%.



Figure 2. Comparison between cases and controls with respect to history of infertility.

Among them, 22 (51.2%) developed ectopic pregnancy while 10 (23.3%) were from the control group. It was found that those with a history of pelvic inflammatory disease have an odd of 3.46 (p value = 0.007, CI = 1.37 - 8.73) in developing ectopic pregnancy as compared to the controls, shown on Table 4. Previous history of ectopic pregnancy was found in only one patient among the subjects and that was in the cases group. Hence odds could not be determined.



Figure 3. Comparison between cases and controls with respect to history of previous ectopic pregnancy.

However, history of prior ectopic pregnancy is a significant risk factor, Figure 3. The number of patients that used intrauterine contraceptive device (IUCD) was 5. Among them, 7% were cases and 4.7% controls and the observed p value was 0.645, which showed no significant risk association. Most patients gave a history of having the IUCD for less than 5 years, i.e. 80% of the total patients. Only one case in the ectopic group had it for 5-10years duration, and none in the control group. Hence the Odds ratio could not be calculated. History of IUCD for more than 5 years is a significant risk factor for ectopic pregnancy, Table 5. History of using contraceptive pills, only 4 subjects had a prior history of taking contraceptive pills, and all were from the ectopic group. None in the control group had a similar history of

oral contraceptive usage; hence Odds ratio could not be calculated.

However, the chi-square test value is 4.195 and the observed p value for this variable was found to be 0.041, which shows a significant association between history of OCP intake and ectopic pregnancy, Table 6 and Figure 4.

Table 5: Comparison for the duration of IUCD.

	Case		Con	trol	Tota	ıl
Years of IUCD	Ν	%	Ν	%	Ν	%
<5 years	2	66.7	2	100	4	80
5-10 years	1	33.3	0	0	1	20
Total	3	100	2	100	5	100

Table 6: Comparison between cases and controls with respect to history of using oral contraceptive pills.

	Case		Control		Total		χ^2	df	р
H/O OCP/POP	Ν	%	Ν	%	Ν	%			
Yes	4	9.3	0	0	4	4.7	4.195	1	0.041
No	39	90.7	43	100	82	95.3			
Total	43	100	43	100	86	100			

Table 7: Comparison between cases and controls with respect to serum Chlamydia IgG antibodies.

	Case		Control		Total		2	JC	-
Chlamydia IgG	Ν	%	Ν	%	Ν	%	χ-	ai	þ
Positive	30	69.8	24	55.8	54	62.8	1.792	1	0.181
Negative	13	30.2	19	44.2	32	37.2			
Total	43	100	43	100	86	100			









Coming to the main objective of this study, there were a total of 54 samples (62.8%) which came out to be positive for Chlamydia IgG. Out of which 69.8% were cases and 55.8% were controls. The Odds of developing ectopic pregnancy in patients with Chlamydia infection is 1.8, Table 7 and Figure 5.

DISCUSSION

This present research was a case-control study, tailored to determine whether *Chlamydia trachomatis* infection is associated with ectopic pregnancy.

According to this study, women above 25 years of age were susceptible to developing an ectopic pregnancy with odds of 2.13. Thus, older women of reproductive age carry an increased risk of ectopic pregnancy compared with those aged 15-25 years. A study done in Brunei by Mridula AB et al showed out of the total 123 ectopic pregnancies encountered there, a majority of patients were from the age group 26-35 years of age, but contrary to the results seen in this study there was no significant difference between the two groups (i.e. cases and controls) with respect to age.⁵ These results were also consistent with a study done by Ashihi et al where women 26-38 years of age were seen susceptible to developing ectopic pregnancy, while in a few others a range of 20-30 were seen.^{6,7,8}

When the socioeconomic statuses of the subjects were compared, it was found that the Upper Socioeconomic Class was more predisposed to develop ectopic pregnancy than the Lower Socioeconomic Class with Odds of 2.16. This was in stark contrast with the study done by Adewunni AA et al in Lagos, Nigeria, where it was found that Lower Socioeconomic Class women are more predisposed to develop ectopic pregnancy (p=0.001, level of education p=0.001).⁹

Comparing the parity between the two groups it was noted that among ectopic pregnancies nulliparas were more in number than the multiparas. But there was no significant difference seen between the two groups (p value-0.946). Similar to the present study done at Shanghai by Cheng Li et al, found that nulliparas more prone to develop ectopic pregnancy but no significant difference was seen between the ectopic group and the intrauterine pregnancy group.¹⁰

In contrast to the findings in this study, multiparas were more predisposed to ectopic pregnancy as seen by Adewunmi AA et al and a significant difference was seen between the ectopic and intrauterine pregnancy (pvalue=0.005).⁹ Similar to this a study done in Mangalore, Karnataka by Shraddha Shetty et al also found that multiparas were more predisposed to develop ectopic pregnancy.⁸

Looking at the number of sexual partners, the maximum seen in either of the groups were 2. Totally 4 subjects had a history of 2 sexual partners among which 2 were cases and 2 controls. Hence on calculating the p value (0.397), no significant difference was seen when comparing the two groups. But it is a well-known fact that multiple sexual partners predispose to sexually transmitted infections thus subsequently increasing the risk for developing ectopic pregnancy. A significant risk association with respect to multiple sexual partners was seen in the study done by Adewunmi AA (p-value= 0.0009).⁹

The mean age of menarche found was 14 years. No significant risk association was found with respect to menarche and menstrual cycle, and ectopic pregnancy in this study. This finding is consistent with previous studies where risk association was not elicited between either of the two variables and ectopic pregnancy.⁹

Next, history of infertility was compared between the two groups and it was found that subjects with a history of infertility had Odds of 3.98 of developing ectopic pregnancy than those without.

This finding is consistent with the studies done by Cheng Li et al where a significant association was found between previous infertility history and ectopic pregnancy, in case of both tubal infertility (OR-8.81, 5.09) and non-tubal infertility (OR-5.51, 2.83).^{10,11} Tubal factor infertility is among the main risk factors for ectopic pregnancy.^{11,12} Similar finding of significant risk association between history of infertility and ectopic pregnancy was also elicited by Shraddha Shetty.⁸

One of the main risk factors predisposing to ectopic pregnancy is pelvic inflammatory disease. A significant risk association was found between patients with history of pelvic inflammatory disease and ectopic pregnancy. In this study, subjects with a history of pelvic inflammatory disease had 3.46 Odds of developing ectopic pregnancy (p value=0.007) than those without a history of PID. Thus, agreeing with the rationale that previous pelvic inflammatory disease can cause damage to the fallopian tubes, leading to subsequent risk of having an ectopic pregnancy. Among the symptoms for prior history of pelvic inflammatory disease, dysuria along with chronic pelvic pain was found to be the commonest symptoms comparing both groups, having a P value 0.048 and 0.026 respectively. While dyspareunia (p=0.167) and discharge PV (p=0.106), were less common.

These findings are consistent with various studies which highlight that the risk for ectopic pregnancy is increased in women with a prior history of pelvic inflammatory disease.^{6,8,10,13} Nearly half the cases seen by Porwal Sanjay et al had a positive history of pelvic inflammatory disease.⁷ Similarly, in Adewunmi AA's study a p value of 0.003 was seen, thus showing a significant risk association between ectopic pregnancy and pelvic inflammatory disease.⁹

In this study, only one subject had a prior history of ectopic pregnancy and that was in the case group, and none were seen in the control group. Although the Odds could not be calculated, a prior history of ectopic pregnancy is a significant predisposing factor for developing ectopic pregnancy. Especially women with two prior ectopic pregnancies have a 10-16-fold increased chance for another ectopic pregnancy.¹⁴ Various other studies also show a similar increased risk association between the two.^{8,10}

History of use of intrauterine contraceptive device is also among the main predisposing factors for ectopic pregnancy. In this study, 5 subjects had a history of IUCD insertion and most of them had it for duration of less than 5 years. One of the subjects though, from the case group, had it for more than 5 years and none in the control group. Hence Odds was not calculated. History of IUCD for more than 5 years is a significant risk factor for ectopic pregnancy. In one study of 61,448 IUD users, 118 contraceptive failures were reported, 21 of these were ectopic.¹⁵ Cheng Li found an increased risk association between IUCD usage and ectopic pregnancy and risk is further increased with increase in duration of IUCD.^{10, 11}

History of prior abdominal surgery, in this study, had no significant association with ectopic pregnancy. This was consistent with the findings in Adewunmi AA's study where no significant association was seen.⁹

In the studies conducted by Cheng Li et al shows that prior abdominal surgery, especially prior adnexal surgery, surgery for ectopic pregnancy and surgery for tubal infertility, increases the risk for developing ectopic pregnancy.^{10, 11}

Looking at other risk factors for ectopic pregnancy, history of oral contraceptive intake was present in 4 subjects, and all of them were from the cases group, none from the control group. Hence Odds ratio was not calculated. In this study the risk associated with prior history of taking oral contraceptives and ectopic pregnancy was shown to be very significant with p=0.041.

These findings were consistent with various studies. Pulkkinen and Talo had described the physiology of tubal ciliary and myoelectrical activity.¹⁶ The static force of the cilia results in fluid flow toward the uterus and the spermatozoa usually overcome this, hence showing that cilia are not obligatory for normal implantation, since patients with congenital inactivity of all cilia (Kartagener's syndrome) are able to have intrauterine pregnancies. It is the myoelectrical activity that plays a vital role in tubal propulsion. In general, estrogens stimulate tubal myoelectrical activity and progesterone inhibits it.¹⁷

The mechanisms of the contraceptive action of levonorgestrel are believed to be multifactorial. Among these mechanisms, alteration in tubal motility may contribute to a delayed arrival of the egg in the endometrial cavity, a fact leading to the occurrence of ectopic pregnancy.¹⁸

Moreover, pharmacologic levels of progesterone may relax tubal myoelectric activity to such an extent that transport through the isthmus does not occur. Similar findings were also seen in the multi centre case control study conducted by Cheng Li, in Shanghai, China where they found a three-fold increase (AOR=3.02, 95% CI, 1.16-7.86) in the risk of ectopic pregnancy with the use of oral contraceptive pills.¹⁰

Using the ELISA kit, it was found that out of 86 samples, 54 were positive for Chlamydia trachomatis infection.69.8% of the ectopic pregnancy cases were serum Chlamydia IgG antibody positive, and among the controls i.e. the normal intrauterine pregnancy group, 55.8% were found to be positive. The Odds of developing ectopic pregnancy in patients with Chlamydia infection is 1.8.

The study conducted by Adewunmi AA et al in Lagos, Nigeria, around 91 cases of ectopic pregnancy among a total of 2468 deliveries giving an incidence of 3.68% or in other words, 1 in 27 deliveries.⁹ The results showed seropositivity of Chlamydia IgG (62.4%) in the cases was significantly higher than that of 29% in the control (p <0.0001). Cheng Li et al in Shanghai, China, also had

shown a three-fold increase in the risk of ectopic pregnancy with respect to previous infection with Chlamydia trachomatis.¹⁰

Rekart et al did a descriptive analysis observing the trends, from 1992 to 2009, for Chlamydia cases, PID and ectopic pregnancy in British Columbia, Canada. It was found that Chlamydia cases substantially increased from 1992 through 2009. Inpatient, outpatient, and total diagnoses of pelvic inflammatory disease and ectopic pregnancy declined from 1992 through 2003. After 2003, PID rates continued to fall, but recent increase in Chlamydia infection was seen from 1996 to 2009 as male Chlamydia urethritis rates increased, causing re-infection and increase incidence again which may contribute to the ectopic pregnancy rates significantly increasing.¹³

A prospective case-control study done in Saudi Arabia by AshshiA M et al, showed a prevalence of 31.8% of sexually transmitted disease among 135 participants and the frequencies were higher in case of ectopic pregnancy. Out of this, Chlamydia trachomatis infection had a higher rate, 27.4%, of frequency in ectopic pregnancy compared to other sexually transmitted disease. But the technique used for testing the Chlamydia infection was by NAAT, multiplex PCR in this study, which is the preferred diagnostic and screening test for Chlamydia trachomatis genital infection.⁶ Though it has high sensitivity and specificity rates, its use in our setting has been limited as these tests are quite expensive and unavailable. But the peptide-based assay, i.e. the ELISA kits, that were used to detect the Chlamydia IgG are well standardized, less expensive and easily available, with 73%-83% sensitivity and 97%-99% specificity.¹⁹

To conclude, as was seen in this study 62.8% of the subjects (54 out of 86 subjects) had Chlamydia IgG positivity which prompts us to be more vigilant indiagnosing, treating and more importantly preventing Chlamydia infection and other causes for pelvic inflammatory disease to prevents its long-term sequels such as ectopic pregnancy.

CONCLUSION

Chlamydia trachomatis infection is one of the most common sexually transmitted disease worldwide. We have seen many adverse outcomes that are caused by this disease, most commonly pelvic inflammatory disease, infertility, and ectopic pregnancy. Out of these complications, we focused on the risk associated between chlamydia and ectopic pregnancy in this study, which produces irreversible tissue damage at the molecular level in fallopian tubes.

As seen in this study there is an increased rate of Chlamydia infection in our population. Hence early diagnosis, treatment especially by syndromic approach, and prevention of vaginal infections and pelvic inflammatory disease is important to prevent these sequels. A rise in ectopic pregnancy has been seen in the last decade which attributable to various factors such as history of pelvic inflammatory disease; history of infertility and history of oral contraceptive intake mainly. Other contributing factors include age more than 25 years, Upper Socioeconomic Class, history of using IUCD for more than 5 years and previous ectopic pregnancy.

Though the preferred diagnostic and screening method of choice for detection of Chlamydia trachomatis infection are the nucleic acid amplification tests or direct fluorescent antibody test, ELISA test may be preferred in developing regions where they are less expensive and easily available with good sensitivity and specificity rates. The increased incidence of ectopic pregnancy among patients with history of oral contraceptive use should prompt us to study further on this aspect so as to reduce the incidence of ectopic pregnancy at the same time decrease the rate of unwanted pregnancy. Looking at the increased number of Chlamydia infection in this study and its increased risk for developing irreversible sequels such as ectopic pregnancy, it is pertinent to vigilantly diagnose, treat and prevent vaginal infection and pelvic inflammatory disease.

Funding: No funding sources Conflict of interest: None declared Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Angela G, Shaila S. Chlamydia infection as a risk factor in ectopic pregnancy: a case control study. Int J Reprod Contracept Obstet Gynecol 2018;7:4254-60.