The etiology of pelvic inflammatory disease with special reference to Chlamydia trachomatis

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Abstract

Introduction: The study was carried out in a tertiary care hospital in India. 178 patients with Pelvic Inflammatory Disease (PID) were considered in the study.

Materials and Methods: IgG ELISA was carried out in all the patients for Chlamydia trachomatis (CT). Cervical swabs were taken and were stained by gram stain and giemsa stain. Also culture of the cervical swabs was carried out. 100 healthy controls were taken.

Results and Discussion: Total number of seropositive cases because of Chlamydia Trachomatis in Pelvic Inflammatory Disease cases were found to be 26.14% which was statistically significant when compared to controls which was 11%. Also the study of the etiology of Pelvic Inflammatory Disease was carried out to study the various other causative organisms of Pelvic Inflammatory Disease.

Conclusion: Chlamydia Trachomatis is an important causative agent of sexually transmitted infections (STI) apart from staphylococcus aureus, Escherichia Coli and Klebsiella pnuemonia.

Keywords: Chlamydia trachomatis, Pelvic inflammatory disease, IgG ELISA, cervical swabs, Giemsa stain, Sexually transmitted infections.

Introduction

Many different bacteria can cause PID. The acute clinical syndrome is most often attributed to ascending spread of microorganisms from cervix to the endometrium, fallopian tubes and contiguous structures. (Eschenbach et al 1975). Chlamydia trachomatis is of specific importance as it is an important organism causing PID. The main symptom of PID is persistent moderate to severe lower abdominal pain. Other symptoms include Increased or abnormal vaginal discharge, with or without odour, bleeding between periods and/or irregular periods, difficulty conceiving (infertility), painful menstruation, with symptoms that worsen with consecutive periods, frequent, painful urination, fever, pain in the upper right abdomen and painful bowel movements. Many women, however, do not have symptoms and are unaware that PID is developing. This is especially common in PID resulting from chlamydial infection. In CT infection, women develop acute urethral syndrome, bartholinitis, mucopurulant cervicitis, endometritis, salpingitis, conjunctivitis and perihepatitis. CT infection leading to PID may result in various complications like infertility, preterm labour, ectopic pregnancy due to salpingitis, perinatal morbidity and post-partum fever. (Chow et al 1974).[13] Chlamydia are non-motile intracellular pathogens. These intracellular pathogens are detected by light or fluorescent microscopy. Chlamydia antibodies in the serum are detected by radioimmunoassay, ELISA and complement fixation test. Antigens present in cervical swab are detected by microimmunofluorescence, countercurrent immunoelectrophoresis, ELISA and radioimmunoassay. For the diagnosis of Chlamydia trachomatis its isolation and culture remains the gold standard. Culture techniques for CT are very expensive and labour intensive. In other words culture of Chlamydia trachomatis requires a complete tissue culture set up which is not available in most hospitals.[6]

Osborne et al (1989) opined that almost in 80% of patients with pelvic inflammatory disease, humoral antibody to Chlamydia trachomatis is present and in considerable percentage a high titre or even a rise in titre is obtained.[56] Thus detection of antigens or antibodies against Chlamydia trachomatis has been recommended as an alternative procedure for the diagnosis. The detection of Chlamydia trachomatis by ELISA is believed to be simple, easy and reliable technique with 100% sensitivity and about 98.5% specificity.

The various other organisms causing PID are Trichomonas vaginalis, Gardnerella vaginalis, Candida albicans, N. gonorrhoeae, Mycoplasma, Mycobacterium tuberculosis. Finding the causative agent of PID is important as its incidence has been increasing in women of the reproductive age group.[14]

In a study by Prabhakar et al (1989), G. vaginalis was found to be an important etiological agent in PID diagnosed in gram film by the presence of clue cells.[61]

Saini et al (2003) reported 73.6% aerobic isolates from endocervical swab culture consisting of E. coli 33.2%, K. pneumoniae 9.5%, Ps. aeruginosa 4.7%, Staph. aureus 14.2% and enterococci 7.1%.[66] Chow et al (1974) isolated 78% aerobic bacteria in cervical swab cultures which mainly constituted streptococci 22%, Staph. aureus 22%, E. coli 7%, proteus 3% and N. gonorrhoeae 13%.[13]

Apart from tubal obstruction and infertility, the risk of developing inclusion conjunctivitis and pneumonia to infants passing although birth canal exists. The various other organisms causing PID are Trichomonas vaginalis, Gardnerella vaginalis, Candida albicans, N. gonorrhoeae, Mycoplasma, Mycobacterium tuberculosis.

The present study was undertaken with following aims & objectives:

1. To find out the microbial profile of the PID cases in sexually active women attending Gynecology Out Patient Department.
2. To study seropositivity for CT antibody (IgM) in cases with PID.
3. To find out the seropositivity of CT antibody (IgM) in relation to risk factors for PID.

Materials and Methods

Patients having PID were selected who had symptoms like leucorrhoea, adnexal pain, fever, cervical motion tenderness and low back pain. Endocervical swab was taken. Also the serum sample of these patients were taken for detecting IgM antibody by ELISA.

The endocervical swabs were transported to the laboratory by amies medium. Also smears were prepared from the swabs and stained by giemsa stain and gram stain. Also culture was done on blood agar, MacConkey agar and chocolate agar.

Giemsa staining was done for inclusion bodies of CT, wet film for trophozoits of T. vaginalis & yeast cells and gram staining for clue cells suggestive of G. vaginalis and C.albicans.

Methods

Microscopy:

1. Giemsa staining for inclusion bodies of Chlamydia trachomatis.
   Smear with minimum 100 epithelial cells was taken as satisfactory. Cloak shaped intracytoplasmic basophilic inclusion bodies seen.[22] (Doris et al 1989)
2. Gram staining for Clue cells and microorganisms.
   Clue cells are squamous epithelial cells coated with large no. of gardnerella bacilli.[16] (Collee et al, 1989)
3. Wet film for trophozoites of Trichomonas vaginalis.

Giemsa Stain:[23]

Smear was fixed in methyl alcohol for 3 min.

1. Mixture of 1 part of giemsa stain and 10 parts of buffer solution (pH 7.0) was kept on the smear for 1 hour.
2. The smear was washed with buffer solution allowing preparation to differentiate for 30 min.
3. Smear was blotted and allowed to dry in air.

Gram Staining: Performed as per Standard laboratory technique.[16]

I. Cervical swab collected in Amies transport medium was inoculated on Chocolate agar, Blood agar, McConkey medium and incubated at 37^0 C in presence of 10% CO_2. Colony morphology was studied and organisms were identified by Standard bacteriology techniques.[16] (Collee et al, 1989)
II. Antibiotic susceptibility of bacterial isolate was done by Kirby-Bauer disk diffusion method (Scott et al 1989) and selection of antibiotics was done as per NCCLS guidelines.[76]
III. Screening for Chlamydia trachomatis (IgG) antibody-

ELISA Test: (Nova Tec, Chlamydia trachomatis IgG ELISA, manufactured by Nova Tec Immunodiagnostica, Germany)

Observation

Cases of PID were subjected to endocervical swab microscopy, culture and Chlamydia antibody (IgG) detection by ELISA. Healthy age matched controls were included to detect the seropositivity for Chlamydia antibody (IgG).

Table 1: Microscopy and PID (n=178)

Microscopy	Number (%)
Bacteria	78 (43.82)
Candida	4 (2.25%)
Clue Cells	8 (4.49%)
Inclusion bodies of Chlamydia	7 (3.33%)
Trichomonas vaginalis	5 (2.80%)

Different bacterial morphological forms in the gram staining were seen in 78 (43.82%) cases. Clue Cells indicating Gardnerella vaginalis were seen in 8(4.49%) out of the total 178 cases, whereas cloak shaped inclusion bodies of Chlamydia trachomatis were found in 7 (3.33%) cases. Trichomonas vaginalis was found in 5(2.80%) cases. Budding yeast cells were seen in 4(2.25%) cases.

Table 2: Cervical swab culture and PID (n=178)

Microbial isolate	No. of isolates (%)
Microbial growth	111 (62.35%)
Staphylococcus aureus	30 (16.85%)
E. coli	20 (11.23%)
N. gonorrhoeae	10 (5.62%)
Klebsiella pneumoniae	10 (5.62%)
Pseudomonas aeruginosa	9 (5.06%)
Enterococcus spp.	7 (3.33%)
Beta hemolytic Streptococci	6 (3.37%)
Proteus spp.	4 (2.25%)
E. Coli + Klebsiella pneumoniae	3 (1.68%)
Klebsiella pneumoniae + Pseudomonas aeruginosa	1 (0.56%)
E. coli + Proteus	1 (0.56%)
Candida albicans	10 (5.62%)
Culture negative	67(37.65%)
Total	178 (100%)

Cervical swabs from 178 clinical PID cases were cultured aerobically. Organisms were isolated from 111(62.35%) cases. Staphylococcus aureus was isolated in 30(16.85%), E. coli 20(11.23%), Klebsiella pneumoniae 10(5.62%), Pseudomonas aeruginosa 9(5.06%), Enterococci spp. 7(3.33%), beta hemolytic Streptococci 6(3.37%), Proteus spp. 4(2.25%), Gonococci 10(5.62%) and Candida albicans in 10(5.62%), Klebsiella pneumoniae+ Pseudomonas aeruginosa, E. coli +Proteus spp. were found in 1(0.56%) case each.

Table 3: Seropositivity for C. trachomatis in PID and control cases

Cases	Total No. of cases	No. of C. trachomatis seropositive cases
Clinical PID	178	47 (26.40%)
Control cases	100	11 (11.00%)

Amongst 178 total clinical PID cases, 47(26.40%) cases were Chlamydia trachomatis (IgG) seropositive, while seropositivity in control cases was 11%. The difference between PID and control cases is statistically significant ($p<0.05$).

Table 4: Age group and C. trachomatis seropositive PID cases (n=178)

Age Group	Total No. of cases of PID (%)	No. of seropositive cases
15 – 20	11 (6.18%)	3 (6.38%)
21-25	46 (25.84%)	16 (34.04%)
26 – 30	65 (36.52%)	20 (42.55%)
31 – 35	27 (15.17%)	6 (12.77%)
36 – 40	29 (16.29%)	2 (4.26%)
Total	178(100%)	47 (100%)

In the present study, seropositivity for Chlamydia antibody was maximally seen between 26-30 years of age.

Table 5: Precipitating factors in C. trachomatis seropositive and total PID cases (n=178)

Precipitating factors	Total No. of clinical PID cases	No. of seropositive cases
Post MTP	6 (3.37%)	1 (0.56%)
Post D & C	13 (7.30%)	2 (1.12%)
Post H SG	13 (7.30%)	1 (0.56%)
IUCD	25 (14.04%)	8 (4.49%)
Total	57(32.02%)	12(6.74%)

Out of the total 178 cases studied, predisposing factors were seen in 57(32.02%) PID cases. Out of 6 post MTP cases 1was seropositive. In post D&C, out of the 13 cases 2 were seropositive. Seropositivity was maximum (4.49%) in IUCD cases; 25 PID cases had IUCD in situ.

Table 6: Clinical diagnosis in PID and C. trachomatis seropositive cases. (n=178)

	No. of PID cases	No. of seropositive cases
Cervicitis	134(75.28%)^0	25(18.65%)^0
Endometritis	26(14.61%)*	12(46.15%)*
Salpingitis	16(8.98%)**	10(62.50%)**
Adnexal Mass	1(0.56%)	0(0.00%)
Cyst	1(0.56%)	0(0.00%)
Total	178(100%)	47(26.40%)

* $p<0.005$
**$p<0.005$
^{0} $p<0.005$

The 178 PID cases were distributed in different clinical groups on the basis of clinical diagnosis. Most cases were of cervicitis 134(75.28%), out of which 25(18.65%) cases were seropositive. This is statistically significant when compared with other PID cases ($p<0.05$).

Endometritis was seen in 26 (14.61%) cases of which 12(46.15%) were seropositive. Salpingitis was seen in 16 (8.98%) cases. Out of 16 cases of salpingitis 10(62.5%) were seropositive. Cervicitis and endometritis also it is statistically significant ($p<0.05$).

Results and Discussion

In the current study maximum number of cases of PID had cervicitis followed by endometritis. In this study 178 PID cases were subjected to endocervical swabs for microscopy, culture and evaluation of serostatus for Chlamydia trachomatis (IgG) antibodies.

In the present study higher prevalence of PID (62.63%) was found in the age group of 21-25 years. This age group is a sexually active and child bearing age group. Gjonnaess (1982) reported 54% PID cases in this age group.[32] Similarly higher prevalence of PID in same vulnerable age group was reported by Westrom (1980)[80] {Table 4}.

It was found that gynaecological & obstretical interference like medical termination of pregnancy (MTP), dilatation & curettage (D&C), histosalpingography (HSG) and intrauterine contraceptive device (IUCD) insertion can be a cause for microbial infection of lower genital tract. These microbes later on ascend to uterus and fallopian tube from cervix. Chronic active infection caused by microbes leads to PID (Eschenbach, 1984).[26]

In the present study, maximum PID cases were seen with IUCD i.e. 25(14.04%). Burkman et al (1981) found 22%, Saini et al (2003) 30% of PID cases with intrauterine device for contraception.[7,66] Westrom (1980) showed that women who use an IUCD are at least 4 times more likely to develop PID than nonusers {Table 5}.[60]

Eschenbach (1984) in his epidemiological study stated that significant past history like sexually transmitted disease to either partner, infertility and complaints of acute PID in past play an important role in PID.[26] Berkman and Women's health study (1981) reported 52% cases of PID with previous history of pelvic inflammatory disease.[8] In the present study 16.85% cases had similar history. Many microbial agents responsible sexually transmitted diseases (STD) are also responsible for PID. Sevgi et al (1991) found 26% cases of PID with history of STD.[71] Nancy et al (1991) reported 31% PID cases with such history.[54] In present study 2.80% PID cases had history of STD.

Infertility is a sequelae of recurrent PID (Echenbach 1984).[26] Our study noted 33(18.53%) PID cases with history of infertility. Aspock et al (1995) reported 11(36.66%) and Westrom (1987) reported 17.4% PID cases with similar history.[3,81] Nancy et al (1991) reported 31% PID cases with history of infertility.[54] Hossain et al (1988) reported 20.9% seropositivity for chlamydia trachomatis in infertile patients.[36] Chlamydia trachomatis plays an important role in causing pelvic inflammatory diseases. These infections lead to tubal obstruction causing infertility Cetin et al(1992).[10] {Table3}

In the present study, 178 PID cases were distributed on the basis of their clinical groups as cervicitis (75.28%), endometritis (14.61%) and salpingitis (8.98%). (Table 6) Lal et al (1999) noted 17.3% PID cases and Lender et al (1991) reported 97% PID cases having cervicitis.[42,43] Cleary et al (1985) indicated 20% PID cases with salpingitis while Paavonen et al (1978) reported 70% cases.[15,58]

Salpingitis is often synonymous with PID; the former term should preferably be used in visually confirmed cases only. The demonstration of the endometritis might be alternative method to identify upper genital tract infection among women who are suspected as salpingitis. Endometritis is an entity associated with PID and most likely represents an intermediate stage between cervicitis and salpingitis.[57] (Paavonen, 1985).Cleary et al (1985) indicated 67% PID cases with endometritis.[15]

In the present study, microscopic examinations of endocervical swabs were performed. Giemsa staining was done for inclusion bodies of C. trachomatis, wet film for trophozoites of T. vaginalis & yeast cells and gram staining for clue cells suggestive of G. vaginalis.

Inclusion bodies of Chlamydia were found in 7(3.33%) cases, clue cells in 8(4.49%) while trophozoites of T. vaginalis in 5(2.80%) cases of PID. {Table 1} Sheela et al (1991) reported 11.60% cases of chlamydia trachomatis inclusion bodies.[73] Clue cells were demonstrated in 51% and 8.5% PID cases by Ison et al (1982) and Gardner et al (1955) respectively.[30,38] T. vaginalis was seen in 3.1% cases of PID by Barbara et al (1986).[4]

Microbial growth was found in 62.35% of endocervical swabs cultures. Staph. aureus 16(16.85%) was the major isolate followed by E. coli 11.23%, gonococci 5.62%, K. pneumoniae 5.62%, Ps. aeruginosa 5.06%, enterococci spp 3.33%, proteus spp 2.25%, candida 5.62% and polymicrobial growth was seen in 5(2.81%) cases. {Table 2}

Saini et al (2003) reported 73.6% aerobic isolates from endocervical swab culture consisting of E. coli 33.2%, K. pneumoniae 9.5%, Ps. aeruginosa 4.7%, Staph. aureus 14.2% and enterococci 7.1%.[66] Chow et al (1974) isolated 78% aerobic bacteria in cervical swab cultures which mainly constituted streptococci 22%, Staph. aureus 22%, E. coli 7%, proteus 3% and N. gonorrhoeae 13%.[13] Choudhary et al (1996) isolated 100 bacterial isolates comprising of Staphylococci 28%, E. coli 23% and Streptococci 14%.[12]

Saini et al (2003) reported polymicrobial flora in 43.2% cases, while Chow et al (1975) reported 23.3% patients of PID with polymicrobial flora.[14,66] In the present study polymicrobial flora was observed only in 2.80% cases. Eschenbach (1984) explained that the majority of infections are caused by bacteria.[26] N. gonorrohoeae, Chlamydia trachomatis and a wide variety of aerobic and anaerobic bacteria are most frequently isolated from cervical swabs of women with PID. These organisms ascend to the uterus and fallopian tube from cervix.

Chlamydia trachomatis is one of the major pathogens responsible for PID. For the diagnosis of C. trachomatis, its isolation & culture remains the "Gold standard". Culture techniques for CT are very expensive & labour intensive i.e. it requires complete tissue culture set up which is not available in most of the hospitals. So in the present study an attempt is made to detect the prevalence of CT in PID cases serologically.

Amongst 178 PID cases, 26.40% were C. trachomatis IgG seropositive while seropositivity in control group was 11%. The difference between seropositivity of clinical PID cases & control group is statistically significant($p<0.05$). {Table 3}

Treharne et al (1979) reported 62% seroprevalence of IgG C. trachomatis antibody in PID cases.[79] Similarly Mardh et al (1981) reported 37%, Gump et al (1983) 38.5%, Sheela et al (1991) 11.6% seropositivity in PID cases.[34,73,45]

Gynaecological & Obstretics procedures and IUCD are responsible for fourfold rise in prevalence of PID cases (Saini et al, 2003).[66] In the present study, the seropositivity for C. trachomatis in PID cases with IUCD was seen in 8 (4.49%) cases {Table 12}. Westrom (1980) attributed increased incidence of PID to IUCD & legal abortions. Women who used IUCD for contraception are at least 2 to 4 times at more risk to develop PID. Guderian et al (1986) reported 47 seropositive cases with IUCD out of 176 cases of PID.

Infertility is a common problem in community. Acute PID if untreated leads to serious complications like tubal blockage and multiple adhesive lesions which cause infertility. (Eschenbach 1984)[26]

In the present study, 33 cases of infertility with PID were reported. {Table 13} Out of these 33 cases 6 (18.18%) were seropositive to CT. Cetin et al (1992) reported 11.6% seropositivity while Hossain et al (1988) reported 20.9% seropositivity in infertility cases.[10,36]

C. trachomatis plays an important role in sexually transmitted diseases. In this study, 2 out of 5(40%) PID patients having history of STD to either partner were seropositive to C. trachomatis IgG antibody. Eckert et al (1997) reported 22.5% chlamydial seropositivity in STD cases.[25]

Eschenbach (1984) in his study reported increase in chlamydial infection in the patients of PID with a history of similar complaints of acute PID in past.[84] In the present study, 8 out of 30 (26.66%) were seropositive and had the history of PID in the past.

In present study, 62.5% cases of salpingitis were seropositive for Chlamydia trachomatis. Paavonen et al (1979) reported 26% seropositivity in cases of salpingitis.[59] Treharne et al (1979) found seropositivity for CT IgG antibody in 62% of acute salpingitis cases.[79] In the present study, 18.65% seropositive cases had cervicitis and 46.15% seropositive cases had endometritis. {Table 6}

Thus, it is observed in the present study that PID is caused by various microbial pathogens. CT is a predominant cause of PID. Intrauterine device is a common method of contraception being used by many women worldwide. The association of C. trachomatis infection in intrauterine device users is found to be higher compared to other precipitating factors leading to pelvic inflammatory disease. Thus, routine monitoring of IUCD user for C. trachomatis should be mandatory.

CT infection leading to PID, especially salpingitis can be a major cause of infertility. This was also evident in the present study. Hence early detection of C. trachomatis infection in the reproductive age group and its treatment can prevent pelvic inflammatory disease & its complications including infertility.

In the present study higher prevalence of PID (62.63%) was found in the age group of 21-25 years. This age group is a sexually active and child bearing age group. Gjonnaess (1982) reported 54% PID cases in this age group.[32] Similarly higher prevalence of PID in same vulnerable age group was reported by Westrom (1980).[80]

In the present study, maximum PID seropositive cases were seen with Intra Uterine Contraceptive Device users.

Significant past history like sexually transmitted disease to either partner, infertility and complaints of acute PID in past play an important role in PID. In the present study, 178 PID cases were distributed on the basis of their clinical groups as cervicitis (75.28%), endometritis (14.61%) and salpingitis (8.98%).

In the present study polymicrobial flora was observed only in 2.80% cases. Apart from CT Staphylococcus aureus and Escherichia coli were found to be the common causative organisms of PID.

Conclusion

For the diagnosis of CT, its isolation & culture remains the "Gold standard". Culture techniques for CT are very expensive & labour intensive and it requires complete tissue culture set up which is not available in most of the hospitals. So, in the present study an attempt is made to detect the prevalence of CT in PID cases serologically.

Thus, it is observed in the present study that PID is caused by various microbial pathogens. C. trachomatis is a predominant cause of PID. Intrauterine device is a common method of contraception being used by many women worldwide. The association of C. trachomatis infection in intrauterine device users is found to be higher compared to other precipitating factors leading to PID. Thus, routine monitoring of IUCD user for CT should be mandatory.

Conflict of Interest: None.

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