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Microbiology Section

IgM ELISA: A Better Choice for the Detection of Active *Chlamydia trachomatis* Infection among HIV Patients

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ABSTRACT

Context: Co-morbid STIs are documented to serve as risk factors that facilitate the acquisition and transmission of HIV. The cryptic plasmid has been linked to Chlamydial virulence and serves as a key target of diagnostic PCR assays. Reports on the higher incidence of *Chlamydia trachomatis* infection and the emergence of plasmid-less isolates in developing countries have raised concern.

Aim: The study was aimed to determine the prevalence of active *C. trachomatis* infection among high risk subjects in South India.

Materials and Methods: Serum and first-void urine samples were screened for *C. trachomatis* using IgM ELISA and COBAS AMPLICOR PCR respectively.

Results: The study participants include, 100 HIV seropositive patients, 51 Non-HIV-subjects attending STI clinic and 25 healthy adults. The incidence of *C. trachomatis* infection among HIV patients (n= 100) as measured by positive IgM ELISA result was 3%, while 0% positivity was observed among the cohort II and III. However, PCR analysis revealed that the cryptic plasmid was not detected in the three EIA positive patient samples.

Conclusions: We report here of low incidence of *C. trachomatis* infection among HIV patients in south India. Discrepancies were noted between the results of IgM ELISA and plasmid- PCR. Our results suggested the existence of plasmid-less/natural variant strains of *C. trachomatis* in South India. Thus, IgM ELISA still remains the method of choice in the laboratory diagnosis and obviates the use of plasmid based NAATs which could only under estimate the incidence of *C. trachomatis* infection among the HIV patients.

Key Words: *Chlamydia trachomatis,* ELISA, PCR, Serology

INTRODUCTION

Chlamydia trachomatis is the most common cause of treatable sexually transmitted infection (STI) worldwide. *C. trachomatis* infections are clinically silent and hence are of major public health concern [1]. A previous South Indian study has reported a high prevalence of female genital chlamydial infection [2]. Untreated co-morbid STIs serve as risk factors that facilitate the acquisition of HIV [3]. Also, early detection and treatment of STIs could reduce the burden of HIV [4]. Condoms confer better protection against genital infections that are transmitted *via* secretions [5]. Diagnosis of STI's in developing countries is essentially based on clinical criteria, symptomatology and physical examination rather than laboratory diagnosis. Hence, we intended to assess the actual prevalence of *C. trachomatis* infection among HIV patients.

MATERIALS AND METHODS

Study Population and Specimens

Sexually active asymptomatic subjects were recruited for the study following ethical clearance by the Institutional Review Board on Human Ethics and oral informed consent of the subjects to participate after explaining him or her the study protocol.

Cohort I included HIV seropositive patients with CD4 count < 500 cells/ cu. mm. Patients on highly active retero-viral therapy were excluded. Cohort II included a high risk population attending a STD clinic that fulfilled the criteria of HIV sero-negativity and not on anti-microbial therapy for the past one month. Cohort III included sexually active, healthy volunteers of the reproductive age group without any symptoms of genital infections.

Blood and first void urine samples were collected from the study participants. The samples were transported in ice to Microbiology laboratory. The serum was separated and stored at –70° C until use. On arrival at the laboratory, well mixed urine samples were centrifuged at 3,000 x g for 30 mins. The deposit was re-suspended in 1 ml of PBS and stored at –70° C until PCR assay.

IgM ELISA: IgM antibodies to *C. trachomatis* was detected in the serum samples by using NovaTec *C.trachomatis* IgM ELISA in accordance with the manufacturer's instructions. Samples were considered positive if the absorbance value was 10% greater than the cut off value and the results were expressed as NovaTec units (NTU).

Plasmid PCR: Urine samples of patients who were positive (n=3) for CT IgM ELISA were subjected to plasmid PCR using COBAS AMPLICOR™ CT/NG kit. Specimens were processed and tested according to the manufacturer's instructions. Specimens that yielded CT or NG signal above the test cut off were interpreted positive, regardless of the IC results. Specimens yielding CT or NG signal below the test cut off were interpreted as negative, only if IC signal was above the required cut off.

RESULTS

Cohort I comprised of 100 seropositive HIV patients hospitalised at Government Hospital for Thoracic Medicine, Chennai. Cohort II comprised 51 Non -HIV patients attending the Sexually Transmitted Infection (STI) clinic of the Institute of sexually transmitted infections, Government General Hospital, Chennai. Cohort III included 25 sexually active apparently healthy subjects without any symptoms of genital infections.

Among 100 HIV patients (male-50, female-48, transgender-2) enrolled in cohort I, the mean age was 36.5 years. Cohort II included 41 males, 10 females with a mean age of 38.5 years and cohort III included 25 healthy adults (13 males and 12 females) and the mean age was 27 years.

Out of 176 serum samples screened, 3 samples showed the presence of anti *C. trachomatis* IgM antibodies by NovaTec ELISA. The overall incidence of *C. trachomatis* infection among HIV patients (n = 100) included in the study as measured by positive CT IgM ELISA result was 3% (females -2, Males -1), while the positive rate was 0% among the cohort II and III. None of the urine samples of the HIV patients (n=3) with active *C. trachomatis* infection (positive by CT IgM ELISA) when subjected to COBAS AMPLICOR™ CT / NG PCR assay, yielded a positive CT amplification signal.

Review of the patient's hospital records revealed that, of the 3 HIV subjects with active *C. trachomatis* infection, 2 subjects (male-1, female-1) had CD4 count < 200 cells/cu. mm. Also, two of the three HIV subjects who were positive for *C. trachomatis* IgM ELISA, were also found to be VDRL reactive, but all the 3 samples were negative for *Neisseria gonorrheae*.

DISCUSSION

Progressive destruction of the immune system by HIV leads to depletion of CD4 cells making the patient highly vulnerable to a variety of opportunistic infections. HIV patients are at a high risk of acquiring other STI's (especially genital *C. trachomatis* infection and syphilis) owing to their promiscuous nature and common route of transmission of these etiological agents. In sexually active men and women, unrecognized and untreated *C.trachomatis* infection can cause excessive inflammation of the genital tract which consecutively appears to increase both the HIV shedding and viral load in genital secretions [6-9] thereby increasing HIV infectiousness [10, 11]. Also, $CD4^+$ T cells are attracted to either the surface of the ulcer [11] or the endocervix [12] in both ulcerative and non-ulcerative STIs, which sequentially disrupts the innate mucosal immunity and potentially enhances an individual's susceptibility to HIV infection. Recently there has been a gradual increase in the global burden of *C. trachomatis* infection. This in part, may be ascribed to the implementation of newer screening protocols, reporting of new chlamydia cases, improved diagnostic tools with a high degree of sensitivity, changes in sexual behaviour. Previous reports have documented a high prevalence of *C. trachomatis* infection in the Indian population and an urgent need for the development of cost-effective, sensitive screening methods [13-17]. The recent Centers for Disease Control (CDC) report shows that among females attending STI clinics aged 15–24 years, *Chlamydia* prevalence ranged from 14.8% to 16.3% from 2004–2008 and in men the incidence ranged from 20.5% to 23.7%. The HP2010 target of CDC was to reduce the overall *Chlamydia* incidence to 3% [18].

Conventional laboratory methods are insensitive, labor intensive and expensive. Accurate laboratory diagnosis remains a challenge; culture has long been considered the gold standard. Nucleic acid amplification methods are currently being adopted as an accurate alternative. However, antibody detection by ELISA is a convenient diagnostic tool in developing countries. Based on our EIA results, the incidence of *C. trachomatis* infection is found to be relatively low (3%) among the HIV seropositive subjects. This is contrary to a previous report from our region, wherein the overall *C. trachomatis* prevalence rate was 30.8% and *C. trachomatis* positivity rate among the HIV positive and HIV negative cases were 54.2%, 26.1% respectively [19]. It could be speculated that there is currently a significant reduction in the CT positivity rate among the HIV cases which could possibly be attributed to increased awareness towards STI among these high risk individuals. Our results indicate that the continuing HIV awareness and control strategies adopted in our region has met the HP2010 target of CDC.

CD4 count is an important surrogate marker for the assessment of the stage of the disease and the initiation of anti-retroviral therapy, the incidence of *C. trachomatis* was relatively common (2/13; 15.4%) in patients with CD4 count <200 cells/cu.mm as opposed to the HIV subjects (1/ 87; 1.1%) with CD4 count > 200 cells/cu. mm.

Molecular detection of *C. trachomatis* by PCR is more sensitive when performed on urine samples though the numbers copy of organism are less compared to that in present urogenital swab specimens. Nevertheless, testing urine has facilitated the use of a non-invasive method of sample collection and has increased the effectiveness, reduced the cost of screening programmes especially among the asymptomatic individuals in whom compliance is a major problem.

C. trachomatis contains in addition to the chromosomal DNA, a cryptic plasmid (7.5 kb) that is common among all serovars *of C. trachomatis,* also viable plasmid-less isolates and variants strains with deletion mutation (377bp) within the plasmids are currently being reported [20-22]. The primers CP 24 and CP 27 that were used in AMPLICOR PCR assay are specific to the DNA sequence approximately 208 bp within the cryptic plasmid of *C. trachomatis.* The plasmid PCR assay could not detect positivity among all the 3 IgM ELISA positive subjects.

George et al., (2003) have documented a decreased positivity by urine PCR (25.2%) as opposed to 32.2% positivity by genital swab PCR. Also, PCR inhibitors were reported in 8.6% of the urine of female patients while, 3.2% among the samples of male subjects [14]. However, the positive amplification signal of the internal control in all the 3 CT EIA positive samples in our study further nullifies the possibility of the presence of PCR inhibitors in the amplification process. Hence, the negativity of AMPLICOR PCR could not be attributed to the presence of PCR inhibitory substances in the urine.

The discrepancies between the two assay results observed in our study i.e. positive IgM ELISA and negative Amplicor PCR results could possibly be due to the occurrence of variant strains of *C. trachomatis* that circulate in our region, which lack the specific cryptic plasmid or possesss deletion mutants. The positive IgM ELISA assay results on plasmid-PCR-negative samples did not appear to be false positives because of the reproducibility of the results when performed in triplicates. These samples appeared to be unique as the presence of anti-*C.trachomatis* specific IgM antibodies suggestive of active infection were detected though

the infecting *C. trachomatis* stains were either plasmid-free or with plasmids having deletion mutations within the target region.

Variant strains of *C. trachomatis* lacking the evolutionarily conserved cryptic plasmid have been previously documented among patients with symptomatic STI which were not detected by AMPLICOR PCR assay, but were detected by rRNA - PCR and RNAse Protection (RNP) assay [23]. Previous studies have documented the incidence of cryptic plasmid-less variants of *C. trachomatis* worldwide [24-28]. Recent Swedish reports have documented the incidence and impact of the new variant of *C. trachomatis* which was described in October 2006. A 377bp deletion mutation in the plasmid target region lead to the negative diagnosis by commercial plasmid based NAATs that eventually resulted in the underestimation of genital Chlamydia infections in the Swedish population [29, 30]. A previous study had reported the incidence of a FVU specimen that was strongly reactive in CRT, but negative with the plasmid based Amplicor PCR (Roche) and ProbeTec ET (BD) assays [31]. Gupta et al., (2008) have raised concern on the existence of a plasmid-less clinical isolate of *C. trachomatis* in a patient from New Delhi, India and has suggested the use of real-time PCR in reviewing the *C. trachomatis* prevalence rates in the Indian population [32]. Also, Sachdeva et al., (2009) has suggested the possible occurrence of plasmid free variants of *C. trachomatis* in the Indian population. In their study, four samples tested positive by Roche AMPLICOR MWP kit were found to be negative by the in–house PCR assay targeting the Phospholipase D endonuclease superfamily gene [13]. A recent Indian study has reported 4 samples that were negative by Plasmid PCR but positive by DFA and FISH assay [33]. Our results suggest that in resource limited settings like India, the use of NAATS (commercial PCR assays, real-time PCR) is questionable and IgM ELISA still stands adequate in the diagnosis of this common STI in the HIV population.

The Advisory Committee for HIV and STD Prevention (ACHSP) [34] and the World Health Organisation (WHO, 2001) [3] have recommended the early detection and prompt treatment of STI's as an effective HIV prevention strategy. Routine, improved screening for asymptomatic STI's especially *C.trachomatis* infection has major public health implications for STI and consequently, would reduce the spread of HIV in this high risk population. In conclusion, we report here the possible occurrence of cryptic plasmid-less or naturally occurring mutant strains of *C. trachomatis* in South India. Failure to detect these variant strains of *C. trachomatis* and hence the infected going untreated would have a substantial impact on the incidence, spread, epidemiology and disease management among the high risk patients (with multiple sexual partners) in India wherein, plasmid based COBAS AMPLICOR PCR has been routinely used for *Chlamydia* screening by the research laboratories. Consistent with other studies, our observations further signify the need for cost effective periodic screening programmes for the detection of active *C. trachomatis* infection and access to treatment among the high risk patients which would substantially reduce the risk of HIV transmission and disease progression [4, 7, 8, 10, 18, 19, 34]. CT specific IgM antibody detection obviates the need for performing plasmid PCR assay and further confirmation by MOMP PCR assay and could also reduce the health care cost. Nevertheless, further studies with a larger sample size and additional molecular methods are still needed to determine the actual magnitude of the problem and would be critically informative in determining whether plasmid-less / deletion mutant variants of *C. trachomatis* circulate in our geographical setting.

REFERENCES

[1] Sexually transmitted diseases treatment guidelines 2002. Centers for Disease Control and Prevention. *MMWR Recomm Rep*. 2002; 51(RR-6):1-78.

[2] Joyee AG, Thyagarajan SP, Rajendran P, Hari R, Balakrishnan P, Jeyaseelan L, et al. *Chlamydia trachomatis* genital infection in apparently healthy adult population of Tamil Nadu, India: a population-based study. *Int J STD AIDS* 2004; 15: 51-55.

[3] World Health Organization. *Global prevalence and incidence of selected curable sexually transmitted infections: Overview and estimates*. Available from: http://whqlibdoc.who.int/hq/2001/WHO_HIV_ AIDS_2001.02.pdf. Accessed Feb 10, 2011.

[4] Gilson L, Mkanje R, Grosskurth H, Mosha F, Picar J, Gavyole A, et al. Cost-effectiveness of improved treatment services for sexually transmitted diseases in preventing HIV-1 infection in Mwanza Region, Tanzania. *Lancet* 1997; 350: 1805-09.

[5] Mindel A, Sawleshwarkar S. Condoms for sexually transmissible infection prevention: politics versus science. *Sex Health*. 2008; 5: 1- 8.

[6] Clemetson DBA, Moss GB, Willerford DA, Hensel M, Emonyi, W, Holmes KK, et al. Detection of HIV DNA in cervical and vaginal secretions. Prevalence and correlates among women in Nairobi, Kenya. *JAMA* 1993; 269: 2860-64.

[7] Atkins MC, Carlin EM, Emery VC, Griffiths PD, Boag F. Fluctuations of HIV load in semen of HIV positive patients with newly acquired sexually transmitted diseases. *BMJ* 1996; 313: 341-42.

[8] Ghys PD, Fransen K, Diallo MO, Virginie ET, Issa-Malick C, Kouadio M.Y, et al. The associations between Cervicovaginal HIV shedding, sexually transmitted diseases and immunosuppression in female sex workers in Abidjan, Céte d'Ivoire. *AIDS* 1997; 11: F85-F93.

[9] Cohen MS, Hoffman IF, Royce RA, Kazembe P, Dyer JR, Daly CC, et al. Reduction of concentration of HIV-1 in semen after treatment of urethritis: implications for prevention of sexual transmission of HIV-1. AIDSCAP Malawi Research Group. *Lancet* 1997; 349: 1868-73.

[10] Wasserheit JN. Epidemiological synergy. Interrelationships between human immunodeficiency virus infection and other sexually transmitted diseases. *Sex Transm Dis* 1992; 19: 61-77.

[11] Spinola SM, Orazi A, Arno J N, Fortney K, Kotylo P, Chen CY, *et al. Haemophilus ducreyi* elicits a cutaneous infiltrate of CD4 cells during experimental human infection. *J Infect Dis* 1996; 173: 394-402.

[12] Levine WC, Pope V, Bhoomkar A, Tambe P, Lewis JS, Zaidi AA, *et al.* Increase in endocervical CD4 lymphocytes among women with non ulcerative sexually transmitted diseases. *J Infect Dis* 1998; 177: 167-74.

[13] Sachdeva P, Patel AL, Sachdev D, Ali M, Mittal A, Saluja D. Comparison of an in-house PCR assay, direct fluorescence assay and the Roche AMPLICOR *Chlamydia trachomatis* kit for detection of *C. trachomatis*. *J Med Microbiol* 2009, 58; 867–73.

[14] George CA, Panchatcharam TS, Paramasivam R, Balasubramanian S, Chakrapani V, Murugan G. Evaluation of diagnostic efficacy of PCR methods for *Chlamydia trachomatis* infection in genital and urine specimens of symptomatic men and women. *Jpn J Infect Dis* 2003, 56: 88-92.

[15] Divekar AA, Gogate AS, Shivkar K, Gogate S, Badhwar VR. Disease prevalence in women attending STD clinic in Mumbai (formerly Bombay), India. *Int J STD AIDS* 2000, 11; 45-48.

[16] Singh V, Rastogi S, Garg S, Kapur S, Kumar A, Salhan S, Mittal A. Polymerase chain reaction for detection of endocervical *Chlamydia trachomatis* infection in Indian women attending gynecology outpatient department. *Acta Cyto* 2002, 46; 540-44.

[17] Singh V, Salhan S, Das BC, Mittal A. Predominance of *Chlamydia trachomatis* serovars associated with urogenital infections in females in New Delhi, India. *J Clin Microbiol* 2003, 41; 2700-702.

[18] Centers for Disease Control and Prevention. Sexually Transmitted Disease Surveillance 2008. Atlanta, GA: US Department of Health and *Human Services*; 2009.

[19] Joyee AG, Thyagarajan SP, Reddy EV, Venkatesan C, Ganapathy M. Genital Chlamydial infection in STD patients: Its relation to HIV infection. *Indian J Med Microbiol*. 2005; 23: 37-40.

[20] Palmer L, Falkow S. A common plasmid of *Chlamydia trachomatis*. *Plasmid* 1986; 16: 52-62.

[21] Peterson EM, Markoff BA, Schachter J, de la Maza LM. The 7.5 kb plasmid present in *Chlamydia trachomatis* is not essential for growth of this organism. *Plasmid* 1990; 23: 144-48.

[22] Herrmann B, Törner A, Low N, Klint M, Nilsson A, Velicko I, et al. Emergence and spread of *Chlamydia trachomatis* Variant, Sweden. *Emerg Infect Dis* 2008, 14; 1462-65.

[23] An QI, Radcliffe G, Vassallo R, Buxton D, O'Brien WJ, Pelletier D A, et al. Infection with a plasmid-free variant *Chlamydia* related to *Chlamydia trachomatis* identified by using multiple assays for nucleic acid detection. *J Clin Microbiol* 1992; 30: 2814-21.
[24] An Q, Olive DM. Molecular cloning and nucleic acid sequencing of *Chlamydia trachomatis* 16S rRNA genes from patient samples lacking the cryptic plasmid. *Mol Cell Probes* 1994; 8: 429-35.
[25] Farencena A, Comanducci M, Donati M, Ratti G, Cevenini R. Characterization of a new isolate of *Chlamydia trachomatis* which lacks the common plasmid and has properties of *biovar* trachoma. *Infect Immun* 1997; 65: 2965-69.
[26] Ripa T, Nilsson P (2006). A variant of *Chlamydia trachomatis* with deletion in cryptic plasmid: implications for use of PCR diagnostic tests. Euro Surveill 11: E0611092, http:www.eurosurveillance.org/ew/2006/061109.asp#2.
[27] Herrmann B. A new genetic variant of *Chlamydia trachomatis*. *Sex Transm Infect* 2007; 83: 253 -54.
[28] Schachter J. The *Chlamydia trachomatis* plasmid deletion mutant -what does it mean to us? *Sex Transm Dis* 2007; 34: 257.
[29] Seth-Smith HM, Harris SR, Persson K, Marsh P, Barron A, Bignell A, et al. Co-evolution of genomes and plasmids within *Chlamydia trachomatis* and the emergence in Sweden of a new variant strain. *BMC Genomics*. 2009, 10; 239.
[30] Unemo M, Clarke IN. The Swedish new variant of *Chlamydia trachomatis*. *Curr Opin Infect Dis*. 2011, 24; 62-69.
[31] Magbanua JPV, Goh BT, Michel CE, Aguirre-Andreasen A, Alexander S, Ushiro-Lumb I, et al. *Chlamydia trachomatis* variant not detected by plasmid based nucleic acid amplification tests: molecular characterisation and failure of single dose azithromycin. *Sex Transm Infect* 2007; 83: 339-43.
[32] Gupta R, Jha R, Salhan S, Eickhoff M, Krupp G, Mittal A. Existence of plasmid-less clinical isolate of *Chlamydia trachomatis* in India is a cause for concern and demands the use of real-time PCR assays. *The Internet Journal of Microbiology*. 2008 Volume 5 Number 2.
[33] Patel AL, Sachdev D, Nagpal P, Chaudhry U, Sonkar SC, Mendiratta SL, Saluja D. Prevalence of *Chlamydia* infection among women visiting a gynaecology outpatient Department: Evaluation of an In-house PCR assay for detection of *Chlamydia trachomatis. Ann Clin Microbiol Antimicrob*. 2010, 9; 24.
[34] HIV prevention through early detection and treatment of other sexually transmitted diseases—United States. Recommendations of the Advisory Committee for HIV and STD Prevention. *MMWR Recomm Rep* 1998; 47(RR-12); 1-24.

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