



Original Research Article

Genital Chlamydial Infection Association with HIV and Syphilis in Female Patients Attending STD-Clinic in a Tertiary Care Hospital

Siva Prasad Reddy Basava^{1*}, Rama Tulasi Sappa³, Sreenivasulu Reddy P¹, Jithendra Kandati¹, Madhavulu Buchineni² and Rama Mohan Pathapati²

¹Department of Microbiology, Narayana Medical College, Nellore, A.P., India

²Department of Pharmacology, Narayana Medical College & Hospital, Nellore, A.P., India

³Department of Dermatology, Konaseema Institute of Medical Sciences, Amalapuram, A.P., India

*Corresponding author

ABSTRACT

Genital chlamydial infection runs an asymptomatic course to a major extent leading to various complications due to primary infection and also promotes the susceptibility of host to other sexually transmitted infections. This study aims at thorough analysis of the clinical features associated with genital chlamydial infection in patients attending Sexually Transmitted Infections outpatient department, comparative evaluation of various diagnostic modalities and study on co-infection with HIV and Syphilis. A total of 180 women in the reproductive age group attending Sexually Transmitted Infections outpatient department with various genitourinary symptoms were enrolled in study. The present study was conducted at Rangaraya Medical College and Hospital, Kakinada from February 2011 to March 2012. Detailed analysis and documentation of presenting symptomatology and associated clinical signs was done. Endocervical secretions were subjected to Chlamydia trachomatis antigen detection by Immunochromatography and Giemsa staining technique. Serum was subjected to ELISA, immunochromatography, rapid plasma reagin test for the detection of IgG antibody to Major outer membrane protein of *Chlamydia trachomatis*, HIV and syphilis, respectively. Genital Chlamydial Infection was found by antigen detection in 8.89% and antibody detection in 14.44% cases. Genital discharge followed by abdominal pain was the predominant symptoms whereas cervicitis and adnexal tenderness were the clinical signs. Co-infection with HIV and syphilis were found to be 18% and 5% respectively. Detection of genital chlamydial infection can be enhanced by screening for both antigen and antibody. Screening of high risk groups as a mass campaign can be done by antigen detection by Immunochromatography. Proper analysis of symptoms and signs can raise suspicion of genital chlamydial infection. Co-infection with other sexually transmitted infections may lead to recurrence and resistance

Keywords

Chlamydia trachomatis, HIV, Syphilis, Immuno chromatography, ELISA, RPR

Introduction

Chlamydia trachomatis is one among the major etiological agents of sexually transmitted infections worldwide (WHO, 2001). It is a curable disease with silent progression, which if untreated results in complications like chronic pelvic pain, pelvic inflammatory diseases, infertility and ectopic pregnancy (Black, 1997). The incidence of Chlamydial infections in women has increased dramatically from 79 to 467 per 1, 00,000 between 1987 and 2003 (CDC, 2004). According to WHO, 101 million Chlamydial infections were detected annually worldwide (WHO, 2011). Up to 40% of untreated Chlamydial infections can ascend to upper genital tract causing PID (Hills *et al.*, 1995).

The prevalence of genital Chlamydial infections in women attending Sexually Transmitted Infections clinics varies from 60% in high risk women to 15% in low risk women (Joshi *et al.*, 1994). Women with genital Chlamydia have a 3–6 fold increased risk of HIV infection (Fleming and Wasserheit, 1999), risk of developing other Sexually Transmitted Infections like Syphilis and risk of developing cervical cancer (Paavonan *et al.*, 1999). Further the co-infection with HIV and Syphilis leads to increased recurrence caused by failure to eradicate the original infection.

Chlamydiae are obligate intracellular bacterial parasites of humans, animals and birds with tropism for squamous epithelial cells and macrophages of the respiratory and gastrointestinal tracts. *Chlamydiae* occur in two forms. The elementary body is a spherical particle with a rigid trilaminar cell wall which is the extracellular infective form. The reticulate body is highly pleomorphic and is the intracellular growing and replicative form. Though considered as

viruses initially due to their filterability and failure to grow on cell free media, they are now considered as bacteria due to the presence of both DNA and RNA, cell wall, ribosomes, replication by binary fission and susceptibility to antibiotics. The present study aims in determining the prevalence of Genital Chlamydia in patients attending STD-OP, associated clinical features and its co association with HIV and syphilis.

Materials and Methods

The present study was conducted at Rangaraya Medical College and Hospital, Kakinada from February 2011 to March 2012. Female patients attending STD op with symptoms (n=180) Voluntary patient participation with written informed consent Sexually active females between 16 and 45 age group Patient accompanied by a responsible guardian were included in the study and Patients on antibiotic therapy, Non willingness, age below 16 yrs and above 45 yrs excluded from the study.

Study design and work up: Detailed history and clinical findings were recorded. With prior permission from the ethical committee and patients consent, endocervical scrapings were collected and subjected to Giemsa staining for inclusion bodies and *Chlamydia trachomatis* antigen detection using immunochromatography based one step Chlamydial antigen test(SD Bioline).

Simultaneously blood is collected and the serum was analysed for IgG antibody against *Chlamydia trachomatis* Major Outer Membrane Protein (MOMP) using Euroimmune Anti *Chlamydia trachomatis* IgG kit. Serum was tested for HIV using immunochromatography kit (SD Bioline) and syphilis by RPR test (Span Diagnostics)

Procedure of antigen detection:

Specimen: Endocervical secretions

The SD BIOLINE Chlamydia is a solid face immunochromatographic assay for the rapid, qualitative detection of *Chlamydia trachomatis* antigen (LPS) which contains a membrane strip pre-coated with mouse monoclonal anti-*Chlamydia trachomatis* antibodies on test band region. The complex of sample including Chlamydia antigen and mouse monoclonal anti-*Chlamydia trachomatis* antibodies- colloid gold conjugate moves along the membrane chromatographically to the test region and forms a visible line as the antigen- antibody gold particle complex forms.

Antibody detection procedure

Kit Used: Euroimmune Anti *Chlamydia trachomatis* IgG kit

Antibody detected: IgG

Antibody against: *Chlamydia trachomatis* MOMP (Major Outer Membrane Protein) antigen

It is an in vitro assay for semiquantitative or quantitative detection of human antibodies of IgG class against *Chlamydia trachomatis* major outer membrane protein in serum or plasma. The test kit contains microtitre strips each with 8 break off reagent wells coated with *Chlamydia trachomatis* antigens.

In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies will bind to the antigens. To detect bound antibodies, a second incubation is carried out using an enzyme labeled anti human IgG (enzyme conjugate) catalyzing a colour reaction.

Calculation of results was done by quantitative method. The standard curve from which the concentration of antibodies in the patients samples can be taken is obtained by point to point plotting of the extinction values measured from the 3 calibration sera against the corresponding units(linear/linear).

On X-axis values are plotted in RU/ml
On Y-axis extinction values (optical density) values are plotted.

The extinction of a serum sample above the value of Calibrator 1(200RU/ml), the result was given as more than 200 RU/ml as recommended by EUROIMMUNE. The upper limit of the normal range of non infected persons (cut off value) recommended by EUROIMMUN is 20 RU/ml.

Results were interpreted as recommended by EUROIMMUN as follows

<16RU/ml : NEGATIVE
>16 TO <22RU/ml: BORDERLINE
>22RU/ml : POSITIVE

Positive and Negative controls act as internal controls for confirmation that kit is working properly.

Screening for syphilis:

By rapid plasma reagin (rpr) test

Kit used: span diagnostics

Sample: Human serum was used for the test. All samples were brought to room temperature before the test

Result and Discussion

No Black Particle lumps -- Non Reactive
Black particle lumps within 6 minutes -- Reactive

Screening for HIV

Kit used: SD BIOLINE HIV 1 / 2 3.0

One step, Rapid Immunochromatographic test for the detection of Anti-HIV 1/ 2 in Human serum, plasma or whole blood.

Specimen – Human Serum, Plasma or Whole blood can be used.

1: A Control Line in the left section of the result window shows that the test is working properly.

2: Color bands will appear in the middle and right section of the result window.

These bands are test line 2 and test line 1(2, 1).

Results and Discussion

In this study Chlamydial infection was found by antigen detection in 8.89% cases (16/180) and by IgG antibody detection in 14.44% cases (26/180). Both were detected in 2.22% cases (04/180). Genital Chlamydial infection is ascertained by considering the detection of either antigen or antibody alone or both. Thus the overall Genital Chlamydial infection in the study group is estimated to be 21.11% (38/180). The positivity detected by antigen and antibody in study group is statistically proved by applying Chi square 46.240 at degree of freedom 1; P value<0.0001 and it is significant. We could not detect any inclusion bodies on Giemsa staining of endocervical secretions. Among the positive cases, Genital discharge was the predominant symptom (100%) followed by pain abdomen (80%), dyspareunia (40%), dysuria (20%), backache (20%) and menstrual irregularities (20%). The predominant signs elicited in positive cases were cervicitis (60%) and adnexal tenderness (60%) followed by PID (40%), cervical erosion (40%) and bleeding on touch (20%). Majority of the positive cases

were in the age group of 16–25 yrs (22.03%) followed by 26–35 yrs (20.27%). No genital Chlamydial infection was detected in the 36–45yrs age group. Co-infection with HIV and syphilis was found in 18% and 5% of cases respectively.

Chlamydia trachomatis is a common genital tract infection in women of reproductive age group. It is worthwhile to screen for this infection especially in high risk group to initiate, prompt and complete treatment to prevent its further spread and other complications. It needs to be emphasized that controlling genital Chlamydial infection may have positive implications in the control of HIV and Syphilis.

Although the infection runs an asymptomatic course to a major extent, thorough analysis of presenting symptomatology and clinical signs may raise suspicion of genital Chlamydial infection. Presumptive treatment of women with mucopurulent cervicitis is a reasonable approach based on the results of local screening programmes or estimate of prevalence.

Antigen detection using immunochromatography based test aids in early diagnosis and can be used as a bedside modality as well as a screening technique in high prevalence areas. In chronic or persistent infections of upper genital tract, indirect evidence of Chlamydial infection using IgG antibody to *Chlamydia trachomatis* is of utmost importance. Combination of antigen detection methods and serological tests will increase the rate of detection of genital Chlamydial infections. Chlamydial positivity by antigen detection (Immunochromatography) in the present study was 8.89% which correlates with the studies of Savita *et al.* (2009) 8.13% and Young *et al.* (1991) 8.8%. The numerical

values in this study were much low compared to studies of Neelam Pandya *et al.* (2011) 12%, Isibor *et al.* (2005) 13.3%, Sheetawy and Abdulla (2007) 17%, and Rukadikar *et al.* (2014) 14%.

The low prevalence rates in my study group may be due to the prevailing traditions and customs like single sexual partner and the test kit used. The numerical values in this study were high compared to studies of Abd Raboh *et al.* (2011) 4.2%, Wang *et al.* (2001) 6.25% and Nwobu *et al.* (2007) 7.5%. This difference may be due to the study group involved and geographical differences.

Chlamydial positivity by IgG antibody detection (ELISA) in the present study was 14.44% which is in line with Meenakshi *et al.* (2008) 10.9%, low compared to studies of Ikeme *et al.* (2011) 29.4% and Rukadikar *et al.* (2014) 56.6% and high compared to Abd Raboh *et al.* (2011) 8%, Ghazi *et al.* (2006) 8.7%. This difference is due to the study group involved.

The prevalence of Genital Chlamydial infection in the STD patients is estimated to be 21.11% (38/180) which is in line with study by Meenakshi *et al.* (2008) 19.9%, Dowe *et al.* (1997) 16%. Present study shows low values compared to that of a

Chennai based study (Joyee *et al.*, 2005) 30.8%. This low positivity rate may be due to irrational usage of antibiotics for other non specific genital infections in this group. Also adequate motivation to attend STD clinics might have resulted in higher detection rates in Chennai based study. However our results were much higher compared to study of Mangalika *et al.* (2014) (8.3%). This variation may be attributed to the geographical differences and the nature of the kit used.

The present study showed genital discharge in all cases (100%) with Chlamydial positivity which is high compared to study of Mahopatra *et al.* (2013) 25%.

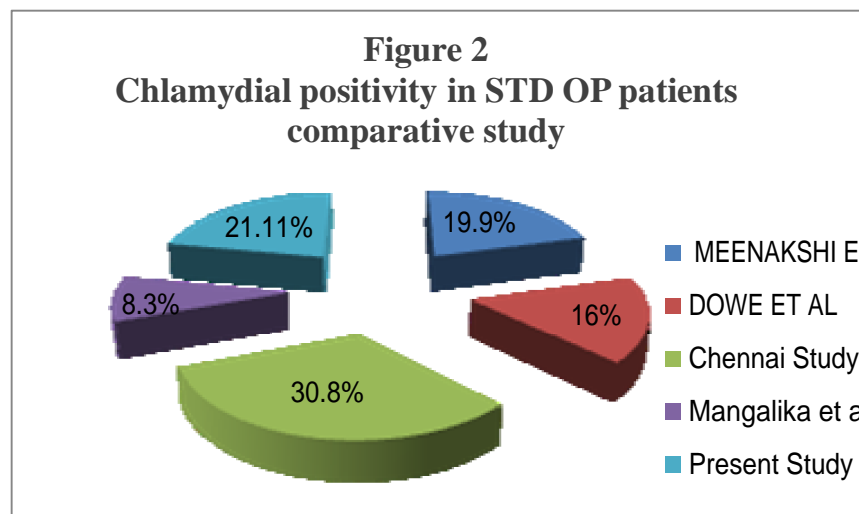
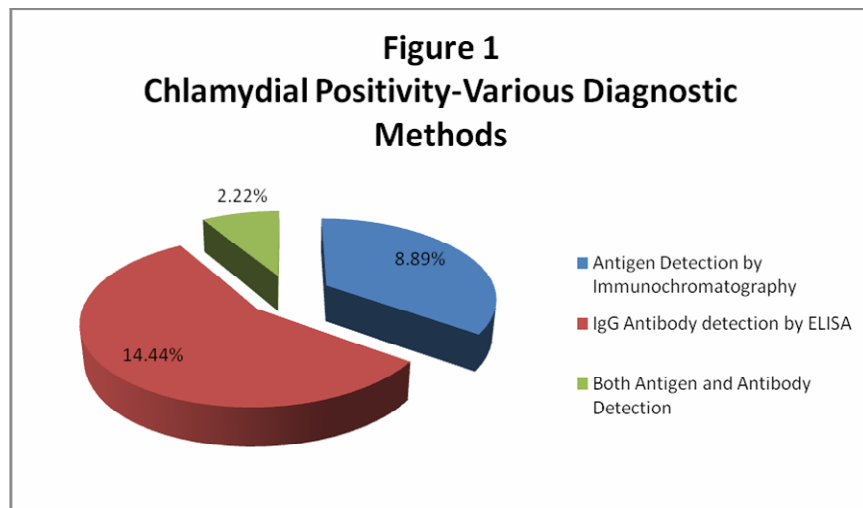
In my study there was high prevalence rate in the younger age group (16–25) compared to the middle age group (26–35) with zero detection in the upper age group (36–45). This could be explained by the anatomic differences in the cervix of the younger women, wherein the squamo-columnar junction, a primary host target for *C. trachomatis*, is everted and thus more exposed. Also there is a higher risk of being behaviorally more vulnerable to STI acquisition, as they generally have a higher number of sexual partners and more concurrent partnerships and change partners more often than older age groups.

Table.1 Detection of Chlamydial infection by various diagnostic modalities

S.No	Test	No.of Positives (N=180)	%
1	Inclusion body - Giemsa stain	00	0
2	Antigen Detection Immunochromotography	16	8.89%
3	ANTIBODY detection ELISA	26	14.44%
4	Both Antigen and Antibody detection	04	2.22%
	Total	28	21.11%

Table.2 Clinical features in relation to Chlamydial infection

Symptom	Chlamydial Positivity	Clinical Entity	Chlamydial Positivity
Genital Discharge	100%	Cervicitis	60%
Pain Abdomen	80%	Adnexal Tenderness	60%
Dyspareunia	40%	PID	40%
Dysuria & Backache	20%	Cervical Erosion	40%
Menstrual Irregularity	20%	Bleeding on touch	20%



Conflict of interest: None

Ethical approval: The study was approved by the Institutional Ethics Committee.

References

- Abd-Raboh, S., Saleh, H.A., Hanafi, N.F., Darwish, H.B. 2011. The detection of *Chlamydia trachomatis* Antigen in cervical secretions and serum antibodies in infertile females undergoing ICSI and its impact on pregnancy success. *Life Sci. J. Acta Zhengzhou Uni. Overseas Edn.*, 8(2): 259–263.
- Black, C.M. 1997. Current methods of laboratory diagnosis of *Chlamydia trachomatis*. *Clin. Microb. Rev.*, 10: 160–84.
- CDC, 2004. STD surveillance 2003 supplement, Division of STD prevention 2004, Department of health and human services, CDC, Atlanta.
- Dowe, G., King, S.D., Brathwaite, A.R., Wynter, Z., Chout, R. 1997. Genital *Chlamydia trachomatis* (serotype D-K) infection in Jamaican street sex workers. *Genitourin. Med.*, 73: 362–4.
- Fleming, D.J., Wasserheit, J.N. 1999. From epidemiological survey to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection. *Sex transm. Infect.*, 75: 3–17.
- Ghazi, H.O., Daghestani, M.H., Mohamed, M.F. 2006. Seropositivity of *Chlamydia trachomatis* among Saudi pregnant women in Makkah. *J. Fam. Commun. Med.*, 13(2): 61.
- Hills, S., Black, C.M., Newhall, J., Walsh, C., Groseclose, S.L. 1995. New opportunities for Chlamydia prevention: application of science to public health practice. *Sex Transm. Dis.*, 22: 197–202.
- Ikeme, A.C., Ezegwui, H.U., Ikeako, L.C., Agbata, I., Agbata, E. 2011. Seroprevalence of *Chlamydia trachomatis* in Enugu, Nigeria. *Nigerian J. Clin. Pract.*, 14(2): 176–180.
- Isibor, J.O., Ugbomoiko, D., Nwobu, G.O., Ekundayo, A.U., Enweani, I.B., Okogun, G.R.A. 2005. Detection of Chlamydia antigen in cervical specimens from antenatal clinic attendees in Benin City, Nigeria. *Afr. J. Clin. Exp. Microbiol.*, 6(3): 208–211.
- Joshi, J.V. *et al.* 1994. The prevalence of *Chlamydia trachomatis* in young women. *Natl. Med. J. India*.
- Joyee, A.G., Thyagarajan, S.P., Reddy, E.V., Venkatesan, C., Ganapathy, M. 2005. Genital chlamydial infection in STD patients: Its relation to HIV infection. *Indian J. Med. Microbiol.*, 23: 37–40.
- Mangalika, G.K., Cankanamge, S.K., Priyadarshana, D., Shamini, P., Sujatha, M., Geeganage, W., Nalaka, A. 2014. Prevalence of *Chlamydia trachomatis* in women attending sexually transmitted disease clinics in the Colombo district, Sri Lanka. *Indian J. Pathol. Microbiol.*, 57(1): 55.
- Meenakshi, M. *et al.* 2008. Prevalence of *Chlamydia trachomatis* and its association with other sexually transmitted infections in a tertiary care center in North India. *Indian J. Sex. Trans. Dis. AIDS*, 29(2): 82.
- Mohapatra, S., Panda, P., Parida, B. 2013. Genital tract infection of women in Southern Orissa with special reference to pelvic inflammatory

- disease. *Indian J. Sex. Trans. Dis.*, 34: 64–6.
- Nwobu, G.P., Agbonlahor, D.E., Turay, A.A., Aloamaka, C.P., Isibor, J.P. 2007. *Chlamydia trachomatis* infection among apparently healthy students of Ambrose Alli University Ekpoma, Edo State, Nigeria. *J. Med. Lab. Sci.*, 14(2): 62–67.
- Paavonan, J., Eggert-kruse, W. 1999. *Chlamydia trachomatis*: Impact on human reproduction. *Hum. Reprod. Update*, 5: 433–47.
- Pandya, N., Pandya, N., Ninama, G.L., Damor, J.R., Javdekar, T.B. 2011. Detection of *Chlamydia trachomatis* antigen directly from endocervical specimen. *Natl. J. Commun. Med.*, 1(1).
- Rukadikar, A.R. *et al.* 2014. Seroprevalence of *Chlamydia trachomatis* in STI patients. *J. Evolut. Med. Dent. Sci.*, 3(7): 1605–1611.
- Savitha, S., Madhavan, S., Raja, V.R. 2009. Incidence of Chlamydial infection in women. *J. Pharm. Sci. Res.*, 1(1): 26–33.
- Sheetawy, A.Z., Abdulla, Z.A. 2007. Detection of *Chlamydia* and other bacteria in cervicitis. *Ann. Coll. Med. Mosul*, 33(1&2): 26–34.
- Wang, *et al.* 2001. Comparison of ligase chain reaction (LCR) and immunochromatography assay (ICA) for the detection of *Chlamydia trachomatis*. *Chin. J. Microbiol. Immunol.*
- World Health Organisation (WHO), 2001. Global prevalence and incidence of selected curable Sexually Transmitted Infections. Overview and estimates. WHO, Geneva.
- World Health Organisation (WHO), 2011. Global prevalence and incidence of selected curable Sexually Transmitted Infections. Overview and estimates. WHO, Geneva.
- Young, H., Moyes, A., Lough, H., Smith, I.W., McKenna, J.G., Thompson, C. 1991. Preliminary evaluation of "Clearview Chlamydia" for the rapid detection of chlamydial antigen in cervical secretions. *Genitourin. Med.*, 67(2): 120–123.