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 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 (SIL) of cervix 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 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 result 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 squeal 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.
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 (Figure 1 and 2).

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 $1.32 \times 10^6$ of sample DNA through thermophilic helicase dependent amplification and $1 \times 10^5$ 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 cases).

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.

### Table 1: Cytological Findings In 22 Detected Cases Of 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                  | Nil                                | Nil                     |

### Table 2: Incidence Of Chlamydial Infection According To 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 3: Incidence Of Chlamydia With Different Gynaecological Diseases.

| 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%)                    |

### 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 ≥ 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 Chlamydia 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 sero-positivity 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 asymptomatic 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.

REFERENCES

1. World health organization global prevalence and incidence of selected curable sexually transmitted diseases: overview and estimates. World Health Organization, Geneva, Switzerland. 2001.
2. Department of Health and Human Services. Sexually transmitted diseases Surveillance 2003 Supplement- Syphilis Surveillance Report. Division of STD prevention. Department of Health and Human Services, CDC, Atlanta, Georgia.2004
3. Paavonen J, Puolokkainen N. et al. Cost-benefit of analysis of first-void urine Chlamydia trachomatis screening program. Obstet Gynecol. 1988; 92: 292-298.
4. Kurman R J. The Bethesda system for Reporting Cervical/ vaginal Cytologic Diagnoses.1994: Springer-Verlag, New York, USA.1994
5. Fernández-Carballo BL, Mc Guiness. et al. Low-cost, real-time, continuous flow PCR system for pathogen detection. Biomed Microdevices. 2016: 18: 34.
6. Bravanan M, Mackay RE. et al. Modular development of a prototype point of care molecular diagnostic platform for sexually transmitted infections. Med Eng Phys. 2016:38: 741-748.
7. Chiriacó MS, Primiceri E. et al. Simultaneous detection of multiple lower genital tract pathogens by an impedimetric immunochip. Biosens Bioelectron.2016: 79: 9-14.
8. Joyce AG, Thyagarajan SP.eal. Need for specific & routine strategy for the diagnosis of genital chlamydial infection among patients with sexually transmitted diseases in India. Indian J Med Res. 2003: 118: 152-157.
9. Manavi K, McMillan A. et al. Genital infection in male partners of women with chlamydial infection. INT J STD AIDS.2006: 17: 34-36.
10. Malhotra M, Sood S. et al. Genital Chlamydia trachomatis: an update. Indian J Med Res. 2013: 138: 303-316.
11. Divakar AA, Gogate AS. et al. Disease prevalence in women attending the STD clinic in Mumbai (formerly Bombay), India. Int J STD AIDS, 2000: 11: 45-48.
12. Goenkar AT, Gogate AS. Chlamydia trachomatis in lower genital tract of women in child bearing age. Indian J Sex Trans Dis, 2002: 23: 31-37.
13. Nandeibam Y, Laisham S.et al. Prevalence of Chlamydia trichomatis in a tertiary care centre in South India. J Med Soc.2016: 30: 31-34.
14. Shafer MA, Beck A. et al. Chlamydia trachomatis:important relationships to race, contraception, lowers genital tract infection and Papanicolaou smear. J Pediar. 1984: 104: 141-146.
15. Quinn TC, Gupta PK. et al. Detection of Chlamydia trachomatis cervical infection: a comparison of Papanicolaou and immunofluorescent staining with cell culture. Am J Obstet Gynecol. 1987: 157: 394-399.
16. Adams EJ, Charlett A.et al. Chlamydia trachomatis in the United Kingdom: a systematic review and analysis of prevalence studies. Sex Transm Infect. 2004: 80: 354-362.
17. Vuylsteke B, Vandenbruaene M.et al,. Chlamydia trachomatis prevalence and sexual behaviour among female adolescents in Belgium. Sex Transm Infect, 1999: 75: 152-155.
18. Gunn RA, Hillis SD. et al. Chlamydia trichomatis infection among Hispanic women in the California-Mexico border area, 1993: establishing screening criteria in a primary care setting. Sex Transm Dis. 1995: 22: 329-334.
19. Behets FM, Ward E.et al. Sexually transmitted diseases are common in women attending Jamaican family planning clinics and appropriate detection tools are lacking. Sex Transm Infect.1998: 74:123-127.
20. Gharsallah, H, Bom RJ. et al.Identification of dominant Chlamydia trachomatis strain in patients attending sexual transmitted infection clinic and female sex workers in Tunisia using a high resolution typing method. Infect Genet Evol. 2016: 44: 444-449.
21. Chandrasekaran L, Davies B. et al,. Chlamydia diagnosis rate in England in 2012: an ecological study of local authorities. 2016: Sex Transm
Infect;2016.

22. Misra JS, Srivastava AN. et al. Cervical Cytology Associated with Pelvic Inflammatory Diseases. J Cytol Histol.2015: 6:343.

23. Pratibha G, Innocent DJP. et al. Prevalence of Chlamydia trichomatis infection in women in Chennai, India. Annals of Biological Research. 2010: 1: 76-81.

24. Misra JS, Srivastava S. et al, Risk-factors and strategies for control of carcinoma cervix in India: hospital based cytological screening experience of 35 years. Indian J Cancer.2009: 46: 155-159.

25. Soper DE. Pelvic inflammatory disease. Obstet Gynecol.2010: 116: 419-428.

26. Hillis S, Black C. et al. New opportunities for Chlamydia prevention: applications of science to public health practice. Sex Transm Dis.1995: 22: 197-202.

27. Miller KE. Diagnosis and treatment of Chlamydia trachomatis infection. Am Fam Physician.2006: 73:1411-1416.

28. Thomas BJ, Evans RT. et al., Sensitivity of detecting Chlamydia trachomatis elementary bodies in smears by use of a fluorescein labelled monoclonal antibody: comparison with conventional chlamydial isolation. J Clin Pathol.1984: 37: 812-816.

29. Dowe G, King SD. et al. Genital Chlamydia trachomatis (serotypes D-K) infection in Jamaican commercial street sex workers. Genitourin Med.1997: 73: 362-364.

30. Paavonen J, Eggert-Kruse W. Chlamydia trachomatis: impact on human reproduction. Hum Reprod Update.1999: 5: 433-447.

31. Malik A, Jain S. et al. Chlamydia trachomatis infection & female infertility. Indian J Med Res.2006: 123: 770-775.

32. Low N, Redmond S. et al. Screening for genital chlamydia infection. Cochrane Database Syst Rev.2016.

33. Angelova M, Kovachev E.et al. Role and Importance of Chlamydia Trachomatis in Pregnant Patients. Open Access Maced J Med Sci. 2016: 4:410-412.

34. Malhotra M, Bala M. et al. Prevalence of Chlamydia trachomatis and its association with other sexually transmitted infections in a tertiary care center in North India. Indian J Sex Transm Dis AIDS.2008: 29: 82-85.

35. Stamn WE. Chlamydia trachomatis infections: progress and problems. J Infect Dis. 1999: 2: 380-383.

36. Kiviart N, Paavonen J. et al. Histopathological manifestation of Chlamydia cervicitis. In: Oriel D, Ridgway G, Schachter J, Taylor-Robinson D, Ward M (eds.). Chlamydia Infections: Proceedings of the Sixth International Symposium on Human Chlamydial Infections. Cambridge University Press.1986: 209: 12.

37. Beatty WL, Morrison RP. et al. Persistent chlamydia: from cell culture to a Paradigm for chlamydia pathogenesis. Microbiol Rev.1994: 58: 686-699.

38. de Sanjosé S, Muñoz N. et al. Sexually transmitted agents and cervical neoplasia in Colombia and Spain. Int J Cancer.1994: 56: 358-363.

39. Ferrera A, Baz MF. A socio- epidemiological study of the relationship between sexually transmitted agents and cervical cancer in Houndra. Int J Cancer.1987: 73: 787-795.

40. Smith JS, Muñoz N. et al. Evidence for Chlamydia trachomatis as a human papillomavirus cofactor in the etiology of invasive cervical cancer in Brazil and the Philippines. J Infect Dis. 2002: 185: 324-331.

41. Gopalkrishna V, Agarwal N. et al. Chlamydia trachomatis and human papillomavirus infection in Indian women with sexually transmitted diseases and cervical precancerous and cancerous lesions. Clin Microbiol Infect.2000 6: 88-93.

42. Girgis SA, Kassem, NN. et al,. Chlamydia trachomatis and Human papilloma virus (HPV) infection in Egyptian Patients with Invasive Cancer Cervix- A Case Control Study. Int J Curr Microbial App Sci. 2015: 4: 937-949.

43. Bhatla N, Puri K. et al. Association of Chlamydia trachomatis infection with human papillomavirus (HPV) & cervical intraepithelial neoplasia - A Pilot study. Indian J Med Res.2013: 137: 533-539.