

Prevalence of *Chlamydia trachomatis* and Other Bacterial Infections in Pelvic Inflammatory Disease - A Cross Sectional Study

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ABSTRACT

BACKGROUND

Pelvic inflammatory disease is a spectrum of diseases affecting the female upper genital tract. It is a poly microbial infection and *Chlamydia trachomatis* is the most common causative agent. We have undertaken this study with the objective of determining the prevalence of *Chlamydia trachomatis* infection in pelvic inflammatory disease (PID).

METHODS

This cross-sectional study consisted of 100 non-pregnant women with clinical features of PID. Endocervical, vaginal swabs and blood samples were collected from them. Polymerase-chain-reaction test (PCR) and enzyme-linked immunosorbent assay (ELISA) for chlamydial detection and Gram's staining & culture for other pathogens were performed. Chi-square and Fisher's exact tests were used to study association between the clinical features and *Chlamydia trachomatis* positivity.

RESULTS

The prevalence rate of chlamydia was 15.1 %. The other organisms isolated were candida species in 12.9 % and *Escherichia coli* in 5.3 % of the study population. There was no association between the various demographic variables, menstrual problems, fever, urinary symptoms, history of infertility, abortions, instrumentations and the presence of *Chlamydia trachomatis*. A significant association was found between the lower abdominal pain which increased during menstruation ($P = 0.013$) and the two clinical signs, cervical motion tenderness and uterine tenderness ($P = 0.001$) with the presence of *Chlamydia trachomatis* and other cervical infections.

CONCLUSIONS

The prevalence rate of *Chlamydia trachomatis* in pelvic inflammatory disease was 15 % and other organisms isolated from the cervical swabs were candida species and *Escherichia coli*.

KEY WORDS

Pelvic Inflammatory Diseases, *Chlamydia trachomatis*

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BACKGROUND

Pelvic inflammatory disease (PID) is an infection affecting the female upper genital tract and is most common among the sexually active women of reproductive age group.¹ The microorganisms from vagina or cervix ascend to the uterus, fallopian tubes or adjacent structures causing endometritis, salpingitis, tubo-ovarian abscess or peritonitis which can result in irreversible damage to these structures. PID if not treated can lead to serious long-term problems like chronic pelvic pain, infertility, ectopic pregnancy etc. Hence, a low threshold is recommended for initiating the empirical treatment in PID.

PID is a poly microbial infection with multiple microbial aetiologies like *Chlamydia trachomatis*, *Neisseria gonorrhoea*, candida species, trichomonas species, bacterial vaginosis, *Staphylococcus aureus*, streptococcus species, *Escherichia coli*, pseudomonas species, klebsiella species, proteus species, *Gardnerella vaginalis*, etc. *Chlamydia trachomatis* is one of the leading causes of pelvic inflammatory disease.

We have undertaken this study to determine the prevalence of *Chlamydia trachomatis* and other bacterial infections in pelvic inflammatory disease through cervical swab culture and serology.

METHODS

This cross-sectional study was conducted in the Department of Obstetrics & Gynaecology in collaboration with the Department of Microbiology, JIPMER, Pondicherry from 2014 - 2016. The inclusion criteria were non-pregnant sexually active women more than 18 years of the age who attended Gynaecology OPD with any one of the following symptoms: lower abdominal pain, white discharge per vaginum, pain in upper right abdomen, abnormal menstrual bleeding, fever and chills, painful micturition, nausea, vomiting, dyspareunia and with presence of any one of the following signs of cervical motion tenderness, uterine tenderness or adnexal tenderness. Pregnant, post-menopausal, women who have undergone hysterectomy with or without bilateral salpingo-oophorectomy and women with gynaecological malignancies were excluded from the study.

Brief Procedure

A total of 100 women were recruited as per the inclusion criteria. After obtaining written informed consent, detailed history including age, demographic profile, symptomatology, menstrual history, history of promiscuity, prior history of PID, prior history of curettage, hysterolaparoscopy and induced abortions were recorded. In general examination, anaemia status, nourishment, body mass index (BMI) was noted. In bimanual examination, the clinical signs like forniceal tenderness, cervical motion tenderness and uterine tenderness were noted. 2 endocervical swabs for chlamydia detection with PCR, 2 endocervical and vaginal swabs for gonococcal and other organism's detection and 5 ml of blood for the serological tests - IgG and IgM ELISA for chlamydia detection were collected from all the recruited population.

Presence of at least 4 - 5 epithelial cells per high power field in microscopy was established before storing the

endocervical swab material at - 20°C for chlamydial PCR until use.

The endocervical and vaginal swabs were inoculated on blood agar, MacConkey's agar and chocolate agar media and endocervical swab was additionally inoculated on Thayer-Martin agar medium. Identification of isolates was based on macroscopical examination of growth colonies, microscopical studies of isolates based on Gram's staining reaction and biochemical characterisation.

Yeast was identified by Gram's stain. Candida species was identified by conventional tests like germ tube test, morphology on corn meal agar, sugar fermentation and assimilation tests.

Polymerase Chain Reaction (PCR) for Chlamydia

DNA was extracted using QIAamp DNA extraction kit, which were then amplified and analysed by 2 % agarose gel electrophoresis and illuminated via UV light in gel documentation machine. *Chlamydia trachomatis* infection was detected by the presence of 517 bp band. Serum was separated and stored at - 20°C until use. It was used to detect IgG and IgM antibodies against *Chlamydia trachomatis* by ELISA kits.

Statistical Analysis

Chi-square and Fisher's exact tests were used to study associations between *Chlamydia trachomatis* positivity and socio-demographic, behavioural and clinical variables. A P-value of < 0.05 was considered statistically significant. All data was analysed using the Statistical Package for Social Sciences (SPSS) version 20.0 PC software.

RESULTS

A total of ninety-three samples were analysed finally (7 samples were rejected due to technical faults). The mean age of women in our study was 31.9 ± 5 years (range 22 - 43 years), with PID being more prevalent in women greater than 30 years. Of the 93 women, 63 belonged to the rural and 30 to the urban population. The majority of the women had completed their secondary education amounting to 52.7 % of the study population according to International Standard Classification of Education. On classifying women into different socio-economic groups, the majority of women belonged to middle class accounting to around 70 %. Around 65 % of the women had normal BMI. About 69 % women had normal menstrual cycles, while 19.4 % had oligomenorrhoea, 12 % had polymenorrhoea.

Symptomatology

Excessive white discharge per vaginum was present in 37.6 %, foul-smelling discharge in 39.8 % and itching in 45.2 % of women. Around 63.4 % of women had increased abdominal pain during menstruation, 85 % had dyspareunia, 28 % had increased frequency of micturition and 25.8 % had dysuria.

Out of the 11 patients who presented with fever, 5 women had high grade fever associated with chills and rigors. 18 % of women had a recurrence of PID. 11.8 % had primary infertility and 4.3 % had secondary infertility. About 36.6 % of women had a history of either one (31.2 %) or two (5.4 %) abortions. Out of 93 women, 38 women had undergone dilatation and curettage either for abortion or infertility workup and 10.8 % of women had history of hysteroscopy. History of extramarital contact was present in 12.9 % of women.

Among the study population, 60 % of women were anaemic, 23 % were diabetic and 5 % were using immunosuppressant drugs (steroids) for conditions like bronchial asthma, arthritis etc.

Clinical Signs

Among the women in our study population almost all women had forniceal tenderness. Cervical motion tenderness was present in 87.1 % and uterine tenderness in 36.6 % of patients.

Prevalence of Chlamydia in Women with PID

In this study, women were considered to have chlamydial PID, when any one the three tests were positive. (1) Positive cervical swab chlamydial PCR, (2) Positive IgM in ELISA, (3) Positive IgG in ELISA. Among the women diagnosed to have PID clinically, 15.1 % had *Chlamydia trachomatis* infection. Acute chlamydial infections identified with chlamydial PCR and IgM ELISA were 6.45 % and chronic or recurrent chlamydial infections with IgG ELISA were 8.6 %. *Escherichia coli* were present in 5.3 % and candida in 12.9 % of the study population.

The organisms isolated from the vaginal secretions were candida species, *Staphylococcus aureus*, *Escherichia coli* and klebsiella species. Among the candidal infections, the common candidal species were *C. glabrata* (14) *C. tropicalis* (7) *C. krusei*, (2) *C. albicans*, (2) and *C. parapsilosis* (1)

Relationship of various demographic variables and prevalence of *Chlamydia trachomatis* and other bacterial infections in PID of the 93 women, 15 % were tested positive for chlamydial infections, out of which 6.5 % were present in 25 - 30 years age group and 5.4 % in 30 - 35 years group. There was no significant association found between the age groups and presence of chlamydial infections or other cervical organisms in PID (the P-value was 0.25). No statistical difference was found between the prevalence of *Chlamydia trachomatis* or other cervical organisms and the residence status (urban and rural population), socioeconomic status and educational status.

Our study showed that chlamydial infection was present in 13 % of the married women, 2.2 % of the divorcees and the widows. Analysis showed no significant difference in the prevalence of chlamydia and other cervical infections and their marital status.

Chlamydia trachomatis was present in 5 % of patients with oligomenorrhoea, 27 % with polymenorrhoea, 21 % with dysmenorrhoea, 25 % with inter menstrual bleed and 27 % with postcoital bleed. Non-chlamydial infections were present in 11 % patients with oligomenorrhoea, 18 % with polymenorrhoea, 19 % with dysmenorrhoea, 12 % with intermenstrual bleed and 18 % with postcoital bleed. There

was no significant relation between the prevalence of chlamydia or other cervical organism and menstrual cycle abnormalities.

All the patients included in this study had white discharge per vaginum and lower abdominal pain as one of their presenting complaints. We didn't find any significant relation between the prevalence of chlamydial and other cervical infections and the various characteristics of white discharge per vaginum like quantity, foul smell, itching etc.

Pain Abdomen	Chlamydial Infections N = 14 N (%)	Other Cervical Organisms N = 17 N (%)	Clinical PID with Negative Microbiology N = 62 N (%)
Increased with menstruation N = 59	11 (11.8)	15 (16.1)	33 (35.4)
No association with menstruation N = 34	3 (3.2)	2 (2.2)	29 (31.1)

Table 1. Relationship of Lower Abdominal Pain with Menstruation and Its Association with Prevalence of Chlamydia trachomatis

The P-value - 0.013

In this study, a significant association was noted between the lower abdominal pain which increased during menstruation with that of the prevalence of chlamydial and other non-chlamydial infections in PID, though lower abdominal pain is not specific to any particular organism.

Dyspareunia was one of the most common symptoms among the women with the clinical diagnosis of PID. *Chlamydia trachomatis* was present in 12.9 % of women with dyspareunia, 15.3 % of women with increased frequency of micturition and 12.5 % of women with dysuria. But there was no association noted between dyspareunia and urinary symptoms and the prevalence of chlamydia and other cervical infections.

During our study period, 11 women presented with fever out of which 5 women had high-grade fever with chills and rigors. Of them, five had history of recent abortions and dilatation and curettage. One patient had undergone hysterosalpingography as a part of infertility work up. One patient had a recurrence of PID, where the previous episode had been treated with azithromycin. These patients were admitted and given inpatient treatment with ceftriaxone intravenous for a week till the acute episode subsided and discharged with doxycycline and metronidazole for 2 weeks. Chlamydia PCR and IgM ELISA was positive in 1 woman, IgG ELISA in 2 women.

Cervical swab culture was positive for *Escherichia Coli* in 3 women and candida species in 1 woman. Vaginal swab culture was positive for candida species in 5 women, *Staphylococcus aureus* in 2 women and *Escherichia coli* in 1 woman. The prevalence of *Chlamydia trachomatis* or other cervical infections and the fever had no statistically significant association.

In this study, 18.3 % had a previous history of PID. In these women with recurrent PID, IgG ELISA for chlamydia was positive in 5 women and candida was present in one woman.

	H / O Infertility		Primary Infertility N = 5	Secondary Infertility N = 4
	Present	Absent		
Chlamydia N = 14	3 (3.2 %)	11 (11.8 %)	0	3 (3.2 %)
Other cervical infections N = 17	5 (5.4 %)	12 (12.9 %)	4 (4.3 %)	1 (1.1 %)

Table 2. Relationship of Infertility and Prevalence of Chlamydia trachomatis and Other Cervical Infections in PID

Chlamydial infection was found to be common among women with secondary infertility than those with primary infertility, though it was not statistically significant. The prevalence of chlamydia and other cervical infections were quite similar in both nulliparous and multiparous women.

		Chlamydial Infections N = 14 N (%)	Other Cervical Organisms N = 17 N (%)	Clinical PID with Negative Microbiology N = 62 N (%)	P Value
History of abortions	Absent N = 59	7 (7.5)	13 (14)	39 (41.9)	0.31
	Present N = 34	7 (7.5)	4 (4.3)	23 (24.7)	
History of dilatation and curettage	Absent N = 55	6 (6.5)	12 (12.9)	37 (39.7)	0.29
	Present N = 38	8 (8.6)	5 (5.4)	25 (26.9)	
History of hysteroscopy	Absent N = 82	10 (10.8)	16 (17.2)	56 (60.2)	0.10
	Present N = 11	4 (4.3)	1 (1.1)	6 (6.5)	

Table 3. Relationship between History of Abortions and Instrumentations and Prevalence of Chlamydia trachomatis and Other Cervical Infections in PID

In this study, no significant association was found between number of abortions, history of dilatation & curettage and hysteroscopy and the prevalence of chlamydia and other cervical infections causing PID.

Out of the 12.9 % of women with the history of an extramarital relationship, chlamydia was present in 1.1 % and other cervical infections in 4.3 %. The history of an extramarital affair had no significant relationship to the prevalence of chlamydia and other cervical infections.

We have found that the prevalence of chlamydia and other cervical infections in PID are not related to the anaemia, obesity, diabetes or nutritional status.

Clinical Signs

Chlamydia was present in 15 % of the women with forniceal and cervical motion tenderness and 10.8 % of women with uterine tenderness. Similarly, other cervical infections like candida species, *Escherichia coli* were present in 18.2 % of women with forniceal and cervical motion tenderness and 9.7 % of women with uterine tenderness.

		Chlamydial Infections N = 14 N (%)	Other Cervical Organisms N = 17 N (%)	Clinical PID with Negative Microbiology N = 62 N (%)
Uterine tenderness	Absent N = 59	4 (4.3)	8 (8.6)	47 (50.5)
	Present N = 34	10 (10.8)	9 (9.7)	15 (16.1)

Table 4. Relationship between the Uterine Tenderness and Prevalence of Chlamydia trachomatis and Other Cervical Infections

The P-value is 0.001

Of the three clinical signs, uterine tenderness was significantly associated with the prevalence of *Chlamydia trachomatis* (P-value 0.000) and other non-chlamydial infections (P-value 0.01) in the clinically diagnosed PID.

DISCUSSION

The prevalence of chlamydial infection in this study was found to be 15.1 %. The prevalence of chlamydia in our study is

comparable to that of the prevalence (22.7 %) noted in the study by Ravindran et al.¹ Another study by Malhotra et al. in Delhi, 2008 reported the prevalence of chlamydia in PID as 23 % in women attending Gynaecology OPD and 19.9 % in women attending STD OPD.² The non-chlamydial organisms identified were *Escherichia coli* (5.3 %) and candida species (12.9 %). But the study by Mohapatra et al. found that the prevalence rate of chlamydia was 26.6 %, gonorrhoea 33 %, *Staphylococcus aureus* 15 %, streptococcus species 8 %, *Escherichia coli* 4 %, trichomonas species 3.3 %, candida species 6 %, mycobacteria species 4 % and ureaplasma species 12 %.³ The organisms detected in Spencer et al. study were streptococcus species (11 %), staphylococcus species (16 %), *Escherichia coli* (10 %), proteus species (3 %) and pseudomonas species (4 %).⁴ This difference in microbiology can be either because the sample size was very small to detect the prevalence of all bacterial infections or the prevalence of *Neisseria gonorrhoea* as the causative organism for PID has decreased, but the candidal infections were present in a significant number of patients. Hence it can be suggested that the empirical treatment for PID can include even an antifungal. A study in Tamilnadu also showed that genital chlamydial infections were present only in 3 % and candida infections in 10 %.⁵

The organisms isolated from the vaginal secretions were candida species, *Staphylococcus aureus*, *Escherichia coli* and klebsiella species. In a study by Mohapatra et al. organisms isolated from vaginal swabs were candida species (9.4 %), bacterial vaginosis (7.8 %), trichomonas species (1 %), *Neisseria gonorrhoea* (0.7 %).³

Nearly 87 % of women in our study were between 25 - 40 years of age. Among the chlamydial positive PID, 50 % of women were less than 30 years and 50 % greater than 30 years of age. This is comparable with the study done by Ravindran et al. where most of the patients with PID (88.6 %) were within the 20 - 40 years age group and 70 % of the chlamydia positive PID patients were in the 20 - 40 years age group.¹ Likewise in the study by Mohapatra et al., chlamydial infections were more prevalent in 20 - 30 years age group.³ Young people are more prone to PID as they are sexually very active, may have multiple sexual partners and increased host susceptibility as the chlamydial antibodies are in lower concentrations, due to larger cervical ectopy and increased permeability of cervical mucus in younger age women.

Around 70 % of women with PID belong to middle class II and III of socioeconomic status. Chlamydia was present in 3.5 % of women belonging to class IV, 4.3 % in class III, 5.4 % in class II. In a study by Ravindran et al., 63.6 % of PID cases were in the lower social classes IV and V and the majority (60 %) of the chlamydia positive patients belonged to the lower social classes IV and V.¹ Increased prevalence of PID in the lower and middle socio-economic group may be due to the poor local hygiene and poor awareness about the prevention of sexually transmitted disease.

In our study chlamydial infections were present in 15 % of the married women and 25 % of the divorcees and widows. In the study by Ravindran et al., 17.5 % of the married, 100 % of widows and 50 % of single women were tested positive for chlamydial infections.¹ Likewise in a study by Simms et al. chlamydial infection was present in 8 % of married women, 22 % of cohabiting women, 13 % in widowed / separated / divorcee and 57 % in unmarried women who were sexually active.⁶ Thus it can be concluded that the people in polygamous

relationships have increased risk of acquiring chlamydial infections.

Chlamydia was present in 15.6 % of women with normal cycles, 13.7 % of women with menstrual cycle irregularities. In the study by Prathiba et al. the prevalence of chlamydia in patients with menstrual irregularities was studied, which showed a 75 % prevalence rate of chlamydia. This contrary in results can be explained, because the aim of our study was to estimate the prevalence of chlamydia in women with PID but the study by Prathiba et al. estimated the prevalence of chlamydia in women with menstrual irregularities.⁷

Chlamydia was present in 11 % of women with dysmenorrhoea, 4 % with intermenstrual bleed and 6 % with postcoital bleed. This is concurrence with the study by Chacko et al. wherein 17 % of the women presented with multiple symptoms like menstrual irregularities, vaginal discharge and pain abdomen.⁸

Chlamydia was present in about 23 % of women with excessive vaginal discharge. This is in comparison with a study done by Ravindran et al., who reported chlamydial positivity in 40 % of patients with foul-smelling vaginal discharge.¹ In the study by Simms et al., chlamydial infection was present in 53.3 % of patients with excessive vaginal discharge.⁶

Around 63.4 % of our women reported increased abdominal pain during first 5 - 7 days of the menstrual cycle. Chlamydia was found in 19 % of the women with such complaints. A significant association was found between the lower abdominal pain which increases during menstruation and the presence of chlamydial and other non-chlamydial infections in PID. This finding can be compared to that quoted in the study by Machado et al., where chlamydia was found in 10 % of PID with increased abdominal pain during menstruation.⁹ This association can be explained by the fact that the peristaltic contractions of the uterus move fluid cephalad, facilitating the ascend of the organisms from the lower genital tract to uterus, fallopian tubes etc.

Dyspareunia was reported by 85 % of women and chlamydia was positive in 15 %. This finding is in comparison to the study done by Ravindran et al. where dyspareunia was present in 72.7 % of PID patients but chlamydia was positive in 20 %.¹

Chlamydia was present in 27 % of women with urinary symptoms. Simms et al. reported that 35 % of the PID patients presented with urinary symptoms.⁶ Urinary symptoms were common among women with PID because most of the organisms causing PID especially chlamydia can also cause urethritis.

Chlamydia was present in 27 % of women with fever. In the study done by Ravindran et al., fever was present in 39 % of the PID patients while none of the chlamydia positive patients had fever.¹ In the study by Simms et al., fever was the presenting complaint in 47 % of PID population.⁶

In our study, about 16 % of women had history of infertility with secondary infertility being more common than primary infertility. Ravindran et al. reported that among the patients with PID 45.5 % had subfertility and 20 % had primary infertility.¹ Secondary infertility is found to be more common than primary infertility in PID because there might be increased chances for the ascending infections to reach the upper genital tract following delivery or dilatation and curettage done for spontaneous or induced abortions.

About 36.6 % of women with PID had history of either one or two abortions. History of dilatation and curettage was present in 40.9 %, history of hysteroscopy was noted in 10.8 % of women. During these procedures there is high chance for the lower genital tract infections to ascend to the endometrium and tubes causing PID. In a study by Simms et al., PID was present in 29 % of women with history of abortions.⁶ In a study by Gogate et al., hysterosalpingography, dilatation and curettage (D & C), laparotomy and laparoscopic procedures were significantly associated with PID.¹⁰

Clinical Signs

Almost all the women in our study population had forniceal tenderness on bimanual examination. Cervical motion tenderness was present in 87.1 % and uterine tenderness in 36.6 % of patients. Chlamydia was present in 15.05 % of the women with forniceal and cervical motion tenderness and 10.8 % of women with uterine tenderness. In the study by Simms et al., the pelvic organ tenderness on bimanual examination was present in 99 % of the patients.⁶ Eggert et al. study showed that PID was diagnosed in 49 % of women with both uterine and forniceal tenderness and in 20 % with uterine tenderness alone.¹¹

CONCLUSIONS

Prevalence rate of *Chlamydia trachomatis* in pelvic inflammatory disease was 15 % and the other organisms isolated from the cervical swabs were candida species and *Escherichia coli* in the clinically diagnosed PID. Increase in lower abdominal pain during menstruation and uterine tenderness were significantly associated with the presence of chlamydial infections.

Data sharing statement provided by the authors is available with the full text of this article at jemds.com.

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