

CYTOLOGICAL DETECTION OF CHLAMYDIAL INFECTION ASSOCIATED WITH DIFFERENT GYNAECOLOGICAL DISEASES

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ABSTRACT

Chlamydia trachomatis is the most common bacterial sexually transmitted diseases (STD) and is associated with gynecological disorders like pelvic inflammatory disease (PID), infertility and habitual abortion.

The present study was carried out in 85 women who had complaints of symptoms like PID, infertility and habitual abortion. These women were recruited from the Gynaecology OPD of GSVM Medical College, Kanpur between January 2008 to September 2009. Both routine Pap smear examination and Chlamydial antigen detection test was performed in the sample collected from the cervix.

Chlamydia was detected in 22 of the 85 cases (25.8%). Inflammatory changes were commonly associated with Chlamydia (77.2%) while squamous intraepithelial lesion of cervix (SIL) was seen in 22.8% of cases. The Chlamydia was very common in the younger sexually active group of 21- 30 years and was associated with PID in 30.7% of cases, with infertility in 21.0% and with habitual abortion in 14.2% of cases.

Routine cytological screening is needed in all symptomatic women especially those complaining of pain in lower abdomen and vaginal discharge and also asymptomatic women to detect Chlamydia to facilitate their timely treatment.

KEYWORDS: Chlamydial infection; SIL; PID; Infertility

INTRODUCTION

Chlamydia trachomatis is the most common bacterial sexually transmitted infection, its prevalence ranging from 8% to 40 %⁽¹⁾. The incidence of chlamydial infection in women has increased drastically in last two decades and according to W.H.O. report, 101 million chlamydial infections are detected annually all over the world.⁽²⁾ Chlamydia is associated with gynaecological diseases like PID, threatened abortion and infertility. In the present study, 85 women attending Gynaecology Out Patient Department of GSVM Medical College, Kanpur were randomly selected who complained of vaginal discharge and symptoms like PID, habitual abortion and infertility. Pap smear was taken in these 85 women and they were simultaneously subjected to antigen detection test.

Chlamydia has been found in a very high percentage of asymptomatic women and may form a reservoir if not detected and treated. The affected women are a potential source of infection to their partners. The chlamydial infection causes a spectrum of diseases like urethritis,

mucopurulent cervicitis and endometritis. The cervicitis can lead to at least three types of complications- PID produced by the ascending intraluminal spread of organism. The descending infection during pregnancy might results in the premature rupture of membrane, chorioamnionitis, premature delivery and puerperal and neonatal infections⁽³⁾The asymptomatic women who are infected with Chlamydia may be at the risk of series of reproductive sequel like ectopic pregnancy and infertility.

Materials and methods

The women selected for this study (85) were registered from the Out Patient Department of Obstetrics. and Gynaecology, Upper India Sugar Exchange Maternity Hospital of GSVM Medical College, Kanpur between January 2008 to September 2009. These subjects had either complaints of vaginal discharge or symptoms of PID, habitual abortion or infertility. The sample from each patient for both routine Pap smear examination and Chlamydial antigen detection was collected from the cervix using Vidas Chlamydia kit.

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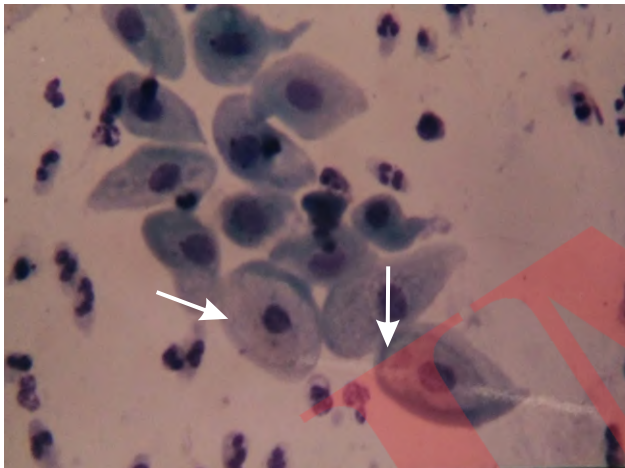
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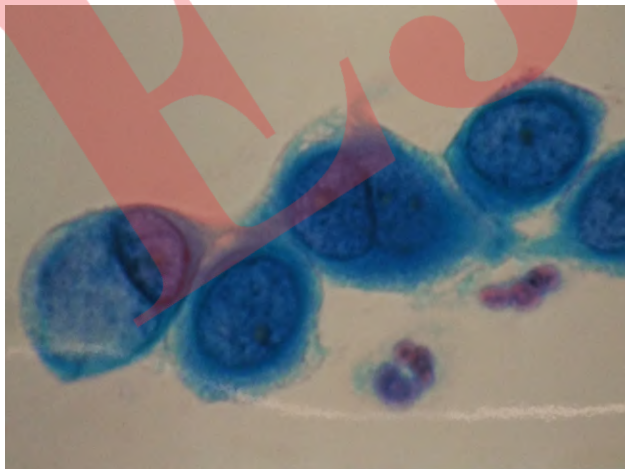
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For Pap smear examination, the sample were collected with the help of wooden spatula from the squamocolumnar junction of cervix and fixed in 95% alcohol and stained according the Papanicolaou's technique. The cytological finding was interpreted according to the Bethesda system of classification of 1993 (4). For the cervical swabs collected for the Chlamydial antigen detection test, the assay principle combined an immunoassay method with a final fluorescent detection enzyme linked fluorescent assay (ELFA).

The Chlamydia trachomatis are seen as characteristic intracellular inclusion bodies in Pap cervical smears (Figs. 1 and 2).



Cervical smear: Inclusion bodies of Chlamydia trachomatis in the cytoplasm and LSIL changes in the nuclei (Pap x 400)



Cervical smear: Inclusion bodies of Chlamydia trachomatis in the cytoplasm and HSIL changes in the nuclei (Pap x 400)

The procedure followed in the study was in accordance with the ethical standards of the Committee of G.S.V.M Medical College, Kanpur.

The other methods of Chlamydia detection include culture followed by immunofluorescent staining direct fluorescent test (DFA) and ELISA. PCR and most sensitive nucleic acid amplification technology (NAAT) are also being currently employed for detection of Chlamydia. Other current technologies recently applied for Chlamydia detection are as follows-

1. Fernandez, et al have demonstrated broad utility of a portable and low cost point of care (POC) PCR system as rapid diagnostic tool for quantitative detection of Chlamydia (5).
2. Branavan et al, have presented a design of a modular point of care test platform which is capable of detection of 1.32×10^6 of sample DNA through thermophilic helicase dependent amplification and 1×10^5 copy numbers Chlamydia trachomatis genomic DNA within 10 minutes through recombinase polymerase nucleic acid amplification test (6).
3. Chiriaco, et al, have reported a biosensing platform based on electrochemical impedance spectroscopy (EIS) that allows multiplexed detection of Chlamydia trachomatis with a single biochip enabling a quick screening of the specific target pathogen (7).

RESULTS

The overall prevalence of Chlamydia in 85 women studied was found to be 25.8% (22 cases) Cytological evaluation of cervical smears showed inclusion bodies of Chlamydia in 19 cases all of which were confirmed by the antigen test. In addition, 3 more cases were detected by the antigen test in which inclusion bodies of Chlamydia were not seen in the cytology smears. Hence, the sensitivity of detecting Chlamydia was slightly higher with antigen test (25.8% - 22 /85 cases) than Pap test (22.3% - 19/85).

Cytological finding in the total 22 detected cases of Chlamydia was found to be as follows.

Inflammatory smears - 17 (77.2%)

SIL-05 (22.8%)

Low grade SIL- 4(18.3%)

High grade SIL-1 (4.5%)

Carcinoma Cervix- Nil

The cytological findings have been summarized in Table 1. The inflammatory changes in the cervical epithelium were found to be commonly associated with chlamydia infection. The SIL was seen in 22.8 % of cases while no case of carcinoma cervix was seen. Of the 5 SIL cases, 4 were LSIL and 1 HSIL. However, number of cases studied is too small (85 women) to

make any statistical analysis of association of SIL with Chlamydia.

Cytopathological lesions of cervix	Number of Chlamydia cases detected	Percentage of Chlamydia
Inflammatory smears	17	77.2%
Total SIL cases	05	22.8%
a) Low grade SIL	04	18.3%
b) High grade SIL	01	4.5%
Carcinoma cervix	Ni	Nil

Table 1. Cytological findings in 22 detected cases of Chlamydia

Distribution of 22 cases of Chlamydia according to age is shown in Table 2. The chlamydial infection was found higher in the younger sexually active women between 21- 30 years and showed decline with increasing age. No case of Chlamydia was seen in older women above 50 years of age.

Age group	Number of cases	Percentage showing Chlamydia
21- 30 years	56	16 (28.5%)
31- 40 years	21	5 (23.8%)
41- 50 years	06	1(16.6%)
Above 50 years	02	Nil

Table 2. Incidence of chlamydial infection according to age

The incidence of chlamydial infection in the three major clinical groups in whom they were mostly associated like PID, habitual abortion and infertility is shown in Table3. The infection of Chlamydia was more frequent in women complaining of PID (30.7%) and the most common symptoms found were pain in lower abdomen and vaginal discharge. The infection was seen in 21.0 % of infertility and 14.2 % of habitual abortion cases.

Type of gynaecological disease	Number of cases	Percentage showing Chlamydia
PID	52	16(30.7%)
Habitual Abortion	14	2 (14.2%)
Infertility	19	4 (21.0%)

Table 3. Incidence of Chlamydia with different gynaecological diseases.

DISCUSSION

The prevalence rate of Chlamydia in the present series was found to be 25.8%. A high incidence of Chlamydia was also reported by Joycee, et al. (8) and Manavi, et al.(9) Chlamydia was detected in 23% of Gynaec OPD patients and 20% of women attending STD clinic by Malhotra, et al in New Delhi (10). A study involving female sex workers and married contacts in Mumbai showed chlamydial positivity in 23.2% (11). However, Goenkar, et al. have reported a low incidence of 8.8% in study carried out in Mumbai (12) and Nandeibam, et al have found 10% of chlamydial infection in a tertiary care centre of Tamil Nadu. (13) Earlier, Mary, et al. and Quinn, et al. have reported a moderate incidence of 12 to 15 % of Chlamydia in their studies. (14, 15).

Health care settings have higher prevalence rate of Chlamydia in the clinics than the population based studies. Adams, et al in UK have found Chlamydia among young women of less than 20 years, the prevalence estimates ranging from 10 -17% in different diagnostic clinics compared to 5% in population based studies (16) . Vuylsteke, et al have reported 7.3 % chlamydial positivity in women attending STD/Genitourinary clinics in Belgium (17). In Latin America, the prevalence rate of Chlamydia ranged from 2 to 4.5% in Chile, Brazil, Peru and Mexico (18) while 12.2% incidence was seen in women attending Family Planning clinics in Jamaica.(19)

Gharsallah, et al, used multilocus sequence typing (MLST) to find out *Chlamydia trachomatis* genetic diversity in Tunisia and have found E (ST3) strain of Chlamydia was dominant in Tunisia and was also highly prevalent in Holland and many other countries.(20). Local Authorities in England have commissioned Chlamydia screening as a part of National Chlamydia screening program to achieve a diagnostic rate of $\geq 23,000$ cases per 1, 000, 00 population aged 15-24 years.(21)

Chlamydia was found associated with SIL in 22.8% in the present series. A high incidence of SIL (28.6%) has also been reported by Misra.et.al.in women with PID.(22) In the present series, Chlamydia was found maximum in younger sexually active group of women between 21-30 years of age. A high percentage of chlamydial positive patients was also found by Pratibha, et.al. in younger women between 21-30 years(23) This is the most vulnerable group for STD acquisition as they mostly have higher number of sexual partners. A high incidence of other STDs namely *Candida albicans* and *Trichomonas vaginalis* have also been found in younger women by Misra, et.al.in a Hospital based cytological screening programme (24).

Chlamydia trachomatis is considered leading cause of PID and infertility all over the world. In USA, about 20-30% of PID cases have been found to be due to chlamydial infection.⁽²⁵⁾ In India, 15-40% of women develop PID following chlamydial infection⁽²⁶⁾. The chlamydial PID is the single most important preventable cause of infertility, PID patients become infertile in 20% of cases, 18% develop chronic pelvic pain and 9% have tubal pregnancy⁽²⁷⁾. Though the majority of women developing infertility are asymptomatic but re-infection/persistent infection leads to more severe tubal damage. Chlamydial infection was found most common in the PID cases by the present workers (30.7%). A high percentage of women ranging from 20-23% have also been reported by Miller, et al developing PID after chlamydial infection in OPD and STD clinics. However, a low incidence of Chlamydia in PID patients have been reported by Thomas et al. and Dowe, et al.^(28,29). The present series showed chlamydial positivity in 21.0% of infertility and 14.2% of habitual abortion cases which is much higher than reported by Paavonen, et al.⁽³⁰⁾. However, *Chlamydia trichomatis* was seen in 28.1% of infertile women in a study conducted by Malik, et al in Aligarh⁽³¹⁾

Low, et al have found utility of Randomized controlled trials (RCTS) on the chlamydial screening and have seen the intervention reducing the risk of PID in the population screened⁽³²⁾. Angelova, et al, have studied the prevalence of Chlamydia among the pregnant women and role of this infection on foetus. They have found that the early diagnosis and treatment of chlamydial infections can prevent the infection to the new born. They have suggested that the preventive examinations should be considered as a priority for early detection of asymptomatic chlamydial infection in the conduct of antenatal care.⁽³³⁾

The Chlamydial infection is also associated with an increased risk for transmission or acquisition of human immunodeficiency virus (HIV) and is also attributed to be a risk factor for development of carcinoma cervix.⁽³⁴⁾ The HIV and Chlamydia have common / behavioral risk factors but may have inter-relationship independent of the sexually transmitted risk factors⁽³⁵⁾. The genital chlamydial infection has been investigated extensively as a potential etiological factor for intra-epithelial neoplasia because of its asymptomatic nature, persistence left untreated and induction of metaplasia and chronic inflammation.^(36, 37) In an epidemiological study on this aspect, chlamydial seropositivity was found a significant risk factor for carcinoma -in -situ in Spain and Columbia after adjusting for HPV-DNA.⁽³⁸⁾ On contrary, Hounduras, Ferrera, et al found no association between chlamydial antibodies and cervical neoplasia after adjusting HPV-

DNA.⁽³⁹⁾ Smith, et al have analysed results from two International Agencies for Research on Cancer in an intraepithelial cervical neoplasia (ICC) case-control studies conducted in Brazil and Manila which indicated a moderate but significant association between chlamydial infection and ICC in the presence of HPV -DNA. Chlamydial positivity was commonly associated with an increased (2 fold) incidence of squamous cell carcinoma in HPV-DNA positive patients.⁽⁴⁰⁾

Gopalkrishna, et. al. have carried out a Chlamydia plasmid –based PCR assay in 50 women with STDs and have found 50% positivity of Chlamydia and 30% positivity of HPV-16 DNA.⁽⁴¹⁾ In women with pre-cancerous and malignant lesions, the rate of HPV-16 positivity was very high (52% and 72% respectively) whereas the frequency of Chlamydia was found to be 12 to 22 %.

Girgis, et. al. in their studies in Egyptian women have found that Human Papilloma Virus (HPV) and Chlamydia was significantly positive in 98% of cancer cervix cases and 4% of normal control cases.⁽⁴²⁾ The co-infection of HPV and Chlamydia was positive in 38% of HPV infected cervical cancer patients and overall presence of HPV in the Chlamydia positive samples was 95%.

Bhatla, et. al. have studied the correlation of HPV and Chlamydia in CIN cases and have found that 89% cases were HPV positive while only 4.3% were Chlamydia positive and 2.2% were positive for both. They have suggested that risk of CIN-2 disease was significantly increased with parity when Chlamydia was seen.⁽⁴³⁾ In our series, Chlamydia was associated with SIL in 22.8 % of cases. However, HPV study could not be done in these cases.

CONCLUSION

The incidence of Chlamydia was found higher in the present series (25.8%) but was comparable to the reports from New Delhi and Mumbai. The association of Chlamydia with PID and other diseases was also comparable with the findings within and outside India.

In view of the association of Chlamydia with HPV infection and a higher rate of Chlamydial infection in the three gynaecological disorders, it is felt that routine cytological screening being cheap and convenient should be carried out at yearly interval in all symptomatic women specially in those complaining of pain in lower abdomen and vaginal discharge as well as in all asymptomatic women for the timely detection of Chlamydia and its treatment. The control of STDs including *Chlamydia trachomatis* has become a public health priority due to their increasing prevalence in last two decades and growing incidence of co-transmission of HIV. Hence, all sexually active women below and above 25 years are at increased risk of Chlamydia

infection and should be routinely screened which should be implemented at all three levels. The primary prevention can be achieved by proper counseling of lifestyle and health education which restricts both exposure and acquisition of chlamydial infection. Safe sex partners should be encouraged and school based health program should be implemented. However, these measures have not been so popular in developing countries like India. The secondary prevention involves the early detection of Chlamydia in asymptomatic women to check their reproductive sequel by timely treatment. Recent detection techniques like PCR and NAAT, etc. are very helpful in detecting the chlamydial infection. The tertiary prevention has no significance as the acute and chronic infection spreads to the upper genital tract leading to the substantial tubal damage.

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