Annexure XXI

Disease Specific Documents for XII Plan

Chlamydiasis

(*C. trachomatis* and *C. pneumoniae*)

High Power Committee to Evaluate the Performance of ICMR, 2012-13

Indian Council of Medical Research, New Delhi

Year of Publication 2014

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Published by the Division of Publication & Information on behalf of the Secretary DHR & DG, ICMR, Ministry of Health & Family Welfare, New Delhi

Designed & Printed at M/s Aravali Printers & Publishers (P) Ltd., W-30, Okhla Industrial Area, Phase-II, New Delhi-110020 Phone: 47173300, 26388830-32

CHLAMYDIASIS (*C. trachomatis* and *C. pneumoniae*)

Following are the ICMR institutes working on *Chlamydia*:

1. National Institute of Pathology (NIOP), New Delhi.
2. National Institute for Research in Reproductive Health (NIRRH), Mumbai.

1. Current situation of disease with contribution to ICMR

Brief Introduction

Genital *Chlamydia trachomatis* infection is an STI of epidemic proportions. Infection with this agent can be asymptomatic in upto 80% of women which can make diagnosis and detection difficult. Left undetected and untreated, *C. trachomatis* can ascend the upper genital tract causing inflammation and scarring in both male and female reproductive tracts and causes serious sequelae including pelvic inflammatory disease(PID), infertility, ectopic pregnancy in females. The agent in the cervix may be transmitted to the neonate passing through the infected birth canal resulting in development of inclusion conjunctivitis in newborn and chlamydial pneumonia in infants. Chlamydial genital tract infection is an important risk factor for HPV-induced cervical neoplasia as well as HIV transmission. In developed countries, there has been a steady increase in the rates of STIs, especially viral STIs and genital chlamydial infection. Therefore screening for genital *C. trachomatis* infection is a high public health priority. However, reliable data on the prevalence of genital *C. trachomatis* infection in India had been scanty. Using conventional serologic and antigen detection assays, a prevalence ranging from 30% to as high as 81% has been reported for *C. trachomatis* in asymptomatic women. Although many diagnostic modalities are present for detection of *C. trachomatis*, none are 100% sensitive and each has its own limitations.

Current situation & disease burden

Globally, *C. trachomatis* causes 30-40% of non-gonococcal urethritis (NGU) in men. The majority of epidemiological studies conducted in India have used the criterion of demonstration of >5 neutrophils in urethral smears or endocervical specimens to establish the diagnosis of NGU, without isolating the causative organism. The prevalence using this criterion varies from 1.5-19% among STI clinic attendees in different parts of the country. In women with symptoms of lower genital tract infection and infertility, who were attending gynaecological clinics in a Delhi hospital between 1990 and 1992, the prevalence was 41% and 36%, respectively. In young women undergoing a routine gynaecological check-up in Mumbai in 1994, genital chlamydial infection was diagnosed in 15% of cases; 53% of cases showed clinical signs suggestive of cervicitis and only 2% had PID. In this study, the contribution of *C. trachomatis* to PID was much lower compared with a study from Nagpur, in which it was responsible for 33% of cases with PID. This infection was detected in 23.3% of attendees of the gynaecological clinic of an outpatient department (OPD) in Delhi in 1994. Among women seeking healthcare for reproductive health complications, chlamydial infection rates of 0.3–3.2% and 23.3–33% have been reported from different parts of India with increasing prevalence.

The wide variation in *C. trachomatis* prevalence could also be due to variety of laboratory methods used for confirmation. The risk factors include a low socioeconomic status, multiple sexual partners and the use of intra-uterine devices, while the protective factors are a higher age group and the use of oral and barrier contraceptives. The variation in reported prevalence also depended on the population sampled. Most of these studies focussed on high risk groups (female sex workers, STI/ infertility patients and HIV-positive women) and were limited by small sample sizes. A study from Tamil Nadu found the prevalence of active genital *C. trachomatis* infection in a healthy adult female population by NAAT of the urine as 1.1%. However, among symptomatic men and women attending a STI clinic, the prevalence of confirmed *C. trachomatis* infection by culture and/ or nested PCR detecting major outer membrane protein (MOMP) was 30.8%. Among antenatal clinic attendees in Delhi in 1999, 21.3% were found to be infected with *C. trachomatis,* with high incidences of stillbirths, prematurity and low birth-weight. Another study from Delhi in 1999 showed the prevalence of chlamydial infection in 17% and 18.6% of the cases during mid-pregnancy and labour. In a study conducted in Vellore in 1993, the prevalence of *C. trachomatis* in pregnant women was found to be 3.3% using EIA which detects the presence of chlamydial antigen. The authors also reported a higher prevalence of *C. trachomatis* in rural women (5.9%) compared to urban women (1.8%). More recently in 2012, the prevalence of genital *Chlamydia* infection amongst pregnant women in a private tertiary care hospital in southern India was found to be only 0.1% in a non-urban setting using NAAT technique.

Using various diagnostic tests with different performance characteristics, the prevalence of chlamydial infection among women in developing countries specifically sex workers (FSWs) varies from 8.5-37.0%. The incidence of chlamydial infection in FSWs in Surat in 2003 was estimated to be 8.5% using PACE2 test while in Ahmedabad, it was almost double. Among the tribal populations (patients/ general population) from central India, although STI was high (36.5%), only 4.0% chlamydial infection was reported during 2007. In a recent study reported from New Delhi in 2010 on symptomatic females, genital chlamydial infection, as detected by Roche Amplicor test, DFA and in-house PCR assay, was 25.2%.

ICMR contributions during XIth Plan:

NIOP, New Delhi

Contributions in Public Health:

In order to have a reliable estimate of female genital *C. trachomatis* infection in urban and slum dwellers residing in Delhi region, the prevalence of infection with this pathogen was determined using gold standard method. Subsequently, a high prevalence (>30%) of genital chlamydial infection was reported in symptomatic women suffering from lower genital tract infection. Secondly, we aimed to identify the serovars of high pathogenecity by genotyping and serovar D was found to be prevalent in females.

- Established culture of Primary cervical epithelial cells from the cervix – cell line deposited in ATCC

- Established a repository of *C. trachomatis* (45*)* at National Institute of Pathology.
- Trained many doctors, researchers and technicians for culture procedures.

Diagnostics

C. trachomatis

- Established the **morphological criteria for localization of intracellular *C. trachomatis* inclusions in cervical smears,** which has the advantage of being a rapid method of screening a large number of women in the field setting.
- We **developed a cost-effective indigenous monoclonal antibody based diagnostic assay for detection of *C. trachomatis*.** This technology has been transferred to the company for commercialization.
- We demonstrated anti-cHSP60 antibodies as prognostic marker in fertile and sub-fertile women and our study suggested that detection of anti-cHSP60 antibodies would help in the early prognosis of sequelae in *C. trachomatis*-infected women. A **cHSP60 based Dot-Blot assay has been developed for prognosis/ diagnosis of immunopathological sequelae in *C. trachomatis* infected women.**

C. pneumoniae

- Prevalence of *C. pneumoniae* in coronary artery disease patients was studied.
- Nucleic acid amplification tests (multiplex, semi-nested and nested PCR) were used for detection of *C. pneumoniae* in the venous blood and atheromatous plaques/ tissue of coronary artery disease patients by PCR and Real time PCR. nPCR showed higher sensitivity and specificity than mPCR and snPCR.
- Demonstrated the presence of *C. pneumoniae* in atheromatous tissue of CAD patients. Beside we also showed the presence of other pathogens (*Helicobacter pylori*, Cytomegalovirus and Herpes simplex virus-1) in atheromatous tissue of CAD patients in different vascular locations, *viz.:* aorta, coronary artery and carotid artery.
- The highest positivity was detected for *C. pneumoniae* followed by *H. pylori*, CMV and HSV-1. Among vascular infection sites, aorta and coronary artery were more prone to pathogen infection compared to carotid artery in CAD patients.

Treatment

- Evaluated new microbicides for their ability to prevent sexual transmission of *C. trachomatis* as treatment (Doxycycline, Azithromycin) failures and recurrent chlamydial infections are common in India. In this regard, the anti-microbial activity of novel polyherbal formulation

BASANT developed by Prof. G.P. Talwar was evaluated *in vitro* for its antichlamydial activity and our results suggested its potential clinical utility for the prevention of *C. trachomatis* infection by the sexual route.

- Further re-infections were determined (23%) in infected female patients and invitro drug susceptibility profiling was done to check the sensitivity pattern of *C.trachomatis* isolates obtained from treatment failure patients. It was found that recurrent *C.trachomatis* infection can be resulted by the heterotypic resistance with decrease drug susceptibility of the isolates. We further studied ygeD gene in *C.trachomatis* clinical isolate having decreased drug susceptibility profile and a point mutation was detected in ygeD gene of *C.trachomatis* isolate In addition our study showed that azithromycin treatment inhibits phosphorylation of ERK which may further be involved in inhibition of IL-8, MCL1 and IAPs suggesting ERK-mediated mechanism in resolution of recurrent chlamydial infection in infertile women.
- Demonstrated that azithromycin treatment inhibited inflammatory cytokines and chemokines, anti-apoptotic genes and phosphorylation of ERK suggesting that azithromycin with its properties apart from anti-bacterial activity may contribute to its therapeutic potential in treatment of chronic recurrent infection in infertile women.

Basic Research

C. trachomatis

- First time reported mucosal (cervical) immune responses to chlamydial infection. Reported downregulation of cell mediated immunity in the cervix of *Chlamydia*-infected women with inhibition of IL-2 locally in the cervix during chlamydial infection.
- *In vitro* study on primary and secondary immune response in cervical lymphocytes of *Chlamydia*-infected women showed that mucosal responses are more helpful in understanding the cytokine responses in female genital tract and are more reliable in understanding the mechanism of the disease.
- Demonstrated role of chlamydial heat shock proteins (cHSPs) and chlamydial inclusion proteins in the induction of pathogenic or protective responses. We showed that IFN-γ could be involved in the modulation of immune response during chlamydial infection directly by causing acute inflammation or indirectly through modulation of cHSP60 expression.
- We also defined combination of local and systemic immune biomarkers (IFN-γ, CRP and cHSP60 antibodies) that can be used for the prediction of women at higher risk of developing fertility disorders due to chlamydial infections.
- Studies suggested that *C. trachomatis* infection in the female genital tract may be regulated by both the synergistic action of the cytokines and the sex hormones estradiol and progesterone which can again be considered as a predictive biomarker for chlamydial pathogenesis.

- Study of various subsets of dendritic cells in the cervix of *Chlamydia*- infected women, showed that both myeloid and plasmacytoid DCs are mobilized to the cervix during chlamydial infection and that plasmacytoid DCs are upregulated in pathologic conditions.
- High titers of antibodies to cHSP60 and cHSP10 were detected in women with PID/ infertility than cervicitis. The present study revealed that cHSP60 antibodies are more reliable serological markers for the sequelae resulting in infertility in sub-fertile patients.

C. pneumoniae

- Our study revealed that seropositivity for specific IgA to *C. pneumoniae* and *H. pylori* was significantly higher in CAD patients, alongwith higher high sensitivity C-reactive protein levels, demonstrating the chronic persistent infection of *C. pneumoniae* and *H. pylori* in CAD patients in India. This suggests the role of burden of bacterial infection in progression of disease; however, *C. pneumoniae* IgA remains the single independent atherosclerotic marker. In addition, level of hsCRP, an independent pro-atherosclerotic marker was also high only in *C. pneumoniae* IgA-positive patients.
- Study on immunopathogenesis of *C. pneumoniae* in CAD showed the role of IL-6 in the pathogenesis of CAD which may get accelerated through *C. pneumoniae* infection. Further circulatory cytokines, namely, IL-4, IL-8 and adhesive molecules like ICAM-1 were enhanced in CAD patients infected with *C. pneumoniae* whereas, in contrast to this, IL-10 and IFN-γ were lowered.
- Demonstrated the role of *C. pneumoniae* HSP60 in etiopathogenesis of CAD .Showed that cHSP60 infection is accelerated through uptake of LDL and cholesterol in CAD patients, as patients positive for cHSP60 have higher levels of lipid transporter (APO-A, B and E) and lipid signalling molecules (LDLR, LPL, LPA) compared to cHSP60-negative CAD patients. Additionally, expression of Bax, caspase 3, 8 and 9 was higher, whereas, the expression of c-FLIP and PPAR-α and γ was lower in cHSP60-positive CAD patients.

NIRRH, Mumbai

1. Detection methods for RTIs/STIs:

i. Specific and sensitive tests to detect Bacterial vaginosis, Candida and Trichomonas infection in women.

ii. Polymerase Chain Reaction (PCR) for diagnosis of *Chlamydia trachomatis* infection using cervical, self collected introital or first void morning urine samples, validated and ready for commercialization.

iii. Significant association of *Chlamydia trachomatis* and Bacterial vaginosis with infertility. 45.6% of women with Chlamydia infection and 40.3% of women with Bacterial vaginosis had history of infertility.

iv. *Chlamydia* infection and Bacterial vaginosis were asymptomatic.

v. Vaginal secretion pH could be used for diagnosis of Bacterial vaginosis.

Under different projects, these tests are in use for screening and treatment of women attending the Obstetrics and Gynecology Out Patient Department of Seth G.S. Medical College and K.E.M. Hospital, Institute clinics as well as in the community studies.

2. Prevalence study on RTIs/ STIs:

Chlamydia trachomatis infection rate varied from 1.96% to 25% in women with different clinical manifestations, highest among women with infertility. In community based study, this infection rate was only 0.4%. Rate of bacterial vaginosis varied from 7.5% to 25.9%, highest in women with infertility, while in community, the rate was 3.2%. In hospital and community based study, *Candida* infection rate was 3.9% and 23.9% respectively. *Trichomonas* infection was observed only in 0.78% of women with history of infertility. HSV-2 IgG antibody positivity was seen in 6.32 % of women.

3. Association of host immunogenetic factors with *Chlamydia trachomatis* infection:

Specific HLA-A*33 seems to be associated with protection from infertility in *Chlamydia trachomatis* infected women.

No role of heterozygous CCR5s 32 gene deletion was observed in prevention of tubal pathology in *Chlamydia*-infected women. Heterozygous deletion in CCR5 gene was observed only in 2% of studied population.

4. Preparation of following guidelines:

(i) National Guidelines on Prevention, Management and Control of Reproductive tract Infections including Sexually transmitted infections.

(ii) Operational Guidelines for Program Managers and Service Providers for Strengthening STI/RTI Services

Institute took a leading role in preparation of these guidelines which are now used by NACO, Ministry of Health and Family Welfare, Government of India. Institute Staff were also involved in training of Doctors, Nurses and Laboratory Technicians on RTI/STI related issues.

2. Important and Essential Research Activities to be continued to XIIth Plan

1. Immunopathogenesis of reactive arthritis/ undifferentiated spondyloarthropathy induced by *Chlamydia trachomatis* (2010-13)

Study highlighted the presence of *C. trachomatis* in joints of Indian patients with genitourinary ReA and uSpA. Evaluation with more samples for cytokine profile is undergoing.

3. New Research activities to be conducted in XIIth Plan

1. **Immunopathogenesis of preterm birth in women with *Chlamydia trachomatis* infection.**

   Earlier studies have shown a significant association between Preterm Birth (PTB) and *C. trachomatis* infection in women. PTB is one of the causes of perinatal mortality and morbidity as preterm activation of the inflammatory cascades can induce premature rupture of membranes and preterm delivery. The objective is to study the immunopathogenesis of PTB and also to find early markers which could be used in routine obstetrical practice to manage and prevent PTB or its negative consequences.

2. **Role of tubal activins and inducible nitric oxide synthase in the pathogenesis of ectopic pregnancy in women with *Chlamydia trachomatis* infection.**

   Tubal Ectopic Pregnancy (EP) is the major cause of maternal morbidity and mortality in early pregnancy. How *C. trachomatis* induces Fallopian Tube (FT) pathology and subsequent development of EP is poorly understood at the molecular level. Study may help to prevent ectopic implantation in patients with prior *C. trachomatis* infection of FT.

3. **Mechanism and effects of *Chlamydia trachomatis* infection in asymptomatic male partners of infertile couples.**

   While the role of *C. trachomatis* in infertile women has been studied, however, there is a paucity of data on *C. trachomatis* infected male partners of infertile women. The ultimate objective is to define cellular and humoral markers predictive of ascension to upper genital tract in asymptomatic male infertile patients in relation to the semen quality.

4. Publications during XI Plan

NIOP, New Delhi

Peer-reviewed journals: 58

NIRRH, Mumbai : 8 (on *C. trachomatis*)

1. Gokral JS, **Mania-Pramanik J,** Meherji P, Mali BN. Potential of introital swab testing for *Chlamydia trachomatis* in resource poor setting: An Indian perspective. *International J. of Fertility and Women's Medicine,* 50(3): 140-143, 2005.
2. **Mania-Pramanik J,** Potdar S, Kerkar S. Diagnosis of *Chlamydia trachomatis* infection. *Journal of Clinical Laboratory Analysis,* 20: 8-14, 2006.

3. Mania-Pramanik J, Kerkar SC, Potdar S, Mehta PB, Salvi VS. Use of vaginal pH in diagnosis of infections and its association with reproductive manifestations. *J Clinical Laboratory Analysis,* 22(5): 375- 379, 2008.

4. Eno E, Okenu D, Mania-Pramanik J, He Qing, Igiet Seme J, Ananaba G, Lyn D, Black C, Eko FA. Vibrio Cholerae ghost-based sub-unit vaccine induces cross-protective chlamydial immunity that is enhanced by CTA2B, the non-toxic derivative of cholera toxin. *FEMS Immunology & Medical Microbiology,* 55: 280-291, 2009.

5. Dwibedi B, Mania-Pramanik J, Sahu P, Kar SK, Moharana T. Prevalence of genital *Chlamydia* infection in females attending an Obstetrics and Gynaecology OPD in Orissa. *Indian Journal of Dermatology,Venerology and Leprology,* 75(6): 614-616, 2009.

6. Mania-Pramanik J, Kerkar SC, Mehta PB, Salvi VS. Bacterial vaginosis: A cause of infertility? *International Journal of STD & AIDS,* 20: 778-781, 2009.

7. Mania-Pramanik J, Kerkar SC, Valhabhadas Anjali, Mehta PB, Salvi VS. Does CCR5 gene-Δ32 deletion protect *C.trachomatis* infected Indian women from tubal pathology? *Microbiology Research*, 3: 19-21, 2011.

8. Mania-Pramanik J, Kerkar S, Sonawane S, Mehta P, Salvi V. Current *Chlamydia trachomatis* infection: A Major Cause of Infertility. *Journal of Reproduction and Infertility,* 13(4): 204-210, 2012.

5. List of Patents

NIOP, New Delhi

- Patent for Development of serovar specific monoclonal antibody to *Chlamydia trachomatis* –Application no. 792/DEL/2003–Granted (patent no.246263)- Licensed to M/S Accurex through BCIL.
- Patent filed for Development of novel primers and method for amplification chlamydial heat shock protein 60 Application No..186/ DEL/2008 ---Technology transfer to BCIL.
- Patent filed for development of Primary cervical epithelial cells Application No.(3563/DEL/2012)

NIRRH, Mumbai

- A patent applications filed; Title: A PCR method for detection of *Chlamydia trachomatis*

  Application no. 834/ DEL/ 2012.

6. Technologies developed/ transferred to the Industry

NIOP, New Delhi

- *Chlamydia trachomatis*-Development of monoclonal antibody based diagnostic assay has been transferred to m/s Accurex for commercialization.
- Technology for cHSP60 dot blot assay has been given to BCIL for commercialization.

NIRRH, Mumbai

- PCR based method for detection of *Chlamydia trachomatis*. This is ready for commercialization.

7. Manpower trained/New human resource generated

NIOP, New Delhi

- 32 personnel including 15 research fellows, 21 M.Sc. students and 5 MD students were trained on various aspects of chlamydial disease mainly on diagnostics of chlamydial infection.
- 7 Ph.D.(s) were awarded during the X1th Plan.

NIRRH, Mumbai

- **Workshops:** Three workshops were conducted to give hands-on training to Doctors & Lab. Scientists (n=34) on detection of RTIs/ STIs.