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Original Research Article

Association between Chlamydia trachomatis infection and recurrent pregnancy loss

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ABSTRACT

Background: Chlamydia trachomatis infection is widely prevalent sexually transmitted infection. Chlamydia trachomatis is associated with pelvic inflammatory disease, salpingitis, infertility and various adverse pregnancy outcomes example- preterm labour, Premature rupture of membranes, spontaneous miscarriage. Association of Chlamydia trachomatis with recurrent pregnancy loss is not yet well understood. The aim of the present study was to determine association of Chlamydia trachomatis infection with recurrent pregnancy loss (RPL) by comparing its prevalence in women with and without RPL.

Methods: This case control study was carried out in the department of Obstetrics and Gynecology at VMMC and Safdarjung Hospital, New Delhi. Total 200 women were recruited on the basis of inclusion and exclusion criteria. Out of these 100 women were in study group with history of RPL and 100 women were in control group with previous successful pregnancy outcomes. ELISA on blood and PCR on urine sample were performed to detect Chlamydia trachomatis and its prevalence was compared in both the groups. All quantitative variables were compared by t test and qualitative variables were compared by chi square test. P value less than 0.05 was considered significant.

Results: Prevalence of Chlamydia trachomatis infection by PCR on urine was found to be 17% in study group and 0% in control with a significant difference in both the groups (p value- 0.0001). Similarly, prevalence of Chlamydia trachomatis infection by ELISA on blood was found to be 22% in study group and 4% in control with a statistically significant difference in both the groups (p value-0.0001). Thus, significantly higher prevalence of Chlamydia trachomatis infection was found in women with recurrent pregnancy loss in comparison to those without it.

Conclusions: This study showed strong association of Chlamydia trachomatis infection with recurrent pregnancy loss. Hence, screening and treatment of pregnant women for C.trachomatis infection may be beneficial to reduce adverse pregnancy outcome.

Keywords: Recurrent pregnancy loss, Chlamydia trachomatis infection, Miscarriage

INTRODUCTION

Pregnancy loss (miscarriage) is spontaneous demise of fetus before the viability period. World health organisation (WHO) defined abortion as termination of pregnancy before viability of fetus that is either 20 weeks of gestation or with a fetus weight less than 500 g.¹ It's generally a

consensus that 15% of pregnant women experience spontaneous sporadic loss of pregnancy. According to European Society of Human reproduction and Embryology (ESHRE 2017) guidelines recurrent pregnancy loss (RPL) is defined as 2 or more consecutive or non-consecutive pregnancy losses before 20 weeks of

period of gestation.² Prevalence of recurrent pregnancy loss falls between 0.6% to 2.3% in various studies.^{2,3}

Approximately 50% of the women have unknown etiology for recurrent pregnancy loss. Known causes of recurrent pregnancy losses are Chromosomal abnormalities, Immunological disorders, Antiphospholipid syndrome, Anatomic abnormalities, Uterine anomalies, Endocrine disorders, Infectious microorganisms, Environmental factors and others like maternal age. Infections known to play role in recurrent pregnancy loss include Mycoplasma, Chlamydia trachomatis, Listeria Monocytogens, Cytomegalovirus (CMV), Toxoplasma and Herpes simplex virus (HSV).⁴

Chlamydia trachomatis is widely prevalent sexually transmitted infection in both men and women causing various types of morbidities.⁵ Prevalence of C.trachomatis infection related recurrent pregnancy loss observed in various studies falls between 17-24%.^{6,7} Majority of Chlamydia trachomatis infections are asymptomatic which if left untreated, can progress to chronic infection, which further can cause pelvic inflammatory disease, salpingitis, infertility. Besides these morbidities Chlamydia trachomatis infection can also cause preterm labor, premature rupture of membrane (PROM) and neonatal complications like Low birth weight, conjunctivitis and pneumonia.^{4,5,8}

The mechanism by which Chlamydial infections cause recurrent pregnancy loss is not well understood. However embryonic rejection may be due to cross reactivity between heat shock protein found in C. trachomatis and embryonic protein in fetus. It can also be due to direct fetal infection which triggers an inflammatory response with release of cytokines causing maternal inflammatory response which causes embryonic rejection.¹⁰ Chlamydia also induces 'toll like receptor 4' which normally mediates immunity to placental cells giving rise to placental trophoblast dysfunction, consequently leading to pregnancy demise.⁵

Chlamydia trachomatis infection can generate an inflammatory response resulting in release of inflammatory cytokines-Interferon alpha, Th1/Th2/Th17 cells activation which may act in pathogenesis of spontaneous abortion.⁷ Chlamydia trachomatis can cause abortion also by causing chorioamnionitis, in which inflammatory responses results in release of proteases. These proteases can cause PROM, resulting in release of prostaglandins due to activation of arachidonic acid pathway causing uterine contractions which leads to preterm labour and abortion. Recently oxidative stress and TNF factor associated apoptosis of decidual cells, also has been found to have role in Chlamydia trachomatis infection associated adverse pregnancy outcomes.^{9,10} Chlamydia trachomatis can also cause endometritis resulting in defective decidualization and miscarriage.⁶

Despite a number of studies, whether Chlamydia trachomatis infection is associated with miscarriage is not clearly understood, because of conflicting results of various studies. Results are also variable due to different methods of detection of Chlamydia trachomatis.

Gutierrez et al, found a significant association between risk of early miscarriage and Chlamydia trachomatis infection.¹¹ Bagheri et al, in a case control study observed a significant association of Chlamydia trachomatis with spontaneous miscarriage when Chlamydia trachomatis detection was done in urine by PCR. They observed non-significant difference in sero-prevalence of C. trachomatis when detection was done by ELISA.¹² Ahmadi et al, observed by PCR observed a significant association of C. trachomatis infection and spontaneous miscarriage.⁴

However some studies observed no association of Chlamydia trachomatis infection with miscarriage.^{13,14} Horne et al, 2020, detected C. trachomatis using PgP3 ELISA, concluded that there is no clear association of Chlamydia trachomatis infection with first trimester pregnancy loss.¹⁴

Thus the results of various studies for association of C. trachomatis with miscarriage are conflicting and they also vary with method of detection of C. trachomatis. Studies on association of Chlamydia trachomatis with recurrent pregnancy loss are limited.

The aim of this study was to determine association of C trachomatis with recurrent pregnancy loss by detection of C. trachomatis by both serological (ELISA) and more sensitive molecular method (PCR) and comparing its prevalence in women with and without recurrent pregnancy loss.

METHODS

This case control study was conducted from November 2019 to September 2021 in the Department of Obstetrics and Gynaecology, VMMC and Safdarjung Hospital, New Delhi in conjunction with ICMR, National Institute of Pathology, New Delhi. Before starting the study, approval was taken from institutional ethics committee VMMC and Safdarjung Hospital. Inclusion criteria was non pregnant women 15-49 years of age attending fetal medicine clinic and Gynaecology OPD divided into cases and control on the basis of history of presence or absence of Recurrent Pregnancy loss. Total 200 women were recruited, study group included 100 women with history of 2 or more than 2 recurrent pregnancy loss. Control group included 100 women with successful pregnancy outcomes.

Exclusion criteria was use of antibiotics within 14 days of taking samples, pregnancy, endocrine or metabolic disorder, smoking, congenital uterine anomaly and history suggestive of chromosomal anomaly in fetus, anti-phospholipid antibody syndrome (APLA). A written informed consent was taken from each participating

woman. Detailed history of enrolled patients was taken. Detailed examination including height, weight, BMI, per abdomen examination, local examination, per vaginal examination was done. All investigations and Ultrasound done by women during time of abortions were noted.

Blood and urine samples were taken under aseptic precautions from all the participants recruited in study. Five ml of non-heparinized blood was taken from each participant in both the Groups in sterilized vials. Serum separated and stored at -20°C and 10-15 ml of urine collected in sterilized falcons, pelleted and stored in -20 degree centigrade. ELISA on blood sample and PCR performed on urine samples for detection of Chlamydia trachomatis. Observations and results were recorded in the on excel sheet and evaluated was done using

Statistical analysis

Statistical package for social sciences (SPSS) version 21. Prevalence of Chlamydia trachomatis was determined in both cases and control group

RESULTS

In the present study age range for both control and cases was between 18-36 years. Mean age of cases and control were 26.08±3.62 years and 26.17±2.7 years respectively. In present study majority of cases and control were in 26-30 years age group,55% and 65%respectively (Table 1). Mean BMI of cases was 21.21 kg /m2 and of controls it was 21.12 kg/m2, difference was not significant.

Majority of women in present study resided in urban area and there was no significant difference in cases and controls. (p value-0.350). In terms of socio-economic status majority (41%) belonged to middle class followed by lower middle class with no significant difference in socioeconomic status of cases and controls. (p value-0.968). In education status majority (51%) women in cases and controls were 8th standard educated and there was no significant difference in education of cases and controls (p value-0.069). Women having symptoms of infection either in present or in past were 36% in cases and 12% in control and this difference was not significant with p value of 0.062 (Table 1).

Majority of abortions in women with recurrent pregnancy loss occurred in first trimester (63%). Chlamydia trachomatis infection affected women had predisposition

to abort in first trimester in comparison to second trimester. (Table 2)

Comparison of prevalence of Chlamydia trachomatis infection in women with and without recurrent pregnancy loss.

In present study 22% of cases were positive on serum ELISA with presence of antichlamydia trachomatis antibodies IgG types. However, amongst control, only 4% patients had antichlamydia trachomatis IgG antibodies. This difference was significant with p value of 0.0001. Similarly, PCR for Chlamydia trachomatis in urine was positive in 17% cases with history of recurrent pregnancy loss but all controls had negative PCR results. This difference was found to be statistically significant with a p value of 0.0001 showing strong correlation of Chlamydia trachomatis infection with recurrent pregnancy loss (Table 3).

Table 1: Sociodemographic characteristics in cases and control.

Demographic characteristics	Cases	Control	P value
Age in years (mean)	26.080	26.170	0.843
Maximum	36	36	
Minimum	18	18	
Mean BMI (kg/m2)	21.21	21.12	0.736
Maximum	26.3	26.2	
Minimum	18.1	18.4	
Education			0.069
Illiterate	14	4	
Upto class 8 th	51	46	
Upto 10 th	28	37	
>10 th	7	12	
Residence			0.350
Rural	32	26	
Urban	68	74	
Socioeconomic status			0.968
Upper	5	4	
Upper middle	20	24	
Middle	34	33	
Lower	41	28	
History of infection (%)	36	12	0.062

Table 2: Correlation of C.trachomatis infection with Gestational age at miscarriages.

Gesational age	No,	%	ELISA No.	%	PCR No.	%
1st trimester	63	63	13	13/63=20.6	12	12/63=19
2nd trimester	6	6	1	1/6=16.6	0	0
1st+2nd trimester	31	31	8	8/31=25.8	5	5/31=16.1

Table 3: Comparison of prevalence of C. trachomatis among cases and control.

ELISA	Case		Control		P value
	N	%	N	%	
Positive	22	22.0%	4	4.0%	0.0001
Negative	78	78.0%	96	96.0%	
Total	100	100.0%	100	100.0%	
PCR					
Positive	17	17.0%	0	0	0.0001
Negative	83	83.0%	100	100	
Total	100	100.0%	100	100.0%	

Table 4: Comparison of various study results.

Author	Year	Study population	Sample size		Investigation	C. trachomatis positive		P value
			Case	Control		Cases%	Control%	
Present study	2021	Indian	100	100	ELISA PCR	22.0 17.0	4.0 0.0	0.001
Horne et al¹⁴	2020	United kingdom	251	118	ELISA	25.9	28	0.71
Gutierrez et al¹¹	2020	Mexican	108	42	PCR	34	4.7	0.004
Bagheri, et al¹²	2018	Iran	97	60	ELISA PCR	4.1 11.3	1.7 0	0.64 0.03
Aliyu et al¹⁵	2018	Nigeria	83	83	ELISA	8.4	3.6	0.192
Ahmadi et al⁴	2016	Iran	109	109	PCR	22.9	11.9	0.03

DISCUSSION

In the present study, the prevalence of Chlamydia trachomatis infection in cases and control was found to be 22% and 4% respectively by ELISA on blood with significant difference in both. Similarly, prevalence by urinary PCR in cases and control was 17% and 0% in cases and control respectively with a statistically significant difference. These results report strong correlation of Chlamydia trachomatis infection with recurrent pregnancy loss.

Sociodemographic characteristics in present study were comparable to other previous studies. Mean age of cases and control were comparable in the present study and similar was found in previous studies. Ahmadi et al, in their study had cases with age range between 19-43 years and mean age was 29.6 years and in control group age range was 19-42 years and mean age was 27.8 years.⁴ Aliyu et al observed that majority of women in cases with miscarriage history and control group were in age >25 years with 28.9% women in cases had age range 25-39 years.¹⁵

In present study there was no significant difference in BMI of cases and control. Similar findings were reported by Horne et al, as they observed mean BMI of cases and

control were 25.6 kg/m² and 26.0 Kg/m² respectively which was not significantly (p value -0.55).¹⁴

In the present study all women were from urban region and majority of women in both cases and controls were 8th standard educated. Ahmadi et al similarly had all women residing in urban areas with no significant difference in socioeconomic status of cases and controls and majority of cases had only primary education.^{5,4}

In the present study, symptoms of infection either in present or in past were 36% in cases and 12% in control which were not significant. Similarly, Ahmadi et al also observed that history of vaginal infection in cases and control was not significant, and was 10.1% and 4.6% respectively.⁴

In present study, 63% cases had history of miscarriage in first trimester. Gutierrez et al similarly observed that mean gestational age of abortion in cases was 9.46 weeks.¹¹ Some previous studies depicted association of Chlamydia trachomatis with miscarriage and some failed to correlate this association.

Gutierrez et al, found significant association between miscarriage and Chlamydia trachomatis infection. They detected C. trachomatis positive PCR in 34% of women

with early abortions and 4.7% women in control group with no miscarriage which was significant with p value 0.002.¹¹

Ahmadi et al, also detected similar results as they found prevalence of *C. trachomatis* using PCR to be 22.9% in cases with history of recurrent pregnancy loss, compared to 11.9% in control group with normal pregnancy had a significant difference (p value- 0.031).⁴

Some studies failed to detect association of Chlamydia trachomatis with recurrent pregnancy loss by ELISA.¹⁶ Horne et al, observed that using Pgp3 antibodies ELISA, Chlamydia trachomatis positivity rate was 28% in cases with miscarriage and 25.9% in control. The difference was not significant.¹⁴ Aliyu et al, observed no significant association of Chlamydia trachomatis infection with spontaneous miscarriage by using ELISA.¹⁵ Bagheri et al, observed no significant difference in anti *C. trachomatis* IgG prevalence in cases with miscarriage (15.2%) versus control group (7.3%).¹² But they observed that PCR results showed high rate of Chlamydia trachomatis infection in miscarriage group compared to control group (11.3% versus 0%, p=0.007). They documented a strong association between molecular evidence of *C. trachomatis* infection and miscarriage.¹²

One of the limitation of our study was that we didn't exclude other infectious agents which can cause recurrent pregnancy loss example- CMV, parvovirus, Mycoplasma, Coxiella Burnetti. Another limitation was we didn't exclude chromosomal anomalies by investigations.

CONCLUSION

Present study compared prevalence of Chlamydia trachomatis infection in women with and without recurrent pregnancy loss and it was observed that prevalence of *C. trachomatis* infection was significantly higher in study group with history of recurrent pregnancy loss in comparison to control with no history of miscarriage. We found that there is significant association of *C. trachomatis* infection with recurrent pregnancy loss. Hence, screening for *C. trachomatis* infection can be beneficial in pregnant women with bad obstetrics outcomes.

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