"PREVALENCE AND LABORATORY DIAGNOSIS OF CHLAMYDIA TRACHOMATIS INFECTION IN INFERTILE WOMEN BY ELISA AND PCR – A ONE YEAR CROSS SECTIONAL STUDY"

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Dissertation

Submitted to the

KLE University, Belgaum, Karnataka

In partial fulfillment

of the requirements for the degree of

DOCTOR OF MEDICINE (M.D)

in

MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY
J.N.MEDICAL COLLEGE, NEHRU NAGAR,
BELGAUM-590010.

MAY 2011

KLE UNIVERSITY, BELGAUM

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LIST OF ABBREVATIONS USED

ARC	**Assisted reproductive centre**
CFT	**Complement fixation test**
DIF	**Direct immuno fluorescence**
DNA	**Deoxyribonucleic acid**
EB	**Elementary body**
EIA	**Enzyme immuno assay**
EP	**Ectopic pregnancy**
Hsp	**Heat shock protein**
IgG	**Immunoglobulin G**
IgM	**Immunoglobulin M**
IF	**Immuno fluorescence**
kDa	**Kilo Dalton**
KLE	**Karnataka lingayat education**
LCR	**Ligase chain reaction**
LPS	**Lipopolysaccharide**
LGV	**Lymphogranuloma venereum**

MIF	**Micro immuno fluorescence**
MOMP	**Major outer membrane protein**
MRC	**Medical research centre**
NAAT	**Nucleic acid amplification test**
PCR	**Polymerase chain reaction**
PID	**Pelvic inflammatory disease**
RB	**Reticulate body**
RNA	**Ribonucleic acid**
STD	**Sexually transmitted disease**
STI	**Sexually transmitted infection**
TFI	**Tubal factor infertility**
TNF	**Tumor necrosis factor**
TWAR	**Taiwan acute respiratory**
VD	**Venereal diseases**
WHO	**World health organization**

ABSTRACT

Background and Objectives: *Chlamydia trachomatis* has currently emerged as the most common sexually transmitted pathogen. It is usually asymptomatic but is known to cause salphingitis, pelvic inflammatory disease, infertility and ectopic pregnancy in females. It is very difficult to diagnose this condition as it does not grow in cell free media. Hence cell culture, ELISA and PCR form the main stay of diagnosing this condition. The present study is done to find out the prevalence of *Chlamydia trachomatis* infection in infertile women by PCR and ELISA and to compare the sensitivity and specificity of ELISA and PCR.

Methods: This study was conducted in the Department of Microbiology, Jawaharlal Nehru Medical College, Belgaum from Jan 2009 - Dec 2009. Blood samples and endocervical swabs were collected from 115 infertile women of reproductive age attending the assisted reproductive centre of KLE's DR. Prabhakar Kore Hospital and Medical Research Centre. ELISA was done to detect anti Chlamydia IgG antibody in serum samples and PCR was done to detect Chlamydial DNA in endocervical swabs.

Results: The prevalence of *Chlamydia trachomatis* infection in infertile women in this study was 2.6% by PCR and 0.9% by ELISA. The sensitivity of ELISA was 33.3% and specificity was 100%. The positive predictive value and negative predictive value were 33.3% and 98.2% respectively. Maximum number of Chlamydia positive cases were in the age group of 35-44 years and maximum number of Chlamydia positive cases had a duration of infertility between 1-5 years.

Interpretation and Conclusion: *Chlamydia trachomatis* infection is known to cause infertility and this needs to be diagnosed at the earliest, for the specific treatment and prevention of complications. ELISA and PCR can be used for the diagnosis of *Chlamydia trachomatis* infection.

Key words: *Chlamydia trachomatis ;*ELISA ;PCR ;Pelvic inflammatory disease ;Infertility

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INTRODUCTION

Sexually transmitted infections (STI) or Sexually transmitted diseases (STD's) also known as venereal diseases (VD) are diseases passed between people through sexual contact and are caused by bacteria,viruses , parasites and fungii. The most common causes of STD's are *Neisseria gonorrhoeae, Chlamydia trachomatis, Treponema pallidum, Ureaplasma urealyticum, Mycoplasma hominis, Calymmatobacterium granulomatis, Human immunodeficiency virus, Herpes simplex virus, Human papilloma virus, Molluscum contagiosum virus* and *Trichomonas vaginalis*[1].

Chlamydia trachomatis (*C. trachomatis*) has recently emerged as the most common sexually transmitted pathogen[2,3,4]. Chlamydia trachomatis is an obligate intracellular gram negative bacilli producing mainly asymptomatic infections in women accounting to 70-80%.

It causes 2 types of genital infections

i. Urogenital syndromes caused by the oculogenital serotypes D-K collectively referred to as genital Chlamydiasis
ii. Lympho granuloma venerum (LGV) caused by serotypes L_1, L_2, L_3[5].

According to world health organization (WHO) there are 90 million Chlamydial infections detected globally each year[6].

It can involve both the lower and upper genital tract of men and women[7].

As many as 25% of men and 70-80% of women identified as having Chlamydial genital tract infection are asymptomatic[1]. It is responsible for a spectrum of diseases including urethritis, mucopurulent cervicitis, with a variety of sequelae like

salphingitis, endometritis, pelvic inflammatory disease (PID), Ectopic pregnancy (EP), Tubal factor infertility (TFI), epididymitis and proctitis[8].

Like other STD's the brunt of this infection is on women, who suffer from its damaging consequences[8].

Infection per se in confined to the epithelial surfaces but an immune mediated host response can cause severe inflammation and damage affecting the deeper tissues, especially after repeated episodes[9] .Left undetected and untreated ,Chlamydia infection can ascend to the upper genital tract, causing inflammation and scarring in the female reproductive tract producing salphingitis and PID[10].

About 8% of women with cervical Chlamydial infection will experience salphingitis as a complication.Infertility is an important complication in 12-13% of women following a first attack rising to 75% after more than 2 episodes[7].

Many of the women who have tubal damage and high levels of antibody to Chlamydia have no prior clinical history of salphingitis. This has led to the concept that a silent salphingitis may occur during what is apparently an uncomplicated Chlamydial infection, and this may be more common than clinically apparent salphingitis[7].

Lower socio economic class and young age are the most readily identifiable risk factor for chlamydial infection[7].

The true prevalence of genital Chlamydiasis is not known in the developing countries as laboratory diagnosis in not widely available. In India, chlamydial infection has been reported in 20-30% of women with mucopurulent cervicitis and 30-60% of those with salphingitis and PID[5].

The fact that the growth of this pathogen does not normally occur outside living cells poses a challenge to its diagnosis[11].

Detecting Chlamydial genital infection and preventing transmission and spread of this infection to the upper genital tract are a challenge for both clinicians and laboratory personelles

C. trachomatis can be diagnosed by

i. Isolating the organism in cell culture
ii. Direct detection of inclusion bodies in clinical specimens
iii. Detection of specific antigens
iv. Serological tests for the detection of antibodies
v. Molecular diagnostic methods[7].

Culture analysis of endocervical swab samples has been considered the diagnostic gold standard for detection of cervical infection in women, but factors like sample collection, transportation time, storage of samples and toxicity of swab can decrease the rate of isolation[12].

Direct antigen detection using direct fluorescent antibody test is expensive and needs equipments like fluorescent microsope with ultra violet light, along with expertise.

Serological methods like ELISA are technically simple to perform and comparitively less expensive. In cases of deep seated upper genital tract chlamydial infections in whom the bacteria are no longer detectable locally, a positive serological test,which detects antibodies in the serum, may be the only indication of chlamydial involvement[13].

Since the association between *C. trachomatis* specific IgG antibodies and tubal pathology has been noted, measuring *C trachomatis* IgG antibodies in serum is used as a screening method for tubal pathology and pelvic inflammatory disease (PID)[4]. In general, determination of antibody titer on a single specimen of serum from adults is unhelpful with the possible exception of cases of pelvic inflammatory diseases, epididymitis and LGV[7].

Chlamydial serology was found to be an inexpensive, non invasive test for tubal factor infertility that matches or surpasses the predictive value of most standard fertility tests[8].

Polymerase chain reaction (PCR) and Ligase chain reaction (LCR) are the two alternatives to conventional methods and have proved useful for the detection of *C. trachomatis* infection in cervical and urethral samples both in symptomatic and asymptomatic women[8]. The introduction of these specific DNA amplification methods allowed the development of diagnostic method for all kinds of microorganisms including fastidious and noncultivable agents[15].

A comparative study of cell culture and nucleic acid amplification tests (NAAT's) observed the later to be more sensitive and recommended NAAT to be considered the new gold standard [8].

In chronic or persistent chlamydial infection the level of Chlamydia is very low and bacteria are often not viable.This results in continuing positive NAAT's but only intermittent isolation of viable Chlamydia and the positive assays for Chlamydial protein antigen detection [9].

NAAT's have become widely used since they show greater sensitivity than other tests as well as good specificity[16].

Chlamydial PID, a major reproductive health care issue in women is the most important preventable cause of infertility and adverse pregnancy outcome like ectopic pregnancy[8].

Early diagnosis is one of the most cost effective means of preventing the long term sequelae of chlamydial infection[8] .But women at highest risk often have the least access to health care facility [17].Therefore there is a need for a rapid simple and accurate test to detect *Chlamydia trachomatis* infection[18]. Since the infection is amenable to antibiotic therapy, screening and appropriate treatment may improve the outcome[18].

The lack of available diagnostic testing makes control of chlamydial gential infection difficult[19].Emphasis should be given to simple cost affective testing strategies such as serology for screening of high risk asymptomatic women for treatment and prevention of various sequelae[8].Gential infections caused by *C. trachomatis* are often asymptomatic and early detection to avoid serious complication is of real value since effective treatment exists.

PCR has recently been introduced for detection of *C. trachomatis* and studies have reported its superior sensitivity in comparision with cell culture, ELISA or direct fluorescent conjugated antibody staining [11].

The NAAT's associated with serology is found to be the best diagnostic strategy[8].

Since we did not have facilities for cell culture and Direct fluorescent antibody tests,we have not used them in the present study.

In view of the sensitivity and broad applicability, we attempted to set up a PCR based method to detect *C. trachomatis* infection in infertile women from endocervical swabs and used this as a gold standard to compare it with ELISA for the detection of Anti Chlamydial IgG antibodies

The sensitivity and specificity of ELISA is also evalualed.

OBJECTIVES OF THE STUDY

1. To study the prevalence of *Chlamydia trachomatis* infection in infertile women by ELISA and PCR
2. To compare the sensitivity and specificity of ELISA and PCR for the detection of *C. trachomatis* infection in infertile women.

REVIEW OF LITERATURE

History

Chlamydiasis is caused by the bacterium *Chlamydia trachomatis*. The word chlamys in greek means a cloak draped around the shoulder and trachoma means roughness. They were described as being draped around the cell nucleus like a cloak and because of their size, thought to be a protozoa. The organism was named chlamydozoaceae, thus the term Chlamydia is a misnomer stemming from the basic misconception. The designation of *C. trachomatis* refers to its etiological role in trachoma[7].

Chlamydiae were first observed in 1907, when intracellular inclusions were detected in conjunctival epithelial scrapings from an orangutan which had been inoculated with material from a patient with trachoma . Subsequently 111 inclusions were seen in epithelial cells from conjunctiva of infants with non gonococcal ophthalmia and from genital specimens of the parents of infected infants[9].

In giemsa stained epithelial cells from human subjects , they observed intracytoplasmic inclusions containing large numbers of minute particles now know as elementary bodies. These workers proved to be correct in suggesting that inclusion bodies represent the causal agents of trachoma[9]. The first genital isolation was reported from the cervix of the mother of a new born infant who had developed inclusion conjunctivitis. *C. trachomatis* had been isolated from the cervix, urethra and from the ducts of Bartholin glands.

Isolation from yolk sac of embryonated eggs in 1957 and from cell cultures in 1965 confirmed its existence as a bacterium[9].

Epidemiology

The natural habitat of *C. trachomatis* is humans. It causes significant infection and disease world wide.

In the United.States *C. trachomatis* is the most common sexually transmitted bacterial pathogen and a major cause of pelvic inflammatory disease. An estimated 3 million *C. trachomatis* infection occurs annually[1].

The epidemiology of oculogenital infection by *C. trachomatis* (sometimes termed paratrachoma) differs markedly from that of classical endemic trachoma[7].

The WHO has estimated that the number of new genital infections caused by Chlamydia has almost reached 100 million annually which makes these infections one of the most prevalent sexually transmitted diseases.

It should however be observed that population based data and reliable surveillance systems are generally lacking .

The number of cases detected ,closely depends on whether screening for chlamydial carriers is performed , as the majority of those who are infected by *C. trachomatis* are more or less asymptomatic and have no reasons to consult health provides[7]. The number of genital infections detected in a region or country is also dependent on whether or not partner notification is performed .

Some of the currently used diagnostic tests have a low sensitivity and / or specificity. This means that either a large proportion of cases remain undetected or are being falsely reported as Chlamydia infected[7].

To sum up, epidemiological data based on population surveys are almost nonexistent and surveyllence data must be interpreted with caution.

Young age is identified as a risk factor to contract genital chlamydial infections and also re-infection. Thus in most societies young teenagers belonging to these age groups should be addressed in preventive work, which has to start as early as school age, to be effective.

As for other sexually transmitted diseases a high number of sex partners is a risk factor. The transmission rate of genital chlamydial infection is, however, poorly documented.

About 8 % of women with cervical chlamydial infection will experience salphingitis as a complication[7].

Genus Chlamydia

The genus Chlamydia belongs to the family Chlamydiaceae in the order Chlamydiales.

It consists of 4 species

1. *Chlamydia trachomatis*
2. *Chlamydia psittasi*
3. *Chlamydia pneumoniae*
4. *Chlamydia pecorum*[5].

They are distinguished by the clinical disease they produce, their staining characteristics, their antibiotic susceptibility patterns, their surface epitopes and their limited DNA homology[20].

1) *C. trachomatis* has 3 biovars

A) Trachoma

B) LGV

C) Mouse pneumonitis

Biovar trachoma has 12 serovars

Servovar A,B,Ba and C causes trachoma

Serovar D,E,F,G,H,I,J,K causes sexually transmissible genital infections, vertically transmissible neonatal infections and adult inclusion conjunctivitis.

Biovar LGV has 3 serovars L_1, L_2, L_3, which causes a mild self limited genital ulceration followed by regional lymphadenopathy and associated with systemic symptoms[9].

Mouse penumonitis: Infection with this biovar of *C. trachomatis* is important as a possible source of error when attempting animal experiments with isolates from humans

Chlamydia trachomatis are also divided into sero groups based on similarities of their surface epitopes.

Serogroup B complex includes serovar B,Ba,D,E, L_1 andL_2

Serogroup C includes serovar A,C,H,J,I

The remaining serovars - G,K,F, L_3 have over laping surface antigens and cannot be readily placed into either serogroup[20].

2) *Chlamydia psittasi* causes disease or asymptomatic infection in a variety of avian and mammalian species. Human infection with *Chlamydia psittasi* is characterized either by predominantly respiratory symptoms or a systemic illness with less prominent respiratory manifestations.

3) *Chlamydia pneumoniae* was initially named TWAR and thought to be a variant of *C. psittaci* but later classified as a separate species on the basis of 10 %

DNA homology with other chlamydial species.It causes a subacute and usually self limited respiratory infection which occurs in epidemics every 2-3 years.

4) The newest species *Chlamydia pecorum* causes various diseases of sheep and cattle including encephalitis, infectious polyarthritis, pneumonia and diarrhoea[9].

Morphology and Development

Chlamydia are non motile and highly specialized gram negative bacteria. Like other prokaryotic cells they multiply by binary fission. For multiplication, Chlamydia are dependent on precursor substances and energy from the host cell. For example amino acids of which isoleucine is essential for chlamydial group[9].

Chlamydia are clearly known to be bacteria and to resemble viruses only in their obligate intracellular nature and their inability to synthesise adenosine triphosphate. In other respects these energy parasites are similar to bacteria , possessing both RNA and DNA, metabolic enzymes and a cell wall similar to that of gram negative bacilli[21].

Chemical composition

Chlamydia trachomatis is composed of approximately 35 % protein and 40-50 % lipids. The genome is composed of a circular double stranded DNA with a molecular weight of approximately 6.6 x 10^8 dalton. In addition, a plasmid that is approximately 4.4 x 10^6 daltons has been identified in all serovars of *C. trachomatis*.

The outer membrane contains a protein that comprises 50-60% of all proteins in the outer membrane. This major outer membrane protein (MOMP) has a molecular weight of 4 x 10^4 daltons. The MOMP gene encodes for nine different amino acid

sequences called regions. There are 5 highly conserved regions that do not vary from serovar to serovar and 4 variable regions that contain distinct amino acid with different serovars (variable domain - VD). Recent investigations have shown that neutralising antibodies correspond to amino acid sequences that are surface exposed.

The VD-3 and conserved regions are believed to be below the surface of the outer membrane[20]. The sequencing of the MOMP has provided an opportunity to establish serotype specific antibody binding, a potential prime site for vaccine development. MOMP is thought to provide rigidity to the chlamydial cell wall by extensive disulphide bond cross linking and to function as a porin, it may also be an adhesin[22].

Chlamydiae have a unique developmental cycle which differentiates them from all other microorganisms. There are two morphological forms, namely elementary body (EB) and reticulate body (RB).

EB's are small spherical infectious particles 250-300nm in diameter, which are relatively hardy and capable of extracellular survival but do not replicate. The rigidity of the EB wall is due to the disulphide linking between MOMP and several cystein rich proteins.

The 3 cystein rich proteins of 62, 40, and 15 kDa seem to confer structural strength to the outer membrane of the elementary body and may compensate for the absence of peptidoglycan in the outer membrane[20].

RB's are the larger (800-1200nm) intracellular , non- infectious , replicative, metabolically active form of Chlamydia whose cell walls lack the disulphide cross linking present in EB's[9].

EB's infect susceptible non –professional phagocytes, including epithelial cells.They adhere to the host cells with the aid of heparin sulphate like glucosaminoglycan molecules which are required for Chlamydia to enter the host cells[7].

A number of substances are known to enhance the contact between EB's and host cells i.e diethyl aminoethyl dextran and idoxuridine, which have been utilized in in-vitro cultures[7].

It is also assumed that one or more specific chlamydial adhesions are involved in attachment to the host cells. Candidate adhesions are defined , in part, by the fact that monoclonal antibodies directed against them inhibit cellular attachment of EB's , they include epitopes in variable domains of MOMP and the 18 and 31-32 kDa eukaryotic cell binding proteins which are expressed in EB but not in RB cell wall[9].

The uptake of EB's into the cell can occur by either phagocytic or pinocytic mechanisms and this is apparently determined by the type of host cell involved and the mode of presentation of the EB's. Within the cell, the EB's appear in membrane bound vacuoles, Chlamydia dependent modification of the endocytic membrane prevents lysosomal fusion and allows development of the typical perinuclear intracellular inclusion body[9].

Both the chlamydial and the host cell membrane carry net negative electric charge at physiological pH giving rise to electrostatic repulsion between the two surfaces .To overcome this ,the addition of DEAE dextran ,which increases the adsorption of *C.trachomatis,*while most cells allow hydrophilic bonds to form and increase the attachment[23,24].

By about 8 hrs, the EB within the endosome undergoes spheroplast like transformation to the large RB's.These RB's begin to divide by binary fission by 12 hrs inside the vesicle.

During the synthesis of substances by the chlamydial cell, the metabolism of the host cell is depressed through the influence of the chlamydia present[7].

By 20- 24 hrs , the pleomorphic progeny show central condensation and are converted to EB's.Binary fission continues till about 40 hrs

The developing Chlamydial microcolony within the host cell is called the inclusion body. The mature inclusion body contains 100-500 EB's , which are ultimately released from the host cell[5].

At the end of the reproductive phase , the Chlamydial inclusion may be so large that it displaces the nucleus of the host cell. This phenomenon may give the impression that the inclusion more or less surrounds the nucleus , like an over coat. This once gave the organisms their name Chlamydia which in greek means overcoat.

In *Chlamydia trachomatis* the mature inclusion appears to be exocytosed in 72-96 hours, the host cell being left with a scar[3].

The EB's thus released will infect other cells.

SPECTRUM OF HUMAN DISEASES CAUSED BY CHLAMYDIA

Species	**Serotype**	**Disease**
C. trachomatis	A,B,Ba,C	Endemic blinding trachoma
C. trachomatis	D,E,F,G,H,I,J,K,	Genital Chlamydiasis , inclusion conjunctivitis , infant pneumonia
C. trachomitis	L_1, L_2, L_3	Lymphogranuloma venerum
C. psittaci	Many serotypes	Psittacosis
C. pneumoniae (Formerly called TWAR strain)	Only one Serotype	Acute respiratory disease
C. pecorum	-	Diseases of sheep and cattle

Risk and Demogrhphic Factors for *C. trachomatis* infection

The most common demographic correlate of infection with *C. trachomatis* is woman with young age (< 20 yrs). The biological basis for this association is thought to be the anatomic differences in the cervix of younger women, where in the squamo columnar junction, a primary host target for *C. trachomatis* is everted and thus more exposed, a condition known as ectopy.

Demographic factors associated with older women includes marital status, multiparity, black race, poor economic condition , higher number of sexual partners, a new sexual partner, lack of use of barrier contraceptive device, and concurrent gonococcal infection are also consistently associated with chlamydial infection. The use of oral contraceptives is associated with cervical chlamydial infection[25].

CAUSES OF GENITIAL TRACT INFECTIONS AND STD[1]

	Group	**Causative agent**	**Clinical picture**
I	Bacteria a) More Common	*Neisseria gonorrhoeae*	Urethritis, cervicitis, salphingitis
		Chlamydia trachomatis	Urethritis, cervicitis, salphingitis , perihepatitis
		Gardnerella vaginalis *Mobiluncus*	Vaginitis Vaginitis
	a) Less Common	*C. trachomatis* L_1, L_2, L_3 *Treponema pallidum* *Haemophilus ducreyi*	Lymphogranuloma Venerum Syphilis Chancroid
II	Viruses a) More common	*Human papilloma virus,* *HSV–2* (less commonly 1) , *HIV* *HepatitisB Virus*	Genital warts ,condyloma and cervical dysplasia Herpes genitalis AIDS Hepatitis
	b) Less common	*Poxvirus,* *Cytomegalo virus*	Molluscum contagiosum, Heterophile negative Mononucleosis and congenital infections
III	Parasites a) More Common b) less common	*Trichomonas Vaginalis,* *Giardia lamblia* *Entamoeba hisolytica*	Leukorrhoea Enteritis, Proctitis Proctitis
IV	Fungi	*Candida albicans*	Vaginitis

INFERTILITY

Infertility implies apparent failure of a couple to conceive, while sterility indicates absolute inability to conceive, for one or more reasons. If a couple fails to achieve pregnancy after one year of unprotected and regular intercourse, it is an indication to investigate the couple. This is based on the observation that 80 % of normal couples achieve conception within a year . Infertility is termed primary if conception has never occurred and secondary if the patient fails to concieve after having achieved a previous conception[26].

The incidence of infertility in any community varies between 5 % - 15%. A total of 14% of female infertility is associated with tubal factor infertility[27].

Pathology of infertility

Male Factors

-Disorders of Spermotogenesis

-Obstruction of the efferent ducts

-Disorders of sperm motility

-Sexual dysfunction .

Semen analysis of men should show the following features when examined within two hours of productions.

Total volume 3-5 ml

Sperm Count 60-120 million / ml

Motility 80-90 % actively motile,Morphology - 80 % or more normal

Female factors

1) Uterine causes

Hypoplasia, malformed uterus and incompetent os cause habitual abortion more than infertility

Endometrial tuberculosis: Asherman's syndrome and Uterine fibroid

2)Cervical causes

Position and patency of cervical canal ,incomplete os

3)Vaginal cause

Gross infection or congenital defects like septum

4)Ovarian causes

Ovarian dysfunction like polycystic ovarian disease (PCOD)

5)Tubal Causes

Tubal block or Adhesion

Peritubal adhesions

Pevlic endometriosis

Chronic ill health can reduce fertility

6)Endocrinal causes

Hypothyroidism

Hypothalamic and pitutary dysfunction

7)Infections by

Neisseria gonorrhoeae

Mycobacterium tuberculosis

C. trachomatis

Clinical features of *Chlamydia trachomatis*[7]

(Biovar trachoma, serovar D-K)

In Females

Although most infections caused by *C. trachomatis* is women are asymptomatic, it can produce the following symptoms.

A] In Non pregnant women

1. Cervicitis
2. Endometritis
3. Salphingitis
4. Pelvic Inflammatory Diseases
5. Periappendicitis
6. Perihepatitis
7. Peritonitis
8. Perisigmoiditis

B] In Pregnant Women

1. Chorio amnionitis
2. Intra uterine infections of fetus

3. Post Partum PID

C] Proctitis

D] Sexually acquired reactive arthritis

E] Reiters syndrome

Sequalae of *Chlamydia trachomatis* in women

(i) Chronic abdominal pain

(ii) Ectopic pregnancy

(iii) Infertility

Lower genital tract infection :-

Heyman (1910) was first to suggest Chlamydial infection of the female genitalia when he claimed to have seen the trachoma inclusions , previously described by Halberstaeder and Von Prowazek in cervical cells[9].

Although initial site of infection is usually the cervix and urethra, rectum may also be infected .

Chlamydial infection of the cervix is found in 15-30 % of women attending clinics for STD's.Carrier rates for this organisms are relatively high in women because up to 70 % of the infection may have neither signs nor symptoms of infection[7].

Lower socio economic class and young age are the most readily identifyable risk factors for chlamydial infection.

The organism is present in the cervix of over 80 % of primary contacts of men with chlamydial urethritis. Approximately 2/3rd of women whose partners have chlamydial urethritis will have positive cervical or urethral cultures for *C. trachomatis*. Some 35-45 % of women with cervical gonorrhoeae have concurrent chlamydial infection i.e. approaching twice the prevalence in men with gonorrhoea. It has been suggested that gonococcal infection may activate latent or subclinical chlamydial infections[20].

Women with post gonococcal cervicitis frequently have persistent chlamydial infection and are at increased risk of developing upper genital tract infections (i.e. salphingitis)

An association between chlamydial cervical infection and hypertrophic cervicitis was noted , but there is no evidence that *C. trachomatis* causes the erosion. Cytological changes are non specific and include degeneration of epithelial cells, an increase in parabasal cells and the presence of polymorphonuclear leucocytes , lymphocytes and large mononuclear cells[7].

In addition to mucopurulent discharge, a history of post coital spotting may be obtained. On examination, ectopy or eversion of the squamocolumnar junction is frequently observed and these findings are associated with greater number of inclusions . The cervix is friable , bleeds easily with placement of speculum or sampling of sthe glandular tissues[20].

The observation of 10-30 polymorphonuclear cells/ oil imersion field on a gram's stain is diagnostic of mucopurulent cervicitis[20].

Women with urethral chlamydial infection may have urinary symptoms. The syndrome of frequency and dysuria without bacteriuria has been associated with urethral chlamydial infection in upto 60 % of women examined. Diagnosis can be confirmed by antigen testing or culture[20].

Upper genital tract infections :-

Chlamydial endometritis does occur. Mid cycle bleeding is often the only abnormal sign associated with chlamydial infection.

The association of *C. trachamatis* with salphingitis has been recognized for many years . Common physical findings include mucopurulent cervical exudates, uterine and adnexal tenderness and pain elicited with motion of the cervix[20].

It is suggested that 8 % of the women with cervical chlamydial infection will experience salphingitis as a complication The organism has been recovered from the fallopian tubes at laproscopy.

Characteristically the patient with chlamydial salphingitis has abdominal pain of longer duration and the ESR is higher than is usual in gonococcal salphingitis. A modest pyrexia is present.At laproscopy ,the tubal damage seen may be more severe than expected from the milder onset. Infertility is an important complication in 12-13 % of women following a first attack , rising to 75 % after more than two episodes. Tubal factor infertility may also be more likely after a severe episode than a mild one.

Ectopic pregnancy is another common outcome of chlamydial salphingtis, reflecting the subsequent tubal damage. Ectopic pregnancy increases 7-10 fold after each episodes of salphingtis.

Seroepidemiogical studies have shown a strong association between high level of antichlamydial antibody and tubal factor infertility and ectopic pregnancy. High level of antibody are not only seen to be specific for chlamydial MOMP antigens but also for Hsp 60. Many of the women who have tubal damage and high level of antibody to Chlamydia have no clinical history of salphingitis.

This has led to the concept that a silent salphingitis may occur during what apparently was an uncomplicated chlamydial infection and that this may be more common than clinically apparent salphingitis[7].

Oral contraceptives can influence the natural history of genital chlamydial infections and symptoms of chlamydial PID.In women with chlamydial salphingitis who are on pills, the spread of the infection into the abdominal cavity is less common than the non users[7].

Extension of infection into the peritoneal cavity results in perihepatitis (Curtis – fitz huge syndrome), characterized by right hypochondriac pain without derangement of hepatic function . At laproscopy , thin fibrinous adhesions (violin strings) are seen between the fallopian tubes and the liver capsule[7].

Adverse Pregnancy outcome

Chlamydial infections are of specific concern in pregnancy. Such infections have been associated with a number of adverse pregnancy outcome, such as early and

late abortions, still birth, prematurity, small for date babies and intra uterine fetal infections.

Pregnant women should be tested for *C. trachomatis* at their first prenatal visit in addition to testing for gonorrhea, syphilis and hepatitis B. Women at high risk for infection should be retested again in the third trimester to prevent transmission to the new born. Women at greatest risk for poor obstetrical outcome are those who are IgM seropositive for *C. trachomatis*[20].Chronic silent chlamydial infection may be the cause for recurrent abortions rather than active cervical infection[28].

A case control study reported a 2-3 fold increased risk for ectopic pregnancy in women with an IgG titre equal to or exceeding 1:64[20].

CLINICAL FEATURES IN MEN

In males, *C. trachomatis* causes urethritis, epididymitis and vaseitis[7].Among heterosexual men chlamydial infection are usually urethral and upto 50 % are asymptomatic[29]. When symptoms do occur , usually 1-3 weeks following exposure, they are indistinguishable from those of gonorrhea (urethral discharge and pyuria). However compared with gonococcal urethritis, Chlamydia in more likely to be asymptomatic. Non gonococcal urethritis is the most common clinical syndromes seen in men in US, caused by *C. trachomatis.*

Non gonococcal urethritis (NGU) is the most common clinical genital syndromes seen in males . Approximately 50-60 % of NGU are caused by *C. trachomatis*.

After an incubation period of 1-3 weeks ,men develop mild dysuria and a white to clear urethral discharge. Although mild meatal erythema may be present, physical signs are frequently absent . Antichalmydial IgM antibody is detected in the serum of men confirmed to have Chlamydial NGU. Chlamydial urethritis is asymptomatic.

Co infection with *N. gonorrhoeae* and *C. trachomatis* occurs more frequently in heterosexual men .Approximately 80 % of co- infected men develop post gonococcal uretritis if treated with penicillin or cephalosporin alone.

Epididymitis in young sexually active men is most often caused by *either C. trachomatis* or *N. gonorrhoeae.*

Unilateral scrotal pain is the predominant symptom. Scrotal swelling , tenderness and fever are common clinical findings.The epididymitis is usually unilateral which may explain, why infertility in males on the basis of past chlamydial infections is uncommon. It is however believed to cause transient subfertility[7].*C. trachomatis* has been recovered from expressed prostatic secretions.

Non LGV serovars of *C. trachomatis* account for approximately 5 % of cases of proctitis occurring more in homosexual and bisexual men . It may be asymptomatic or present with symptoms such as tenesmus, rectal pain, diarrhoea, mucus like discharge or rectal bleeding[20].

Lympho granuloma venerum (LGV)

LGV is caused by serovars L_1,L_2,L_3 of *C. trachomatis* . It is usually found in tropical and subtropical countries[5].

Transmission occurs by sexual contact, the incubation period is 1-3 weeks. In men, the primary lesion is a vesicle or ulcer on the penis. Rectal infection occur in homosexuals . In women the commonest site is the fourchette. The lesions may pass unnoticed .Healing of the primary lesion is rapidly followed by the second stage , which is characterised by swelling of the inguinal lymph nodes. Enlargement of the nodes both above and below the inguinal ligament sometimes results in a characteristic groove sign .The nodes become matted , fluctuant and fixed to the skin, they may break down and discharge through multiple sinuses.

If untreated , the disease may progress after some years to third stage, which is usually more serious in women than men. Ulceration , proctitis , rectal stricture and rectal or rectovaginal fistulae may occur. The vulva may become grossly affected by ulceration and granulomatous hypertrophy (esthiomene).

NEONATAL INFECTIONS :-

Approximately 50-60% of infants exposed to Chlamydia at delivery demonstrate serologic evidence of infection .Significant common clinical manifestations include inclusion conjunctivitis and a late onset pneumonia occurring as late as 4 months after delivery. Approximately 20% of infants showing serological evidence of infection remain asymptomatic. Conjunctivitis may be subclinical, symptoms usually begin within 1 week of delivery and consists of edema of the eyelids, conjunctival erythema and a mucopurulent discharge. Most cases of *C. trachomatis* pneumonia occur within 2 months of delivery.

Rhinitis, the initial symptom, is followed by cough and tachypnea. Typically the infants are afebrile with slightly diminished breath sounds on ascultation. The diagnosis is confirmed by culture, antigen detection of a pharyngeal aspirate or the

appearance of IgM antibodies to *C. trachomatis* .Rectal and vaginal colonization of the newborn is described but not clinically significant[20].

IMMUNRESPONSES IN CHLAMYDIAL INFECTIONS

Infections with any species of Chlamydia can vary in severity from transient asymptomatic mucosal infection to chronic diseases associated with severe inflammation and long term tissue damage. This variation is apparently due to differences in host response rather than in the virulence of different Chlamydial strains[7]. It is stated that attachment and entry into the host cell is the critical stage in infection as for other obligate intracellular parasites.

The endocytosed Chlamydia possesses the ability to inhibit the lysosomal fusion with the enclosing vesicle membrane.

ANTIBODY RESPONSE:

Chlamydia species specific antibodies of the IgM, IgG and IgA classes appear in both, the serum and local secretions. The nature and magnitude of the responses vary with the type and duration of infection.

Antibody classes :

Specific IgA (mostly secretory IgA) is more constantly demonstrable in secretions from infected mucus membranes than in serum, where as the reverse is true of IgM antibody. Some or all the IgG antibody detectable in secretions may be derived by transudation from the blood. IgM and IgG titres are liable to be higher in

generalized diseases such as LGV or PID, than in localized infections such as trachoma.

IgM antibody appears early in the serum, but may persist alongside IgG for relatively long periods, especially if reinfection occurs.

Individuals with complicated infections tend to have higher levels of antibody than do individuals with uncomplicated localized infections. For example women with salphingitis have higher levels of antibody when compared to women with cervical infection. And men with epididymitis have high levels compared to men with uncomplicated urethritis.

Antibody function :

It is much easier to measure Chlamydia specific antibodies than to define their role in protection and recovery from infection.

In the sera of patients with proven Chlamydial infections of the genital tract IgG, IgM and IgA antibodies directed against the Major outer membrane protein and various other polypeptides of EB and RB are detected.

Serotype specific epitopes on some of the polypeptides elicit neutralizing antibodies which if produced at mucosal surfaces, may play some part in protecting against reinfection ,atleast in the short term.

Antibodies against a variety of specific Chlamydial proteins are capable of neutralizing infectivity in cell culture systems, but it has not been possible to relate them to protection against infection in vivo[7].

A single chlamydial antigen, a 57 kDa protein was shown to be a member of the 60 kDa family of Heat shock protein (Hsp).Analogous Hsp are also present in mammalian cells, their production is markedly increased under conditions of stress such as increased temperature or immune system activation. As member of this protein family exists in man and shares considerable homology with the chlamydial 57 kDa protein, Hsp antibody production can cause localized inflammatory reaction, example in the uterine tubes[30].

RB's may escape the immune responses and thereby explain persistent infection.

Formation of new EB's from RB's can induce an immune response and inflammatory reaction, resulting in a cyclic process of scarring of the infected tissues. This may lead to partial or total obstruction of the fallopian tubes causing tubal factor infertility or subfertility. Such a pathogenicity might also explain the lower success rate of in vitro fertilization in women with evidence of a passed genital chlamydial infection[7].

Cellular Responses

1) Polymorphonuclear leukocytes

   In vitro ,these cells readily inactivate Chlamydia, but in vivo their early appearance and comparatively rapid dimunition in numbers in the face of persisting infections suggests that their part in the recovery process is limited.

2) Mononuclear Phagocytes.

   The relation between these cells and Chlamydia is complex. Experiments showed that viable elementary bodies are phagocytosed, but by contrast with what

happens in permissive cells, the phagosomes fuse with lysosomes and the EB's are degraded with loss of infectivity.

3) Lymphocytes :

Experiments showed that both *C .trachomatis* and *C. psittaci* bind to and cause polyclonal stimulation of murine B lymphocytes .Invitro effector molecule does not appear to be the lipopolysaccharide, which is common to both species, but it may be the MOMP. By contrast to B lymphocytes, T cells showed specific clonal stimulation by *C.trachomatis* with class II HLA restriction. In these experiments, primed blood monocytes served as antigen presenting cells.

The 60 kDa Hsp is one of the first proteins produced by a developing embryo. The sensitization of T. lymphocytes to the chlamydial Hsp may result in the generation of lymphocytes that can also react with the analogous human Hsp that can be expressed by the embryo or maternal cells in the pregnant uterus. The resultant immune system activation might directly destroy the embryo or interfere with immune regulatory mechanisms[30].

The role of T cell mediated cytotoxicity in Chlamydial infection may depend upon the Chlamydial species.

It has become obvious in a number of experimental systems that resistance to re infection and the ability to clear primary infection is in large part dependent on T cell functions.

Both CD_4 and CD_8 cells have been shown to have a protective role.

Natural killer cells may also play a role.

The cellular infiltration leading to scarring, that characterises many chronic or repeated ocular or genital infections has been ascribed by a number of workers to immunopathological mechanisms with a strong delayed hypersensitivity component.

INTERFERONS

The induction of cytokines by infection may play an important role in determining the outcome of chlamydial infection and may be important not only in defensive and protective responses, but in pathogenesis. Interferons are active against Chlamydia and the most important one appears to be interferon γ (IFN γ). Chlamydial infections induce the production of IFN γ and the organisms are sensitive to its action. In the presence of IFN γ (which act by depleting tryptophan levels in cells), chlamydial replication is inhibited at the reticulate body stage. This may result functionally in dramatic reduction in chlamydial infection and as some infected cells may be sloughed, the infection may be abrogated.

However the infected cells may also be excreting chlamydial antigen that can induce further sensitization and contribute to pathogenic mechanisms. Thus it is likely that the delayed type hypersensitivity reaction to Chlamydia species represents a double edged sword with both protective and immunopathological implications.

Tumor necrosis factor (TNF) may also play a role either independently or by enhancing IFN-γ.

In summary, the immune responses to chlamydial infections are relatively ineffective in promoting either recovery or immunity to reinfection. On the contrary

cell medicated immune responses may contribute significantly to tissue damage especially in chronic or repeated infections[7].

COMPLICATIONS

After a single episode of PID, the risk of tubal factor infertility is approximately 10% , each repeated episode doubling the risk.

The intense and chronic inflammation elicited and maintained by re- infection or persistent infection with *C.trachomatis* leads to damaging sequelae such as infertility and it can apparently cause more severe tubal immunopathology than other agents, inspite of the absence of overt symptoms[8].

It can also lead to ectopic pregnancy and repeated abortions

Clinical variables associated with chlamydial infection include new sexual partner in the past 2 months or more than 1 partner in the preceding 6 months, cervical ectopy, a friable cervix, endocervical swab with greater than 20 PMN cells / high power field and the presence of WBC's in the vaginal secretions[20].

LABORATORY DIAGNOSIS

Accurate and early laboratory diagnosis is necessary to ensure appropriate therapy and epidemiological control. The diagnosis is most commonly done by culture or by direct detection of specific chlamydial antigens or DNA is specimens collected from the site of infection. Serological tests are also available.

PCR has been described for the diagnosis of all types of chlamydial infections. Its role in routine diagnosis is yet to be defined but it has significant advantages over

conventional methods. It is more sensitive and faster than culture ,there is no risks of laboratory acquired infection if appropriate precautions are taken to avoid contamination, cost appears to be the only significant disadvantage at present.

Infection with *C. trachomatis* may be diagnosed by:-

1) Direct detection of inclusion in clinical material (by microscopy of stained specimen)
2) Isolating the organism in cell culture.
3) Detection of specific antigens
4) Nucleic acid detection
5) Serological methods for detection of antibodies[7].

1) Direct detection of inclusion in clinical specimens by microscopy:

Cytological examination of smears using Giemsa, iodine or Papanicolaou stains were commonly used in the past for rapid detection of chlamydial inclusions in epithelial cells.

These methods are non specific, insensitive and require expert interpretation. They have been used successfully for examination of conjunctival smears but are unsuitable for examination of genital infections[9]. EB's are eosinophilic and RB's are basophilic

Dark field microscopy can be used to detect chlamydial organisms where EB's appear as yellow bodies due to their natural auto fluorescence. It is rare to detect chlamydial inclusions in clinical samples

Collection of specimen : The endocervial swab is spread evenly on a clean glass microscope slide. The smear should ideally be not more than one cell thick and should provide for examination atleast 500 epithelial cells. The smears are air dried and should be fixed by using absolute methanol as soon as possible after collection.

The smear is stained by Giemsa and iodine staining method.

Giemsa staining and examination by bright field microscopy is traditionally the method of choice for detecting inclusion bodies. Cell nuclei stains red ,the cytoplasm stains blue, while inclusion body which is usually adjacent to the nucleus may consist of blue stain RB or purple stained EB or a mixture of the two depending on the stage of development.

Iodine staining has been widely used to detect glycogen mass in *C. trachomatis* .It is faster and simple than Giemsa staining but less sensitive.

They have now been replaced by immunofluorescence, Enzyme Immuno Assays or other commercial kit based methods for rapid detection of specific chlamydial antigen[9].

2) Isolating the organism in cell culture:

Chlamydia can be isolated in the laboratory only in living cells, this means either embryonated eggs or cell monolayers. Until recently cell culture has been the accepted gold standard for diagnosis of infection due to *C. trachomatis.*

However, cell culture is relatively slow , expensive and the results depend on the correct methods of collection , transport and storage of specimens.

C.trachomatis will grow in only a limited number of cell lines and has exacting growth requirements. It can be inhibited by certain components of body fluids, swabs, reagents , containers and its viability is reduced by incorrect storage of specimens[9].

It has been shown that in single passage experiments ,vial culture is more likely to detect infectious particles without passage than microdilution well cultures[23].

Collection and transport of specimens for culture :-

The organism can be recovered from or detected in infected cells of the urethra, cervix, conjunctiva, nasopharynx, rectum and from material aspirated from the fallopian tubes and epididymitis.

For collecting specimens from the endocervix, the specimen for *C. trachomatis* culture should be obtained after all other specimens Eg. those for gram stained smear, *N. gonorrhoeae* culture or Papanicolaou smear[1] .

Wood tipped swabs and swabs made of calcium alginate should be avoided. If tipped with calcium alginate the number of inclusions which may be detected is reduced either by swab toxicity or by readsorption of the organism on to the swab[32]. Some cotton swabs have been toxic to Chlamydia. Dacron or rayon material is preferred.

Cervical swabs are obtained after exposing the cervix with a bivalve speculum. The cervix should be cleaned with a sterile gauze to remove all secretions. The appropriate swab is inserted into the cervix past the squamo columnar junction

about 1-2 cm deep and rotated against the wall for 10-30 sec ,withdrawn without touching any vaginal surface and then placed in appropriate transport medium or swabbed onto a slide prepared for Direct Fluorescent antibody (DFA) testing[1]. Cytological brushes collect more cells than swabs and No. 1 are thought by some investigators to improve isolation rate. But the brushes are more invasive and induce bleeding, which may inhibit some non culture tests and cannot be used for pregnant women.

Urethral specimens should not be collected until 2 hours after the patient has voided.

A urogenital swab is gently inserted into the urethra (females 1-2 cm and males 2-4 cm) rotated atleast once for 5 seconds and then withdrawn. Again swabs should be placed into the appropriate transport medium or onto a slide prepared for DFA testing[1]. The pooling of urethral swab specimen with endocervical swab specimen, increases culture sensitivity by 23%

C. trachomatis are found intracellularly in epithelial cells, the specimen should contain many of these cells rather than the exudates which is less often positive on culture.

Antibiotic treatment given to patients before swabs are taken can affect isolation. Obviously ,antibiotics such as Tetracycline and Erythromycin will reduce the chances of isolation, but other antibiotics which do not affect Chlamydia in vivo may also reduce the likelihood of isolating the organisms.

Treatment of patients with penicillin or cephalosporin before taking the samples for chlamydial isolation, increases the chances of negative results in primary culture.

Chlamydiae are relatively labile, viability can be maintained by keeping specimens cold and minimizing transport time to the laboratory. For successful culture, specimens should be submitted in a chlamydial transport medium such as 2SP (0.2M sucrose phosphate transport medium with antibiotics), a number of transport media are commercially available.

Specimens should be refrigerated upon receipt and if they cannot be processed for culture within 24 hours, they should be frozen at -70^{o}c[1] .

Recent reports have shown a delay of only 24 hours results in a significant decrease in the isolation rate for chlamydiae. Compared with immediate inoculation, the best recovery occurred after the specimens were held at 4^{0}C for 24 hours[33]. Freezing specimen is likely to result in atleast a 20% loss of viability. Addition of 2-5% fetal bovine serum is favoured by some investigators.

All media used for storage and transport of Chlamydia should contain antibiotics which will inhibit contaminating microorganisms but will not interfere with chlamydial isolation. Such antibiotics are Gentamycin (10-20 gm/ml) for bacterial contaminants. Amphotericin B (2.5 -4 gm/ml) or Nystatin (25-50 gm/ml) for fungal contaminants are most commonly used.

Swabs in transport medium should be shaken for 1-2 min on a vortex mixer or sonicated , and the swab removed before storage.

Semen and tissues may be toxic and cell culture should be inoculated undiluted and after 1 in 10 and 1 in 100 dilution.

Culture of urine has been attempted but is generally much less sensitive than urethral culture, presumable because urine contains few epithelial cells.

For tissue culture ,the specimen is placed in 2 ml of Eagles minimum essential medium in Earle salts containing 10% fetal calf serum, 1% L – glutamine, 10 gm of gentamycin / ml, 100µg of vancomyin / ml, 10 U nystatin / ml and 0.003 m mol of glucose / ml[34].

The first reported isolation of *C. trachomatis* was in irradiated McCoy cells grown on cover slips in flat bottomed glass tubes after centrifugation of the specimen on to the cell monolayer to enhance infectivity. After incubation, the cells were stained with iodine to demonstrate chlamydial inclusions[9]. Although chlamydiae are bacteria ,they are obligate intracellular pathogen[35].

Cell lines which support the growth of Chlamydia include McCoy (Mouse fibroblast line), HeLa 229 (derived from human cervical carcinoma), BHK 21 (derived from baby hamster kidney) and Buffalo green monkey (BGM) cells[9]. Cycloheximide treated McCoy cells are commonly used.

HeLa 229 cells have been used for culture of *C.trachomatis* since 1966.Pre treatment of these cells with DEAE – dextran enhances the infectivity of *C.trachomatis* .Prior to inoculation ,clinical specimen should be sonicated to disrupt host cells and inclusions and to separate Chlamydial elementary bodies[36].

Inhibition of cellular protein synthesis by irradiation or treatment with antimetabollites such as cycloheximide increases the size of inclusions and hence the sensitivity of culture of *C.trachomatis*, by preventing competition for nutrients and energy by the host cell.

Cycloheximide inhibits the protein metabollism of the host cell, there by favoring growth and multiplication of Chlamydia organisms, as being energy

parasites stealing ATP from the host cells. More inclusions were detected in cycloheximide treated cells than in other cells ,even though there were no differences in the isolation rate.

A recent study has suggested that mitomycin may be preferable in term of increased sensitivity. Trypsinisation of cycloheximide treated HeLa cells may also improve sensitivity[20].

Cell monolayers are grown on cover slips in shell vials on microtitre plates[9]. After shaking the clinical specimen with 5mm glass beads, centrifugation of the specimen onto the cell monolayer presumably facilitates adherence of EB's[1].Following inoculation of the specimen, centrifugation of cell monolayer significantly enhances culture sensitivity[37].

The effect of centrifugation increases upto 15,000 g but, in practice 1500-6000 g is satisfactory and practicable. The efficacy of centrifugation is optimal at 22-37^{0}c which requires the use of a temperature controlled centrifuge[9]. Isolation of bacteria can be optimized if a force of 3000g for 60 min can be achieved .

Polybrene, a poly cation that has been used to enhance infectivity of retroviruses, also enhances the number of chlamydial inclusions and the rate of isolation from clinical specimens[26]. Inclusion bodies of *C.trachomatis* contain glycogen which may be stained with iodine after incubation for 48-72 hours .The cover slips are removed and stained with iodine or, for greater sensitivity with a fluorescinated monoclonal antibody (by their typical apple green fluorescence against a red counter stain)

Species specific monoclonal antibody directed against OMP and genus specific antibodies directed against lipopolysaccharide are available .Chlamydial inclusions are well demarcated cytoplasmic structures. Additional sensitivity may be achieved by sub culturing growth in a vial if the culture is negtive after a single passage, but the expense of the procedure is increased considerable[35].

Although its specificity approaches 100%, the sensitivity of culture has been estimated between 70-90% in experienced laboratories[1].

Micro methods for culture have been described in which 96 wells, microplates are seeded with McCoy cells either before or at the time of inoculation with the clinical specimen. The wells are examined microscopically after staining with iodine or fluorescent conjugated antiserum. The microplates are considerably more economical than the individual shell vials and have been found to be of equivalent sensitivity by several investigators[35].

Recovery can be improved in women by performing both endocervical and urethral cultures in both men and women by blind passage. Although multiple passage may be impractical for most clinical specimens, a single blind passage, can substantially increase recovery with a minimal increase in cost[31]. Blind passages of sonicated monolayers following 48 hours of incubation have been reported to result in the recovery of an additional 3-10% of isolation[38]. Culture may serve the purpose of allowing rapid test of microbial cure after antibiotic therapy, that is during the 2nd and 3rd weeks after finishing treatment.

DNA tests may then still be positive due to the presence of dead but not yet shed EB's. The use of culture as a diagnostic method also offers the possibility of

performing antibiotic susceptibility tests of isolated strains and detecting non – plasmid containing strains[7].

Only Chlamydia cultures should be used in situations with legal implications (Eg:sexual abuse) when the possibility of a false positive test is unacceptable[1].

Cell culture is considered the gold standard for detection of *C.trachomatis* but is generally considered to be only 70-80% sensitive[39].

3) Direct detection of specific antigens:

Commercial availability of reliable rapid tests for detection of *C.trachomatis* has increased the availability of specific diagnostic tests and improved the management of sexually transmitted chlamydial infection. Immunofluorescence (IF) and enzyme immuno assay (EIA) are the two methods used most commonly and a large number of different kits are available. Each has advantages and disadvantages but, overall, the sensitivity and specificity of the two methods are comparable. The procedure used for specimen collection for these tests are similar to and as important as for culture. However, since they do not rely on the presence of viable Chlamydia, transport and storage of specimen is less critical. For optimal results, specimen must be collected and stored, and the tests performed according to manufactures instructions.

Direct antigen tests should not be used alone when a positive result can have medicolegal implication Eg in a case of sexual abuse. Culture should be done in these circumstances.

First catch urine specimen have been used successfully to detect *C. trachomatis* antigen by both IF and EIA[9].

Poorly collected specimen may contain levels of antigen below the threshold of antigen detection, they may however have a sufficient number of viable chlamydial cells to permit reproduction of the organism to a detectable level in culture[40].

Direct immunofluorescence (DIF)

DIF was the first commercially available method for rapid diagnosis of *C. trachomatis* infection .Different systems used Fluorescin Isothiocynate – labelled antibodies (usually monoclonal) against either species specific MOMP of *C. trachomatis* or the genus specific LPS. IF is most commonly used for diagnosis of oculogenital *C. trachomatis* infection but anti LPS antibody based method can also be used for examination of sputum, tracheal aspirate or tissue for diagnosis of suspected chlamydial respiratory infection[9].

Compared to anti LPS antibodies, anti MOMP antibodies gives brighter and more consistent staining of free EB's, which appears as bright apple green punctate structures, 300 nm in diameter with a smooth edge. EB's are less brightly stainted and their edges less regular when stained with anti LPS antibody, which also stains free LPS and may cross react with other gram negative LPS. Artifacts are easily mistaken for EB's by inexperienced microscopist[9].

Clinical material can be examined by direct immunofluorescence for the presence of elementary bodies, a species specific monoclonal antibody directed against the 40 kDa mol wt MOMP is described. They described the appearance of EB, rather than inclusions in DIF stained smears of clinical material.With the use of

monoclonal antibody reagent specific for MOMP of *C.trachomatis* as antigen the sensitivity of DIF is 80-90% and the specificity is 98-99% compared to culture when both are performed optimally[41].

Previous antibody preparations were of insufficient sensitivity to detect anything other than inclusions.

Early reports recommended that 10 or more elementary bodies should be observed before the specimen is reported as positive. But the sensitivity is reduced if this criterion is used[7].

IF has many advantages. It can be done rapidly (< 1hr) on specimens from individual patients if a rapid diagnosis is required and is suitable for small batches.

Direct inspection of the smear allows assessment of its quality. Ideally there should be atleast 20 epithelial cells on the stained area. A significant number of polymorphs (due to purulent secretion) or red blood cells (due to trauma during collection) suggests that the specimen has not been properly collected. These can obscure EB's if present or produce artifacts, the result is unreliable and another specimen should be requested. The main disadvantages of IF are the level of skill required, the need for a high quality fluorescence microscope and its unsuitability for batch testing of large number of specimens.

Studies found cytobrush to yield an increase in the number of epithelial cells, chlamydial elementary bodies and increased positivity rate in DIF[34].

If performed according to the manufacturers instructions by an experienced microscopist, the specificty is high (>95%) compared with culture. Apparently false positive IF results may actually be due to false negative cultures. The sensitivity is

relatively high in patients with symptoms consistent with *C. trachomatis* infection (>90%) but generally too low (60-80%) in asymptomatic individuals to recommend the use of IF for screening of low prevalence populations[9].

Enzyme immuno assay (EIA)

EIA offers the potential advantages over DIF of objectivity and ease of mechanization.

There is an even greater variety of EIA tests available for diagnosis of chlamydial infection using monoclonal and polyclonal antibodies against LPS. This test is based on detection of soluble LPS antigen captured by antibody attached to a solid surface (plastic bead, microtitre well or membrane) and then diluted with an enzyme labeled detector system and a chromogenic substrate. A positive result is indicated by a colour change which is read spectrophotometrically or visually.

The only special equipment required is the EIA plate reader. No special expertise is required and the methods are suitable for large scale batch testing and automation. The sensitivity of EIA is generally similar to IF. False positive results are common. They may be due to cross reaction with LPS of other bacteria[9].

They should be used with caution in low prevalence settings because of the effect of false positive results on the predictive value of a positive result.

Blocking assays to confirm positive results have improved specificity of these tests to greater than 99% so that when confirmatory assays are used EIA can be performed in low prevalence settings.

Both EIA and DIF are less sensitive than cell culture and have a sensitivity of only 75-85%, thus a major portion of infected individuals will not be detected. DIF and EIA tests perform better in high prevalence settings than in low prevalence population.

4) Nucleic acid detection tests:

i) DNA hybridization probe :

The first commercial molecular test applied to *C. trachomatis* was a DNA probe against rRNA.

Radioactive DNA probes for detection of *C.trachomatis* in cell culture and cervical smears were first used in research laboratories in the early 1980's and have become commercially available in 1989, but were relatively insensitive and impractical. More recently a chemiluminescence assay has become available. It uses an acridum labeled single – stranded DNA probe which is complementary to r RNA of *C.trachomatis*

The labeled DNA- RNA hybrid is detected in a luminometer which measures, in relative light units, light emitted by the acridium ester label. The sensitivity has

been increased by adding another step to remove unbound label (PACE -2) and it is atleast as high as antigen detection tests[42]. It is more sensitive than culture (99% Vs 80%)[43].

PACE 2 has been reported to be more sensitive than culture when a true positive specimen was defined as one which was culture positive or positive by two different non culture methods.

The specificity can be increased to > 95% by a probe competition assay in which the test is repeated in the presence of excess unlabelled probe. If the initial result was a true positive, the relative light units reading is significantly reduced[9].

DNA probe is about 1 log unit more sensitive than EIA's.

Grossly bloody specimen can produce false positive results in the PACE 2 test due to autofluorescence.

Recently Gen probe developed another probe test that can simultaneously detect both *C.trachomatis* and *N.gonorrhoeae* from a single specimen (PACE -2) .A positive result indicates the presence of either or both organisms and requires additional testing specific for detection of *C.trachomatis* and *N.gonorrhoeae* for identification and confirmation of the precise etiology of infection.

In preliminary evaluation of PACE -2 with culture as a standard ,sensitivities were reported to be > 89% and specificities were >95% for detection of *C.trachomatis* and *N.gonorrhoeae* infection in a high prevalent population of men and women.

ii) Nucleic acid amplification tests(NAAT)

The second generation of molecular tests are amplification tests of several kinds. 4 commercial assays using NAAT are FDA approved for laboratory diagnosis of *C.trachomatis* infection. These assays use different formats PCR, LCR, standard displacement amplification and transcription mediated amplification. The first 3 assay formats amplify the target DNA sequence present in the chromosome or cryptic plasmid where as the last format amplifies ribosomal RNA sequence.

Various amplification targets have been used, including the common endogenous plasmid, the OMP- 1 gene which codes for MOMP and the 16SrRNA gene. The plasmid based PCR methods are generally more sensitive than those based on amplification of chromosomal DNA. This difference is due to the presence of multiple copies (7-10) of the plasmid per bacterial cell, compared with single OMP-1gene and 2-3 tandem repeats of the 16SrRNA gene.

A possible disadvantage of chlamydial plasmid based amplification is the potential occurence of a plasmid free variant of *C.trachomatis* which would give a false negative result[16].

MOMP gene is a single copy gene and this assay may therefore be less sensitive than a PCR procedure which targets the endogenous multicopy plasmid[29]. Because of the increased sensitivity of detection, first voided urine specimens from symptomatic and asymptomatic men and women are acceptable specimens to detect *C. trachomatis*, thereby affording a non invasive means of chlamydia testing.

PCR assays on urethral / cervical swabs for the laboratory diagnosis of genital Chlamydial infection in symptomatic men and women show sensitivities 89-100% and specificities 89-100% compared with traditional culture[45].For urine specimens ,PCR assays show sensitivities between 87-100%[46].

The various amplification methods appear to function in a similar fashion. All are more sensitive than other methods but they are also more expensive.

Price appears to be the most important factor that has deterred laboratories from adopting what is clearly the most sensitive approach to diagnosis. In addition to sensitivity, the amplification method also offer convenience. They can be applied to urine, which is more acceptable alternative for male patients and for women who do not require a pelvic examination for other reasons.

In general endocervical specimen are marginally more sensitive than urine in women, but urine is more sensitive than urethral swabs in asymptomatic men.

Cross reactions have not been demonstrated with transcription mediated amplification.

Most assays for *C.trachomatis* are performed in the absence of clinical symptoms and are therefore screening tests.

A significant problem for amplification tests is the presence of inhibitors of amplification in clinical specimens. If a substance normally present in the specimen (eg β globulin) is not amplified, a negative result must be considered suspect. Unfortunately the cost of the test is increased by this manipulation.

Transcription mediated amplification appears not to be affected by inhibitors[35]. There were initial problems with Taq polymerase inhibitor and DNA contamination during sample handling, particularly after amplification.

Clinicians being in charge of patients who mainly belong to low prevalence group for genital Chlamydial infection are recommended retesting of all patients with a positive test result to avoid false diagnosis.

A positive result can be confirmed by repeating the PCR on the original specimen using a different primer set. PCR using OMP 1 gene as the target and restriction fragment length polymorphism (RFLP) analysis of the amplified product can be used for rapid serotyping.

Each serotype produces a distinctive RFLP pattern. Despite these advantages PCR was limited until recently to research laboratories because it required complex procedure including DNA extraction, gel electrophoresis and southern hybridization using radio active labels.

The PCR detects only a small part of the genome of a microorganism and is therefore not necessarily a measure of viability[15].

In LCR tests, swabs from the vaginal introitus yields a somewhat higher positivity rate than samples collected from the cervix of the same women. Self swabbing of the introitus by women themselves resulted in an even somewhat higher percentage of positive cases than when the sampling of the same women were performed by medical personnel[7].

5) Serological tests for chlamydial antibody

Tests for antichlamydial antibodies have made use of both chlamydial LPS and MOMP as antigens. Chlamydial LPS is shared by all chlamydial species. As LPS of *C.trachomatis* cross reacts with *C.pneumoniae* and *C. psittaci* and was easy to produce, it was in the past made use of in complement fixation tests (CFT) used for diagnosing LGV and ornithosis /psittacosis.

Recombinant MOMP and recombinant OMP -2 have been used to detect antichlamydial antibody. A species specific peptide has been identified, which avoids

cross reactivity problems in serological analysis of chlamydial infection by the various species[7].

Serological diagnosis usually depends on demonstration of seroconversion or a significant increase in IgG or total antibody level and/ or the presence of specific Ig M antibody. Antibody tests are also useful in seroepidemiological studies of *C.trachomatis* infection in acute salphingitis and its complication such as ectopic pregnancy or infertility in which chlamydial antibodies have been found more commonly and at higher titre than in controls with normal fallopian tube[9].

Serology is therefore rarely helpful in the diagnosis of acute chlamydial genital infection because infection is confined to the mucus membrane.

More over reinfection which is more likely to be clinically apparent is usually not associated with a rising antibody level.

Micro immuno fluorescence (MIF)

The standard method used for detection of *C. trachomatis* antibody is MIF using EB's of standard serovar to detect serovar specific antibody. Alternative methods use RB's as genus specific antigens or inclusions in cells grown on the slide which can detect both genus and type specific antibodies to *C.trachomatis.*

More recently MIF has also been used to detect specific antibody to *C. pneumoniae*.

The indirect IF test is more sensitive than the CFT and has detected antichlamydial antibody in upto 91% of patients with clinically and microbiologically proved ocular infections.

Although the MIF test is rapid to perform ,the production of the whole range of chlamydial serotype is time consuming and laborious.

About 20-70% of people have antibody by MIF. This high back ground rate makes MIF less useful diagnostically in patients with non systemic infections (*C. trachomatis* A-K) but quiet useful for epidemiological surveys[21].

This test is more sensitive than CFT and most people with past or current chlamydial infection including superficial genital infection, have detectable antibody[21].

Complement fixation test (CFT)

CFT detects complement fixing antibodies that recognize the genus specific LPS antigen and is not specific for any chlamydial species.

Historically, high quality antigen for the CFT is produced in embryonated hens egg infected with *C.psittaci*. Infected egg yolk is extracted with an organic solvent to enrich the carbohydrate containing LPS antigen called group antigen.

Although a single serum specimen with a CFT tire of >1 in 64 can be consistent with a diagnosis of infection due to any of the chlamydial species, the predictive value of this titre on a single specimen is low. Treatment with antibiotics can delay or diminish the production of CFT antibody and will reduce the sensitivity.

The antigen used in CFT is a heat stable large acid polysaccharide found in all chlamydial species.

A small percent of uninfected people may have titres greater than or equal to 1:16 and 5.-20% of those with genital or ocular *C.trachomatis* may have this ratio. Women with salphingitis may show a rise in CFT titre or have CF titre of greater than or equal to 1:64[21].

VACCINES

The surface of Chlamydia does not contain proteins that are distinctive enough to induce a full immune response. The cell wall does contain an exo glycolipid antigen that induces a weak immune response (for reasons unknown, the immune response is weaker to carbohydrate antigens). This is the basis for a recent vaccine developed by researchers at John Hopkins University .The researchers are developing a protein version of the antigen by injecting *C.trachomatis* into mice, isolating and amplifying the antibodies and then using these antibodies to mold a protein resembling the exoglycolipid antigen. The next step is to adopt the procedure in humans.

ANTIBIOTIC SUSCEPTIBILITY

Until recently, antibiotic susceptibility tests have played an important role in the management of infections by *C.trachomatis* in the belief that all strains were susceptible, at least to erythromycin, azithromycin and tetracycline drugs.

However there are now reports of strains with high minimum inhibitors concentration (MIC) values to erythromycin (ie > 1 µg/ml) and tetracycline (ie > 4µg/ml)

Single strains with a reduced susceptibility to azithromycin and to quinolones eg ofloxacin, have been linked to therapeutic failure.

Chlamydia organisms lack muramic acid, but the presence of 2 penicillin binding protein may explain their susceptibility to β lactam antibiotics, provided enough high doses are given for atleast 2 weeks.

Chlamydia positive cases at retest have usually been regarded as cases of reinfection or due to compliance problems rather than to antibiotic resistence

Under antibiotic pressure ,*C.trachomatis* may, in invitro tests, develop decreased susceptibility to the drug under test. Such strains usually lose their antibiotic resistence when propogated in absence of the antibiotics. Such heterotypic resistence has, however not been connected to therapeutic failure

Sub inhibitory concentration of β lactam antibiotics may induce RBs which are insensitive to this group of antibiotics. This might explain relapses after use of β lactam antibiotics.

RBs are naturally also insensitive to some other antibiotics, which may be another reason for relapse after antibiotic therapy.

TREATMENT

Protein inhibitors eg tetracycline, erythromycin and azithromycin are all first line alternatives in the therapy of chlamydial infection, apart from tetracycline in pregnancy

Other macrolides such as roxithromycin seem to have a some what lower activity than erythromycin with higer dose and / or prolonged courses.

Azithromycin is a derivative of erythromycin classified as an azilide antibiotic.It has the advantage that it is rapidly and efficiently taken up by eukaryotic cells where it can reach chlamydial organisms.

Another advantage is that azithromycin can be given as a single dose regimen ie. With 1 gm. The drug also persists in the body for a period similar to a standard multidose regimen with tetracycline. It can be used in pregnancy.

Azithromycin also has good invitro activity against *Ureaplasma urealyticum*[47].

Doxycycline should be given with 200 mg/day both to men and women, in men for atleast 7 days and in women for 10-14 days.

Amoxicillin is tested as a second line therapeutic alternative with a dose regimen of 500 mg , 3 times a day for 7 days.

For pregnant women, the best alternative is 1gm azithromycin or amoxicllin 500 mg for 10 days.

Quinolones are chlamydicidal in action. These are 2nd line because of low efficacy and are contra indicated in pregnancy.

Co infection with *N. gonorrhoeae* and *C.trachomatis* should be considered in any case in which such infections might be involved. It is essential to note that sex partners should be involved in treatment regimen as well .

The added benifit of prescribing ofloxacin is the treatment of concomitant gonococcal infection. Uncomplicated gonococcal infection are eradicated by a single 400 mg dose. However ,*C.trachomatis* requires a full 7 day therapy[20].

A repeat culture for test of cure has not been recommended to date[20].

The treatment regimens for PID are

Regimen A[20]

Cefoxitin 2 gm IV every 6 hours or Cefotenan IV 2 gm every 12 hours.

plus

Doxycycline 100 mg every 12 hours orally

After discharge from hospital, continue Doxycycline orally 100 mg BD X 10-14 day

Regimen B[20]

Clindamycin IV 900 mg every 8 hours

plus

Gentamycin loading dose (2mg/kg) plus maintainence every 8 hours

After discharge

Doxycyline 100 mg BD X 10-14 days.

Seven day, multidose regimen of tetracycline, doxycycline or erythromycin are the most frequently used treatments for genital chlamydial infection but many patients do not complete the course of therapy[47].

MATERIAL AND METHODS

This study was conducted in the Department of Microbiology, Jawaharlal Nehru Medical College, Belgaum.

Source of data: Infertile women of reproductive age attending the Assisted reproductive centre (ARC) of KLE'S DR. Prabhakar Kore Hospital and Medical research centre, Belgaum.

Study design / duration :

One year cross sectional study from 1st January 2009 – 31st December 2009.

Method of collection of data.

Inclusion criteria :-

All women of reproductive age group (15-45 yrs) with primary or secondary infertility were included in the study.

Infertility is defined as inability to conceive for more than a year despite regular unprotected intercourse.

Primary infertility is defined as those cases in whom conception has never occurred.

Secondary infertility is defined as those cases where there is inability to conceive after a previous successful conception.

Exclusion criteria

Patients on antibiotics within past 10 days

Sample collection:

Blood sample was collected for detection of Anti-Chlamydial IgG antibody and Endocervical swab was collected for detection of Chlamydial DNA .

Sample size:

$$n=\frac{4pq}{d^2}=\frac{4x28x72}{(8.4)^2}=115$$

Allowing 30% sampling error and taking 28% prevalence

n = sample size

p = prevalence

q = 100-p

d = 30% of p

Methodology :- Written informed consent was taken

Sample collection from all patients for ELISA.

3 ml of blood was collected in a sterile vial by venepuncture with all aseptic precaution

Blood was allowed to clot at room temperature for half an hour, then it was centrifuged at 3000 rpm for 5 min.

The serum was separated and transferred into vials. The vial was labelled with patients name, OP number and sample number. Samples were kept in the freezer of the refrigerator along with other samples in a vial holding box.

EUROIMMUN Kit was used for the detection of Anti-Chlamydial IgG Antibody.

Lot numbers: E090401AF and

E100119AD

The kit was obtained from Konark, Hubli

Photograph 1: ELISA kit used for detection of anti-Chlamydia trachomatis IgG antibody

Photograph 2: Contents of the EUROIMMUN kit

Principle of the test:

The ELISA test kit provides a semiquantitative or quantitative in vitro assay for human antibodies of the IgG class against *C. trachomatis* in serum or plasma. The test kit contains microtitre strips each with 8 break off reagent wells coated with *Chlamydia trachomatis* antigen (cell lysate of BGM cells infected with *C. trachomatis* of the serotype. K.). In the first reaction step, diluted patients samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also Ig A and Ig M) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate), which is capable of promoting colour reaction

Contents of the kit

1. Micro plate wells
2. Calibrator 1 (200 RU / ml)
3. Calibrator 2 (20 RU / ml)
4. Calibrator 3 (2 RU / ml)
5. Positive control
6. Negative control
7. Enzyme conjugate (Peroxidase labelled anti human Ig G)
8. Sample buffer
9. Wash buffer
10. Chromogen / substrate solution

    TMB / H_2O_2
11. Stop solution

    0.5 M H_2SO_4

Test Procedure

1. Bring the micro well plate and reagents to room temperature (20-25^{0}c)
2. Dilute the samples with sample diluent 1:101 and use within one working day
3. Place enough wells in the frame plate and mark it –

   Cal 1

   Cal 2

   Cal 3

   Positive control

   Negative control

   Sample 1

   2 etc
4. Add the 100µl of the respective calibrators, controls and diluted samples and incubate at room temperature for 30 min
5. Empty the micro well plate and wash it with 300 µl of wash buffer per well. Reaction time 30-60 seconds / washing for 3 times
6. Add 100 µl of enzyme conjugate and incubate at room temperature for 30 min. Empty the microwell plate.
7. Wash it with wash buffer as in step 5
8. Add 100 µl of chromogen substrate and incubate at room temperature for 15 min.
9. Add 100 µl of stop solution in each well
10. Carry out photometric measurement at 450 nm.

Calculation of results:-

Semi quantitative:- Results can be evaluated semi quantitatively by calculating the ratio of the extinction value of the control or patients sample over the extinction value of the calibrator 2

Calculate the ratio according to the following formula.

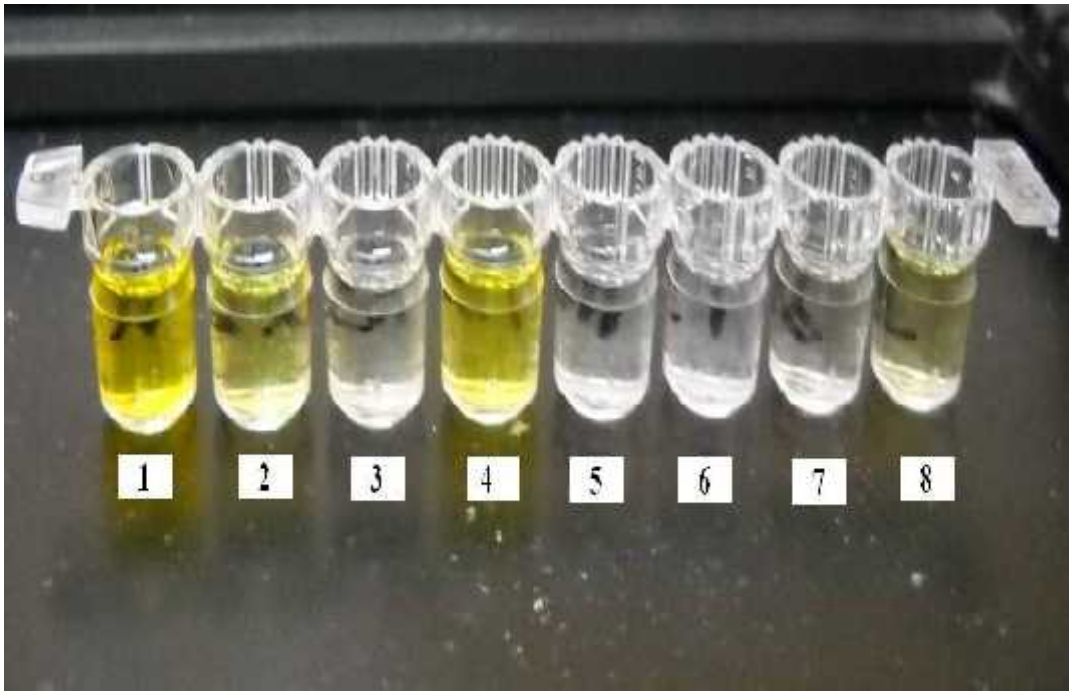
$$\frac{\text{Extinction of the control or patients sample}}{\text{Extinction of calibrator2}} = \text{RATIO}$$

EUROIMMUN recommends interpreting the result as follows.

Ratio < 0.8	Negative
Ratio > 0.8 to < 1.1	Borderline
Ratio > 1.1	Positive

In case of borderline test results, an additional sample in taken within 7 days.

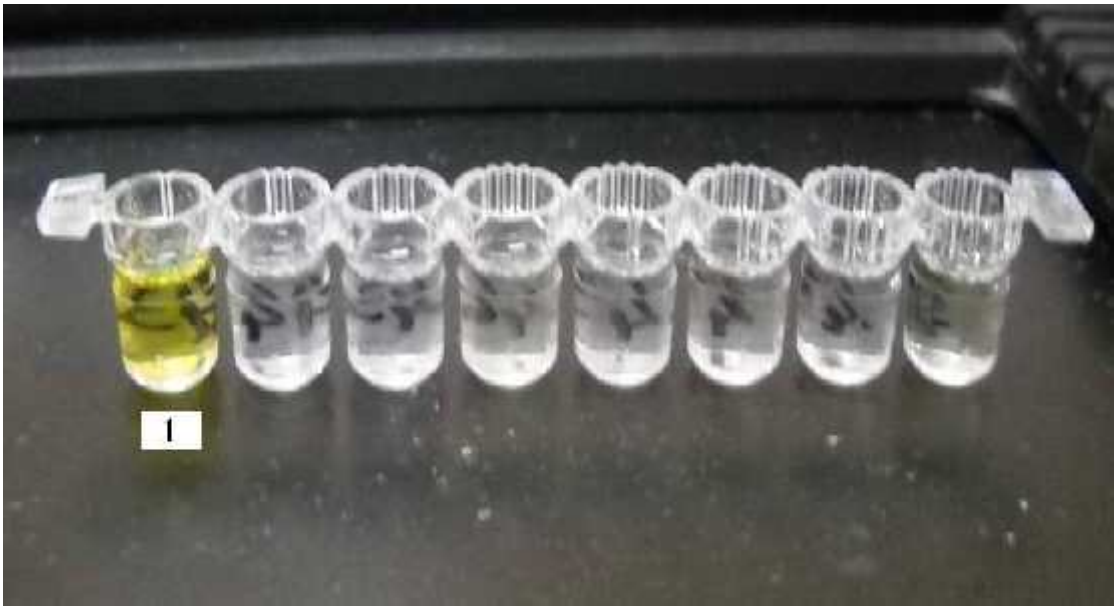
Photograph 3: Colour development after adding stop reagent in microtitre wells



The numbers in the above photograph represents the following:

1. Calibrator 1 (200 RU/mL)
2. Calibrator 2 (20 RU/mL)
3. Calibrator 3 (2 RU/mL)
4. Positive control
5. Negative control
6. Sample number 1
7. Sample number 2
8. Sample number 3

Photograph 4: Sample positive by ELISA



In the above photograph the microtitre well labelled 1 shows the positive sample and rest all others are negative.

Sample collection for PCR.

For PCR the endocervical samples were sent to a Private Lab.

First the endocervical mucus is cleaned with the help of a cotton swab.

Then it is scraped with the spatula present in the kit.

Next the cytobrush is used to collect the scraped material from the endocervical area. And this is placed in the kit containing normal saline

This was sent to the private lab in a cool ice pack where it was processed .

The DNA is extracted using the Fast prep® instrument

The 16S rRNA gene on the chlamydial DNA is targeted

The Following Primers are used.

Sense 5' – GGCGTATTTGGGCATCCGAGTAACG – 3'

Antisense 5' – TCAAATCCAGCGGGTATTAACCGCCT – 3'

The amplification was made in the thermocycler, using the following 30 cycle programme;

Denaturation at 92^0c for 1min

Annealing at 62 ^{0}c for 1 min

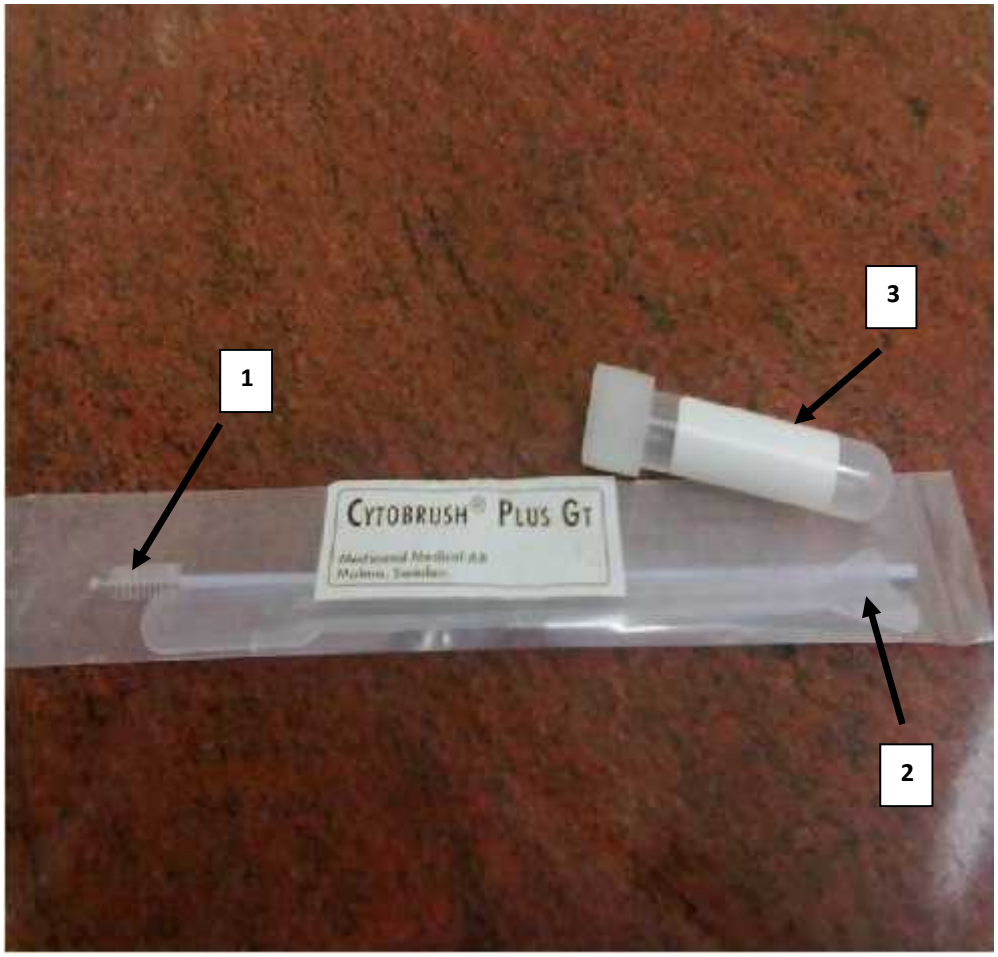
Polymerization at 72^0c for 1 min

Followed by a final extension at 72^0c for 4 minutes

The PCR products were analysed by electrophoresis in a 1.5% agarose gel.

Sterile distilled water and Chlamydia trachomatis species (ATCC 434) were used as negative and positive control respectively.

5: Kit used for collection of endocervical samples for PCR

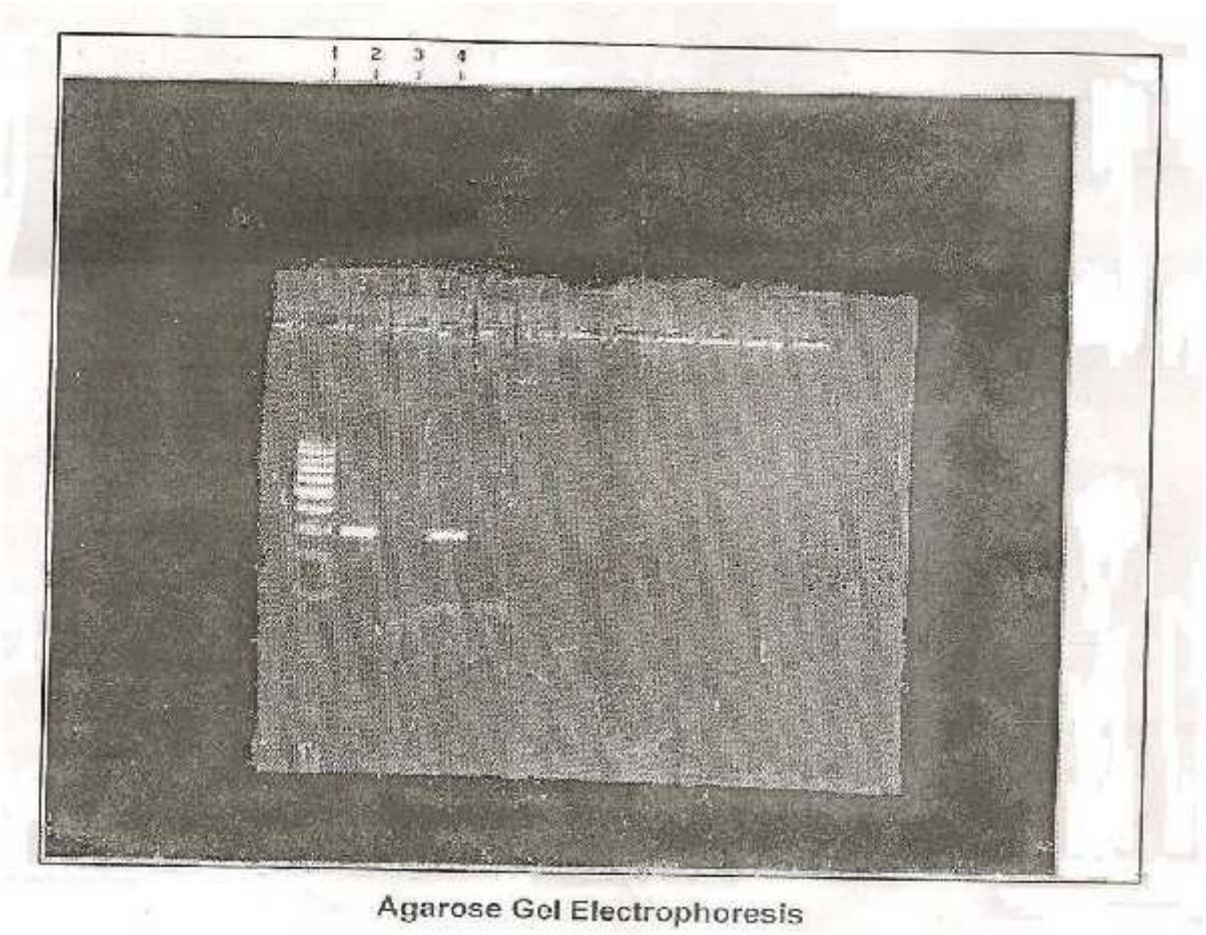


1.Cyto brush

2 Spatula

3 Sample collection vial

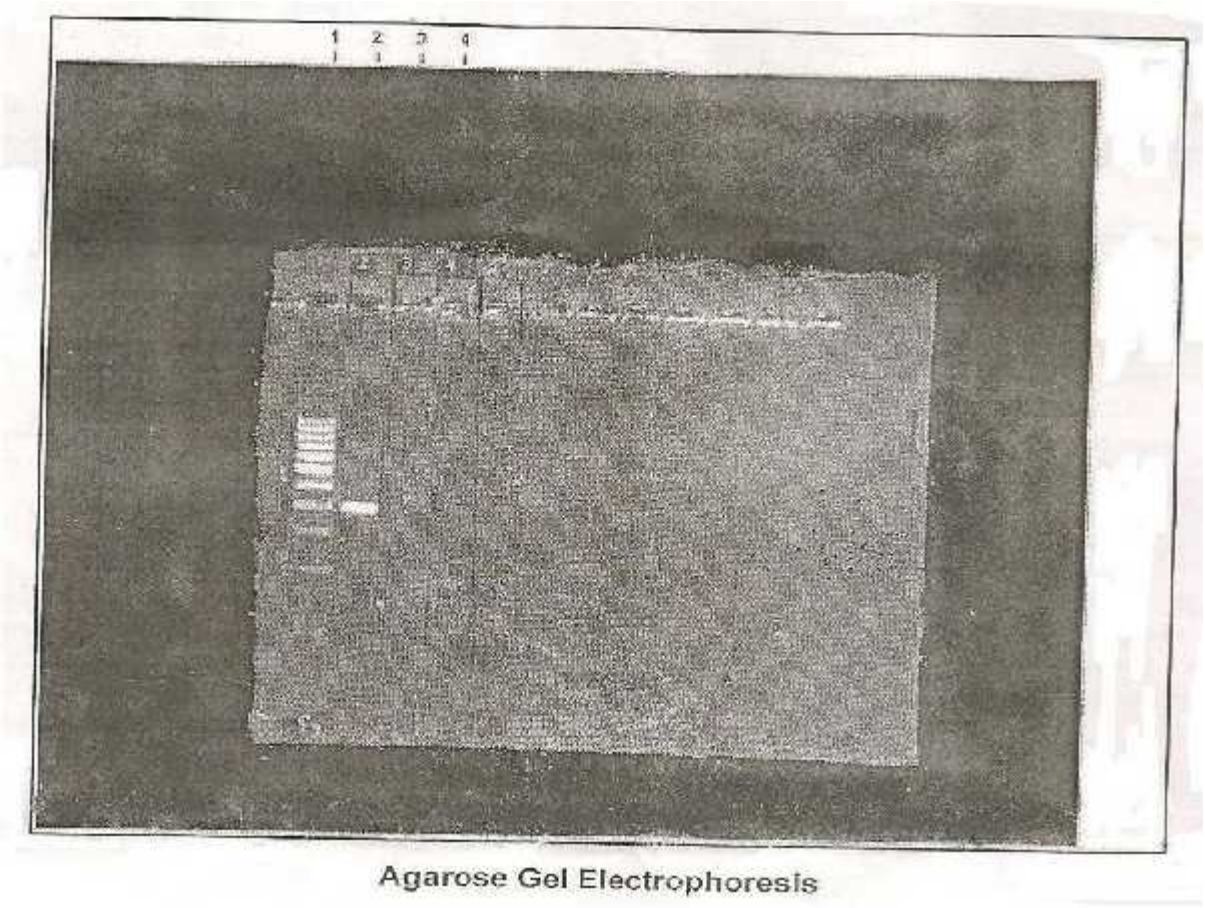
Photograph 6: Sample positive for Chlamydia DNA



The numbers in the above photograph represents the following:

1. DNA ladder
2. Positive control
3. Negative control
4. Sample positive for Chlamydia DNA

Photograph 7: Sample negative for Chlamydia DNA



The numbers in the above photograph represents the following:

1. DNA ladder
2. Positive control
3. Negative control
4. Sample negative for Chlamydia DNA

RESULTS

Table 1 Prevalence of *Chlamydia trachomatis* infection detected by PCR in different age groups.

Age group	Number of cases (%)	Number of positive cases(%)
15-24 Yrs	27 (23.6%)	00
25-34 Yrs	71 (61.8%)	02 (2.8%)
35-44 Yrs	17 (14.9%)	01 (5.9%)
Total	**115**	**03 (2.6%)**

The above table shows that maximum number of *Chlamydia trachomatis* positive cases ie 5.9% were in the age group of 35-44 Years. Followed by 2.8% cases infected in the age group of 25- 34 Years.

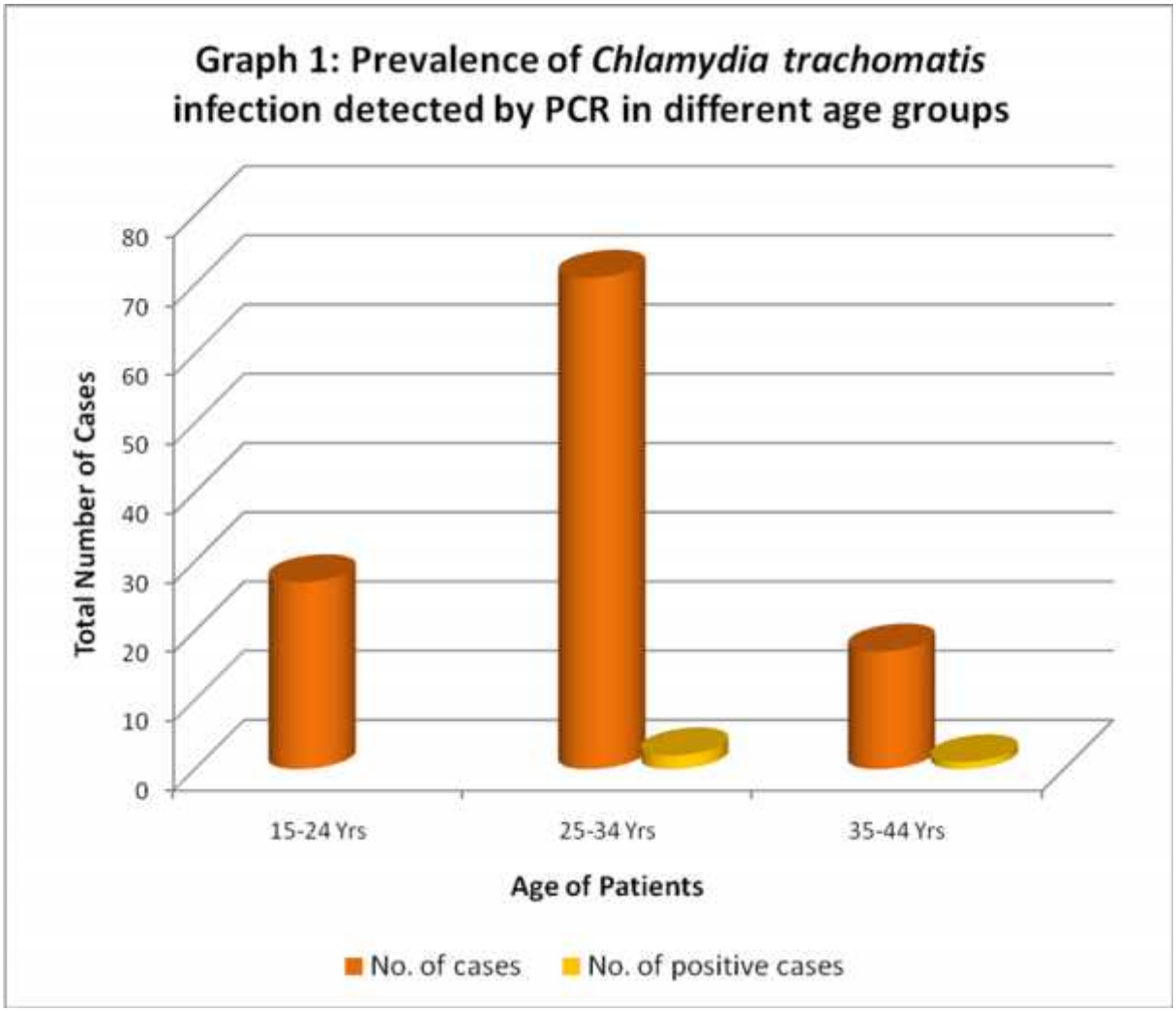

Graph 1: Prevalence of Chlamydia trachomatis infection detected by PCR in different age groups
Total Number of Cases
80
70
60
50
40
30
20
10
0
15-24 Yrs
25-34 Yrs
35-44 Yrs
Age of Patients
No. of cases
No. of positive cases

Table 2 Showing the results of PCR and ELISA for the detection of *Chlamydia trachomatis* infection

TEST	POSITIVE	BORDERLINE	NEGATIVE	TOTAL
PCR	03 (2.6%)	NA	112 (97.4%)	115
ELISA	01 (0.9%)	01 (0.9%)	113 (98.2%)	115

The above table shows that of the total 115 endocervical samples tested for *C. trachomatis* DNA, 3 samples ie. 2.6% were positive and 112 samples ie 97.4% were negative.

And of the 115 Blood samples screened for Anti Chlamydia IgG antibody 1 was positive and 1 was in the Borderline range. The remaining 113 sample ie (98.2%) were negative.

Table 3 Showing sample positivity by ELISA and PCR

	ELISA Positive	**ELISA Negative**	**Total**
PCR Positive	01	02	03
PCR Negative	00	112	112
Total	**01**	**114**	**115**

The above table shows that out of the 3 samples that were positive by PCR, ELISA was positive for only one sample.

Of the two sample that were PCR positive and ELISA negative one was in the borderline range by ELISA

All the 112 sample that were negative by PCR were also negative by ELISA.

Table 4 Comparison of PCR and ELISA for *Chlamydia trachomatis* infection in relation to the type of infertility

Type of infertility (%)	Total no% infected	Both (PCR & ELISA)	PCR Alone	ELISA Alone
Primary infertility 92 (80%)	02 (2.2%)	00	02 (100%)	00
Secondary infertility 23 (20%)	01 (4.3%)	01 (100%)	00	00

The above table shows that 92 patients (80%) had primary infertility and among these 2 (2.2%) were infected with *C. trachomatis*, These 2 case were positive by PCR only.

23 patents ie.20% had secondary infertility and among these only one (4.3%) was infected with *Chlamydia trachomatis* infection. This case was detected by both ELISA and PCR.

Table 5 Prevalence of *Chlamydia trachomatis* infection in relation to duration of infertility

Duration of infertility	Number of cases(%)	Number of positive cases(%)%
1-5 Yrs	57 (49.6%)	02 (3.5%)
5-10 Yrs	36 (31.3%)	01 (2.8%)
10-15 Yrs	20 (17.4%)	00 (0.0)
15-20 Yrs	02 (1.7%)	00 (0.0)

The above table shows that the number of Chlamydia positive cases was maximum ie. 3.5% in patients who had the duration of infertility between 1-5 Years and 2.8% of Chlamydia positive cases had the duration of infertility between 5-10 Years.

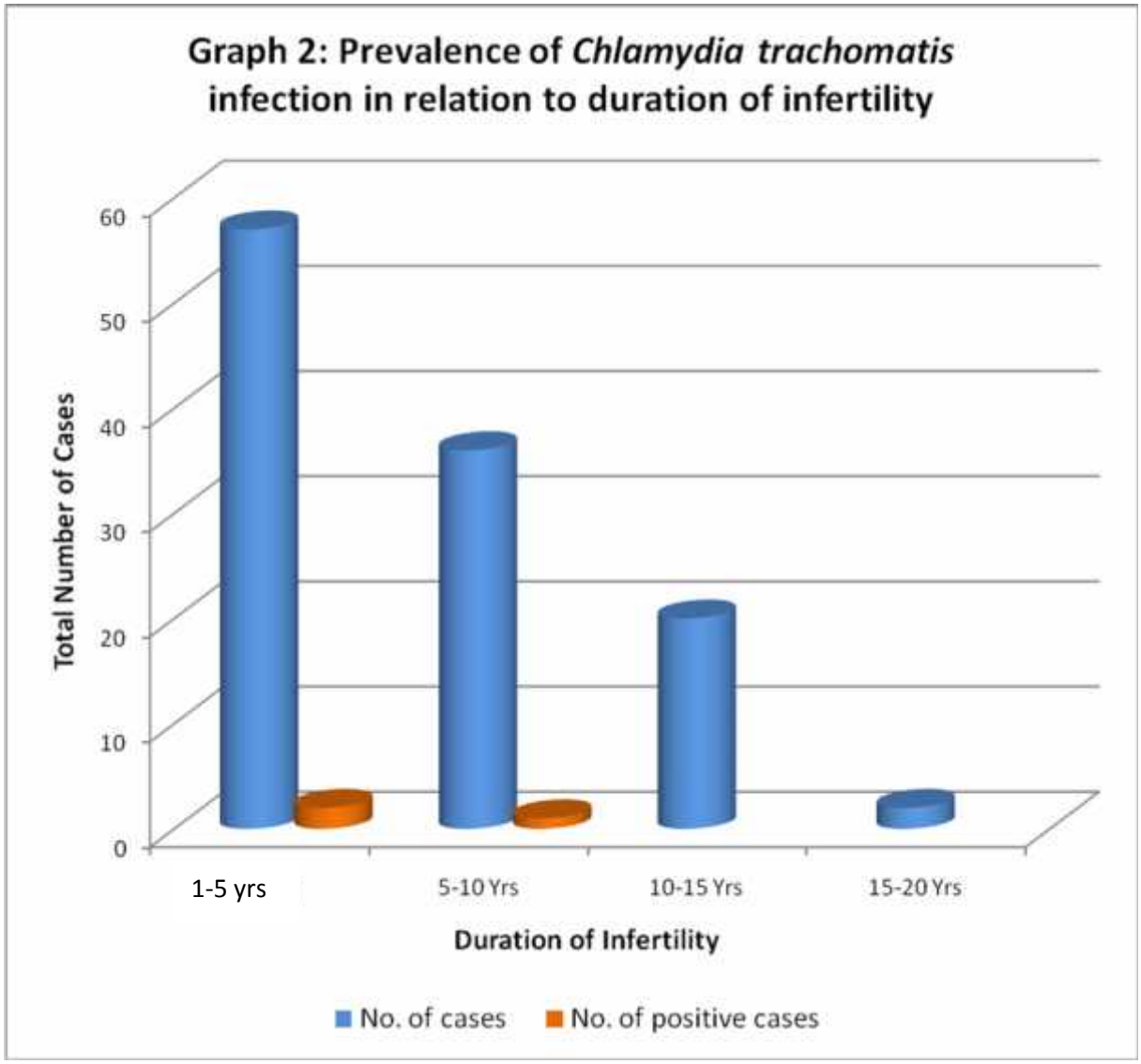
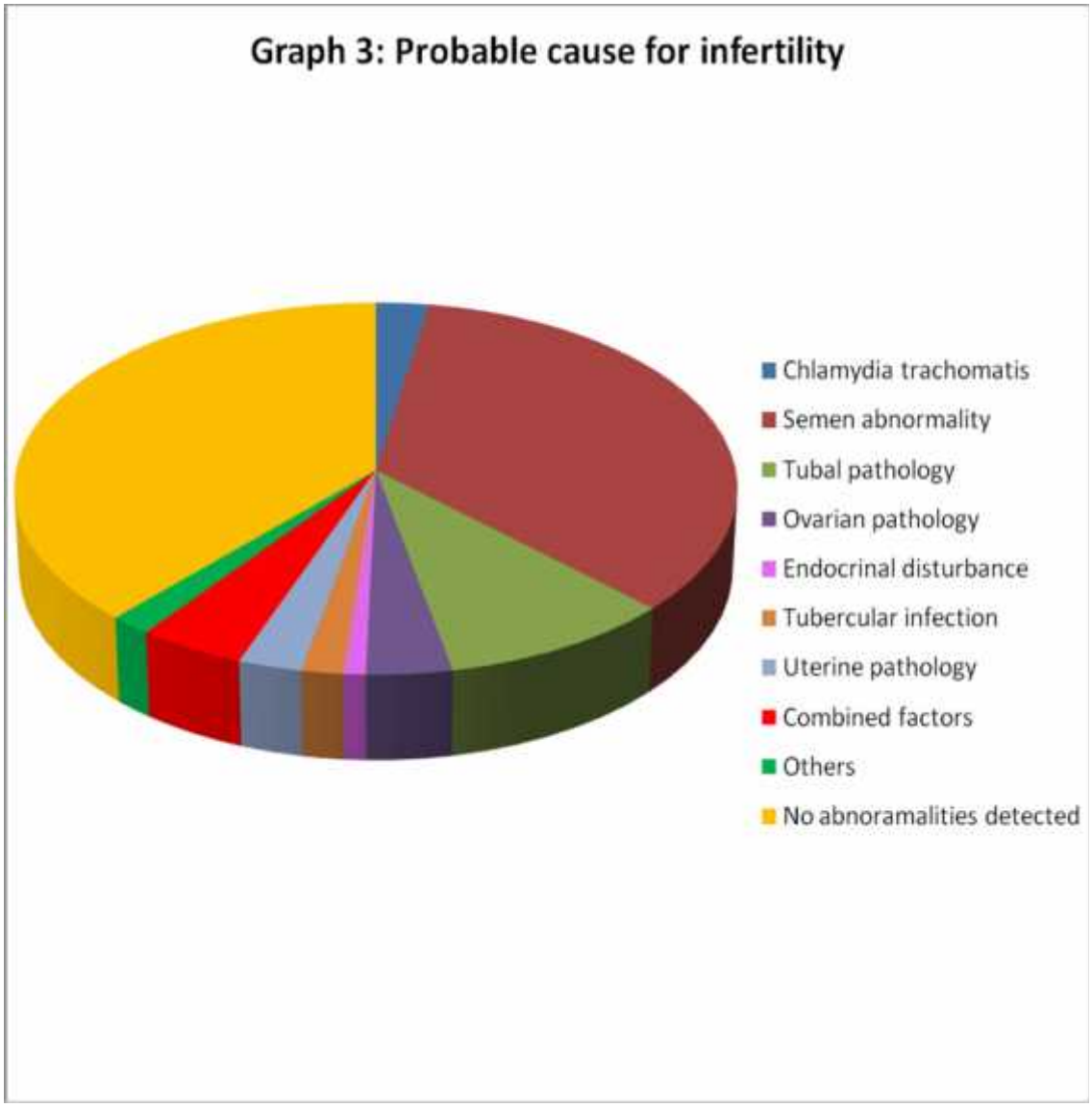

Graph 2: Prevalence of Chlamydia trachomatis infection in relation to duration of infertility
Total Number of Cases
60
50
40
30
20
10
0
1-5 yrs
5-10 Yrs
10-15 Yrs
15-20 Yrs
Duration of Infertility
No. of cases
No. of positive cases

Table 6 Showing the probable cause for infertility

	Probable cause	**Number of cases affected**
1.	*Chlamydia trachomatis* Infection	03 (2.6%)
2.	Semen abnormality	40 (34.7%)
3.	Tubal pathology	11 (9.6%)
4.	Ovarian pathology	04 (3.5%)
5.	Endocrinal disturbance	01 (0.9%)
6.	Tubercular infection	02 (1.7%)
7.	Uterine pathology	03 (2.6%)
8.	Combined factors	05 (4.4%)
9.	Others	02 (1.7%)
10.	No abnormalities Detected	44 (38.3%)
	Total	**115**


Graph 3: Probable cause for infertility
Chlamydia trachomatis
Semen abnormality
Tubal pathology
Ovarian pathology
Endocrinal disturbance
Tubercular infection
Uterine pathology
Combined factors
Others
No abnoramalities detected

DISCUSSION

The members of the order Chlamydiales are obligate intracellular bacteria that are proven or suspected pathogens of vertebrates. Recent studies from the United States and Europe reported that the prevalence of *Chlamydia trachomatis* ranges from 5-20% in sexually active persons . Among all women infected with *C. trachomatis* 75-80% are infected at the cervix, 70-80% of cervical infection are without associated symptoms or signs[6].

When present, the symptoms are non specific. Among them 20-30% have specific mucopurulent cervicitis including mucopurulent endocervical discharge, oedematous cervical ectopy with spontaneous or easily induced endocervical bleeding.

Among women, the consequences of the disease include PID, ectopic pregnancy and infertility, sequelae often accompanied by a substantial economic impact[6].

C. trachomatis is known to be one of the most frequent causes of sexually transmitted diseases and could seriously affect public health. Therefore, effective epidemiological control, starting with an adequate method for correct and sensitive diagnosis is required[48].

A truely effective Chlamydia control programme must be aimed at reducing the reservoir of infected asymptomatic individuals who make up the bulk of prevalent infections and are responsible for maintaining transmission of the infection within the community[49].

C.trachomatis is an attractive etiological agent for tubal occlusive disease for the following reasons:-

i) Chlamydial infections are commonly subclinical, a feature of the infection preceding tubal infertility.

ii) Epidemiological data document that non goncoccal salphingitis more frequently culminates in infertility than does gonococcal salphingtis, and *C. trachomatis* is a major cause of non gonococcal salphingtis

iii) Fibrosis is a principal pathological feature of tubal infertility and is a feature of infections due to *C. trachomatis*[50].

In the present study PCR results have been compared with ELISA for the diagnosis of *C.trachomatis* as this was readily available and facilities for cell culture and DFA were not available.

In our study the prevalence of *C.trachomatis* infection in infertile women was found to be 2.8% by PCR and 0.9 % by ELISA

A WHO study reported the current Chlamydia infection in infertile women to be 18-20%[2].

A previous study in a community of urban and rural adult population of Tamil nadu determined the prevalence of Chlamydia as 3.9%[8].

It is reported that PCR is superior in its sensitivity to ELISA.

According to one study, when PCR was compared with a direct EIA using 375 cervical specimens, PCR had a sensitivity of 100% while ELISA had a sensitivity of 58.8% and specificity of 100%[11].

In our study ELISA had a sensitivity of 33.3% and specificity of 100%. The positive predictive value and negative predictive value of ELISA was 100% and 98.2% respectively .

According to one study, out of the 91 patients studied there were no *C. trachomatis* infected case, in a group of infertile women who were evaluated by ELISA and PCR as routine protocols for the investigation before assisted reproductive treatment procedure[51].

In a study from Australia, the prevalence of Chlamydia infection was high (45%) in the infertile group and the number of infertile patients , who suffered from other types of infertility problems was fewer than the women with tubal occlusion[51].

In India, *C. trachomatis* was detected in 28% of infertile women after excluding other causes of infertility[2].

The duration of infertility in the Chlamydia positive cases in our study was approximately 1-5 years which corresponds well with other reports[2].

The incidence of *Chlamydia trachomatis* was more common in women with secondary infertility. This increased susceptibility could be due to their longer period of active sexual life, thus enhancing their exposure to Chlamydial infection.Secondary infertility associated with higher rates of Chlamydial infection have been reported earlier by other studies[2].

According to one study the highest frequency of *C. trachomatis* cervical infection was shown in women aged 28-30 years old but in our study, the highest *C. trachomatis* cervical infection frequency was found in women aged between 35-44 years

PCR is considered to be the most sensitive and specific diagnostic method for detection of infectious agents, including *C. trachomatis*.

On the contrary, another study showed that chlamydial etiology was identified more often using serology in comparison with PCR in PID patients[13].

The analysis of anti Chlamydia IgG titre estimation in single serum specimen has shown elevated anti chlamydia IgG levels in the female upper genital tract disease patients. Thus, it appears that a single serum IgG titre estimation may be diagnostically helpful to identify deep seated upper genital tract infection with *C. trachomatis* in the absence of paired serum specimens[13]. Although IgG antibodies are markers of a previous infection, they do not reflect an on-going chronic inflammatory property[52].

Chlamydia antibody testing has been incorporated in fertility work up on a large scale and has been shown to be a non invasive and cost effective screening method for tubal factor subfertility.

The persistently high Chlamydia IgG antibody titre may be due to reinfection by Chlamydia[53].

Women with positive serological test most likely have tubal disease and are candidates for directly proceeding to laproscopic evaluation and therefore by-passing hysterosalphingography, a procedure that may have a higher risk of infection in women who have previously had salphingitis[50].

To limit the complications of *C.trachamatis* infection in the population, a well planned disease management strategy from STD care providers, using highly sensitive

and specific diagnostic techniques and specific antibiotics for treatment of the patient and his /her partner according to the standard treatment regimens are mandatory[8].

Emphasis should be given to simple cost effective testing strategies such as serology for screening of high risk asymptomatic women[8].

The CDC has previously recommended confirming NAAT positive samples in population of low prevalence through supplemental testing ,which can increase positive predictive value and decrease the possibility of false positives[54].

The prevalence of *C.trachomatis* varies with the population under study and the sensitivity of the Laboratory methods used. Our study suggests that all infertile women should be screened for *C. trachomatis*.

In the absence of requisite infrastructure and skills for culture and for direct fluorescent assay, ELISA can play a significant role in screening for *C.trachomatis* in infertile women. Screening of infertile women for *C.trachomatis* is recommended in the first year of infertility itself so that early therapeutic intervention can be instituted to allow the woman to conceive naturally. Studies with larger sample size should further elucidate the extent of infertility caused by *C. trachomatis.*

CONCLUSION

In the present study we have screened 115 infertile women for *C.trachomatis* infection by ELISA and PCR.

1. The prevalence of *C.trachomatis* infection in infertile women by ELISA was found to be 0.9% and by PCR the prevalence was 2.6%

2. The sensitivity of ELISA was 33.3% and specificity was 100%

   The positive predictive value and negative predictive value of ELISA were 100% and 98.2% respectively

3. Maximum number of Chlamydia positive cases were in the age group of 35-44 years

4. Maximum number of Chlamydia positive cases had a duration of infertility between 1-5 yrs

SUMMARY

This study was conducted in the Department of Microbiology ,Jawaharlal Nehru Medical College, Belgaum from 1st Jan 2009 – 31st Dec 2009.

One hundred and fifteen infertile women of reproductive age attending the Assisted reproductive centre of KLE's DR Prabhakar Kore Hospital and Medical research centre were included in the study.

These infertile women were screened for *C. trachomatis* infection by ELISA and PCR.

For ELISA blood sample were collected and serum was used for the detection of Anti Chlamydial IgG Antibody.

For PCR, endocervical samples were used for detection of Chlamydial DNA.

Out of the 115 samples screened for *C. trachomatis*, PCR detected Chlamydia DNA in 3 samples and ELISA detected Anti Chlamydia IgG Antibody in 1 sample and ELISA was indeterminate for one sample

The samples which were positive and borderline by ELISA were positive by PCR . PCR was positive in 3 samples.

The prevalence of *C. trachomatis* infection in infertile women was 2.6% by PCR and 0.9% by ELISA

The sensitivity of ELISA was 33.3% and specificity was 100%

The positive predictive value and negative predictive value of ELISA was 100% and 98.2% respectively.

BIBLIOGRAPHY

1. Forbes B, Sahm D, Weissfeld A. Bailey and Scotts Diagnostic Microbiology. 10th edition. Missouri: Mosby Elsever; 1998: 510-8.

2. Malik A, Jain S, Hakim S, Shukla I , Rizvi M. *Chlamydia trachomatis* infection and female infertility. Indian J Med Res 2006; 123: 770-5.

3. Lan J, Walboomers J, Roosendaal R, van Doornum G, MacLaren D, Meijer C. Direct detection and genotyping of *Chlamydia trachomatis* using polymerase chain reaction and restriction fragment length polymorphism analysis. Journal of Clinical Microbiology 1993; 31: 1060-5.

4. Santos C, Teixeira F, Vicente A. Detection of *Chlamydia trachomatis* in endocervical smears of sexually active women in Manaus-AM, Brazil, by PCR. Braz J Infect Dis 2003; 7:1-6.

5. Ananthnarayan R, Paniker CKJ. Text book of Microbiology. 8th edition. Hyderabad: Universities press; 2009: 415-22.

6. Sattari M, Zeighami H, Peerayea SN. Detection of *Chlamydia trachomatis* in endocervical smears of women with abortion. Research Journal of Biological Sciences 2008; 3: 214-6.

7. Borriello P, Murray PR, Funke G. Topley and Wilsons Microbiology and Microbial Infections. 10th edition. USA: ASM press; 2005: 2006-26.

8. Ray K. *Chlamydia trachomatis* and infertility. Indian J Med Res 2006; 123:730-4.

9. Collee JG, Doguid JP, Fraser AG, Marmion BP. Mackie and McCartney. Practical Medical microbiology. 14th edition. USA: Churchill Livingstone; 1989: 621-33.

10. Keyhani AH, Nazer M, Mirsalehian A. Evaluation of serum Chlamydia IgG antibody in women with tubal factor infertility. Research Journal of Medicine and Medical Sciences 2006; 1: 150-3.

11. Mania-Pramanik J, Donde UM, Maitra A. Use of polymerase chain reaction (PCR) for detection of *Chlamydia trachomatis* infection in cervical swab samples. Indian J Dermatol Venereol Leprol 2001; 67:246-50.

12. Das V, Agarwal M, Agarwal J, Jain A, Agarwal A, Pandey A. Prevalence of genital *Chlamydia trachomatis* by first void urine polymerase chain reaction test in women attending out patient clinic.J Obstet Gynecol India 2006; 56:511-3.

13. Joyee AG, Thyagarajan SP, Reddy V, Rajendran P, Venkatesan C. Diagnostic utility of serologic markers for genital chlamydial infection in STD patients in Chennai, India. J Assoc Physicians India 2007; 55:777-80.

14. Hartog JE, Land JA, Stassen FRM, Kessels AGH, Bruggeman CA. Serological markers of persistent *C. trachomatis* infections in women with tubal factor subfertility. Human Reproduction 2005; 20: 986-90.

15. Ossewaarde JM, Rieffe M, Rozenberg M, Ossenkoppele PM, Nawrocki RP, van Loon AM. Development and clinical evaluation of a polymerase chain reaction test for detection of *Chlamydia trachomatis*. J Clin Microbiol 1992; 30: 2122-8.

16. Fontana C, Favaro M, Cicchetti O, Minelli S, Pistoia E, Favalli C. Performance of strand displacement amplification assay in the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Jpn J Infect Dis 2005; 58: 283-8.

17. Malenie R, Joshi PJ, Mathur MD. *Chlamydia trachomatis* antigen detection in pregnancy and its verification by antibody blocking assay. Indian J Med Microbiol 2006; 24: 97-100.

18. Yasodhara P, Ramalakshmi BA, Naidu AN, Raman L. Prevalence of specific IgM due to toxoplasma, rubella, CMV and *C. trachomatis* infections during pregnancy. Indian J Med Microbiol 2001; 19: 79-82.

19. Tam MR, Stamm W, Handsfield H, Stephens R, Kuo C, Holmes K et al. Culture-independent diagnosis of *Chlamydia trachomatis* using monoclonal antibodies. N Eng J Med 1984; 310: 1146-50.

20. Pearlman MD, Mcneeley SG. A review of the microbiology, immunology, and clinical implications of *Chlamydia trachomatis* infections. Obstetrical and Gynecological survey 1992; 47: 448-61.

21. Howard BJ, Klaas J, Weissfeld AS, Rubin SJ, Tilton RC. Clinical and Pathogenic Microbiology. The CV Mosby Company; 1987: 835-41.

22. Lampe MF, Suchland RJ, Stamm WE. Nucleotide sequence of the variable domains within the major outer membrane protein gene from serovariants of *Chlamydia trachomatis*. Infection and Immunity 1993; 61: 213-9.

23. Wyrick PB, Choong J, Davis CH. Entry of genital *C.trachomatis* into polarised human epithelial cells. Infection and Immunity 1989; 57: 2378-89.

24. Prain CJ, Pearce JH. Ultrastructural studies on the intracellular fate of *Chlamydia psittaci* (strain guinea pig inclusion conjunctivitis) and *Chlamydia trachomatis* (strain lymphogranuloma venereum 434) : modulation of intracellular events and relationship with endocytic mechanism. J Gen Bacteriol 1989; 135: 2107-33.

25. Cates W. Jr, Wasserheit JN. Genital Chlamydial infections : epidemiology and reproductive sequelae. Am J Obstet Gynaecol 1991; 164: 1771-81.

26. Howkins and Bourne. Shaw's textbook of gynaecology. 12th edition. New Delhi: Churchill Livingstone; 1999: 156-161.

27. Veenemans LMW, van der Linden PJQ. The value of *Chlamydia trachomatis* antibody testing in predicting tubal factor infertility. Human reproduction 2002; 17: 695-8.

28. Rao S, Narayan PK, Shivananda PG. *Chlamydia trachomatis* antigen from cervix by ELISA in women with recurrent abortions. J Obstet and Gynaecol 1997: 164-7.

29. Zelin JM, Robinson AJ, Ridgway GL, Ailason-Jones E, Williams P. Chlamydial urethritis in heterosexual men attending a genitourinary medicine clinic; prevalence, symptoms, condom usage and partner change. Int J STD AIDS 1995; 6:27-30.

30. Witkin S, Ledger W. Antibodies to *Chlamydia trachomatis* in sera of women with recurrent spontaneous abortions. Am J Obstet Gynaecol 1992; 167: 135-9.

31. Jones RB, Katz BP, Van Der Pol B, Caine VA, Batteiger BE, Newhall WJ. Effect of blind passage and multiple sampling on recovery of *Chlamydia trachomatis* from urogenital specimens. J Clin Microbiol 1986; 24; 1029-33.

32. Mardh PA, Zeerberg B. Toxic effects of sampling swabs and transportation test tubes on the formation of intracytoplasmic inclusions of Chlamydia trachomatis in McCoy's cell cultures. Br J Vener Dis 1981; 57: 268-72

33. Mahony J, Chernesky M. Effect of swab type and storage temperature on isolation of *Chlamydia trachomatis* from clinical specimens. J Clin Microbiol 1985; 22: 865-7.

34. Moncada J, Schachter J, Shipp M, Bolan G, Wilber J. Cytobrush in collection of cervical specimens for detection of *Chlamydia trachomatis*. J Clin Microbiol 1989; 27; 1863-6.

35. Winn WC, Allen S, Janda W, Koneman E, Procop GW, Schreckenberger PC, et al. Koneman's Colour Atlas and Textbook of Diagnostic Microbiology. 6th Ed. Philadelphia: Lippincott Williams & Wilkins; 2006: 1403-11.

36. Warford AL, Carter TL, Levy RA, Rekrut KA. Comparision of sonicated and nonsonicated specimens for the isolation of *Chlamydia trachomatis*. Am J Clin Pathol 1985; 83: 625-9.

37. Rota TR, Nichols RL. *Chlamydia trachomatis* in cell culture-Comarision of efficiencies of infection in several chemically defined media, at various pH and temperature values, and after exposure to diethylaminoethyl dextran. Appl Microbiol 1973; 26:260-5.

38. Barnes RC. Laboratory diagnosis of human Chlamydial infections. Clin Microbiol Rev 1989; 2: 119-36.

39. Loeffelholz MJ, Lewinski CA, Silver SR, Purohit AP, Herman SA, Buonagurio DA et al. Detection of *Chlamydia trachomatis* in endocervical specimens by polymerase chain reaction. J Clin Microbiol 1992; 30: 2847-51.

40. Kellogg JA, Seiple JW, Stroll ES. Direct fluorescent-antibody confirmation of chlamydial antigen below the detection threshold of the chlamydiazyme enzyme-linked immunosorbent assay. J Clin Microbiol 1993; 31: 1646-7.

41. Chermesky MA, Mahoney JB, Castriciane S, Mores M, Stewart IO, Landis SF et al. Detection of *Chlamydia trachomatis* antigens by enzyme immunoassay and immunofluorescence in genital specimens from symptomatic and asymptomatic men and women. J Infect Dis 1986; 54: 141-8.

42. Clarke LM, Sierra MF, Daidone BJ, Lopez M, Covino JM, McCormack WM. Comparision of enzyme immunoassay and Gen Probe PACE-2 with cell culture for diagnosis of *C.trachomatis* infection in a high prevalence female population . J Clin Microbiol 1993; 31: 1646-7.

43. Warren RB, Dwyer M, Plackett K, Petti N, Rizvi N, Baker AM. Comparitive evaluation of detection assays for *Chlamydia trachomatis*. J Clin Microbiol 1993; 31: 1663-6.

44. Goessens WH, Kluytmans J, Toom N, van Rijsoort-vos TH, Niesters BG, Stolz E et al. Influence of volume of sample processed on detection of *Chlamydia trachomatis* in Urogenital samples by PCR. Journal of Clinical Microbiology 1995; 33: 251-3.

45. Wiesenfeld HC, Uhrin M, Dixon BW, Sweet RL. Diagnosis of male *Chlamydia trachomatis* urethritis by polymerase chain reaction . Sex Transm dis 1994; 21: 268-71.

46. Chermesky MA, Jang D, Lee H, Hu H, Sellors J, Tomazic-Allen SJ et al. Diagnosis of *Chlamydia trachomatis* infections in men and women by testing first-void urine by ligase chain reaction. J Clin Microbiol 1994; 32: 2682-5.

47. Martin DH, Mroczkowski TF, Dalu ZA, McCarty J, Jones RB, Hopkins SJ et al. A controlled trial of a single dose of azithromycin for the treatment of chlamydial urethritis and cervicitis. N Eng J Med 1992; 327: 921-5.

48. Jenab A, Golbang N, Golbang P, Chamani-Tabriz L, Roghanian R. Diagnostic value of PCR and ELISA for *Chlamydia trachomatis* in a group of asymptomatic and symptomatic women in Isfahan, Iran. International Journal of Fertility and Sterility 2009; 2: 193-8.

49. Schachter J, Stamm WE, Quinn TC, Andrews WW, Burczak JD, Lee HH. Ligase chain reaction to detect *Chlamydia trachomatis* infection of the cervix. J Clin Microbiol 1994; 32: 2540-3.

50. Brunham R, Maclean I, Binns B, Peeling R. *Chlamydia trachomatis*: Its role in tubal infertility. The Journal of Infectious Diseases 1985; 152: 1275-82.

51. Kalantar S, Kazemi M, Sheikhha M, Aflatoonian A, Kafilzadeh F. Detection of *Chlamydia trachomatis* infection in female partners of infertile couples. International Journal of Fertility and Sterility 2007; 1: 79-84

52. Land JA, Gijsen AP, Kessels A, Slobbe M, Bruggeman C. Performance of five serological chlamydia antibody tests in subfertile women. Human Reproduction 2003; 18: 2621-7.

53. Gijsen, Anna P, Jolande AL, Valere G, Marlea EPS, Cathrien AB. Chlamydia antibody testing in screening for tubal factor subfertility : the significance of IgG antibody decline over time. Human Reproduction 2002; 17: 699-703.

54. Masek BJ, Arora N, Quinn N, Aumakhan B, Holden J, Hardick A et al. Performance of three nucleic acid amplification tests for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by use of self collected vaginal swabs obtained via an internet based screening program. Journal of Clinical Microbiology 2009; 47: 1663-7.

CONSENT FOR PARTICIPATION IN RESEARCH

We are requesting you to enrollee yourself in study titled **"PREVALENCE AND LABORATORY DIAGNOSIS OF CHLAMYDIA TRACHOMATIS INFECTION IN INFERTILE WOMEN BY ELISA & PCR. A ONE-YEAR CROSS SECTIONAL STUDY"** conducted by Reg no BI0108001, Postgraduate student in Microbiology , JNMC, Belgaum under KLE Academy of Higher Education and Research Centre, Belgaum.

You have been requested to participate in research because you are into the study group. During the study you will be asked some questions and you are supposed to answer to the best of your knowledge.

Your participation in research is voluntary. Your decision whether or not to participate in the study will not affect your relationship with JNMC. If you decide to participate you are free to withdraw at any time.

The purpose of research is to diagnose *C.trachomatis* infection in infertile women.

PROCEDURE INVOLVED :

Endocervical swab and blood collected from infertile women.

RISKS AND BENEFITS

There are no extra risks and benefit will be that treatment can be started early against the infection if diagnosed.

PRIVACY AND CONFIDENTIALITY

The only people to know that you are a research subject are members of the research team. No information about you or provided by you during research will be disclosed to others without your written permission, except :

1. In emergency to protect your rights and welfare.
2. If required by law.

AUTHORIZATION TO PUBLISH RESULTS

When the results are published or discussed, in a conference no information will be displayed that would disclose your identity. Any information that is obtained in connection with this study and that can be identified with you will remain confidential.

FINANCIAL INCENTIVES FOR PARTICIPATION

You will not be paid / offered any free gifts for participating in the research. You will not be reimbursed for expenses.

I undersigned _____ have been explained in my vernacular languageabout the study and my participation in the study is voluntary. If I want, I can withdraw at any time. Also I have been given enough time to clear my doubts and rights as study participant.

In case you have any questions about your rights as a study participant, you can contact Dr. V. D. Patil.

CONSENT STATEMENT

Signature or left hand thumb print of participant or legally authorized representative.

Participants Name _____ Signature _____

Witness Name Signature _____ Signature _____

Experimenters Name Signature _____ Signature _____

Date :

Place :

PROFORMA

TITLE: PREVALENCE AND LABORATORY DIAGNOSIS OF CHLAMYDIA TRACHOMATIS INFECTION IN INFERTILE WOMEN BY ELISA and PCR. A ONE-YEAR CROSS SECTIONAL STUDY.

Name : Age:

Parity :

Socioeconomic status :

Educational status :

Occupation :

Husband's occupation :

History of consanguinity :

History of present illness :

Past history :

Investigations done :

ELISA IgG for *Chlamydia trachomatis* infection

Results:

Interpretation:

PCR for *Chlamydia trachomatis* DNA

MASTER CHART

S. No.	Age	O. P. No.	Diagnosis	Duration of infertility	Sample index of Elisa	Results	PCR	Probable cause of infertility
1.	38 yrs	1030489	Secondary infertility	7 yrs	0.24	Negative	Chlamydia DNA Not detected	Azoospermia
2.	36 yrs	997496	Secondary infertility	11 yrs	0.31	Negative	Chlamydia DNA Not detected	Oligospermia
3.	25 yrs	936271	Primary Infertility	6 ½ yrs	0.29	Negative	Chlamydia DNA Not detected	B/L tubal block
4.	29 yrs	1070126	Primary Infertility	3 ½ yrs	0.10	Negative	Chlamydia DNA Not detected	No abnormality detected
5.	29 yrs	1086531	Primary Infertility	9 yrs	0.64	Negative	Chlamydia DNA Not detected	Azoospermia
6.	36 yrs	Private	Primary Infertility	11 yrs	0.19	Negative	Chlamydia DNA Not detected	No abnormality detected
7.	25 yrs	1011783	Primary Infertility	7 yrs	0.29	Negative	Chlamydia DNA Not detected	Oligoastheno teratozoospermia
8.	35 yrs	1091418	Primary Infertility	7 yrs	0.92	Borderline	Chlamydia DNA detected	Chlamydia trachomatis

9.	25 yrs	922457	Primary Infertility	3 yrs	0.23	Negative	Chlamydia DNA Not detected	Azoospermia
10.	23 yrs	970326	Primary Infertility	1 yrs	0.23	Negative	Chlamydia DNA Not detected	Endometriosis
11.	28 yrs	1092733	Secondary Infertility	5 yrs	0.13	Negative	Chlamydia DNA Not detected	Oligospemia / polycystic ovarian syndrome
12.	33 yrs	1089087	Secondary Infertility	10 yrs	0.11	Negative	Chlamydia DNA Not detected	polycystic ovarian syndrome
13.	26 yrs	982297	Primary Infertility	2 ½ yrs	0.32	Negative	Chlamydia DNA Not detected	Tubal adhesions
14.	38 yrs	1111878	Primary Infertility	8 yrs	0.25	Negative	Chlamydia DNA Not detected	Oligorpermia
15.	38 yrs	1086312	Secondary Infertility	11 yrs	0.30	Negative	Chlamydia DNA Not detected	Tubal block
16.	20 yrs	1083000	Primary Infertility	2 yrs	0.68	Negative	Chlamydia DNA Not detected	No abnormality detected
17.	32 yrs	1109472	Primary Infertility	7 yrs	0.19	Negative	Chlamydia DNA Not detected	No abnormality detected
18.	31 yrs	115472	Primary Infertility	17 yrs	0.23	Negative	Chlamydia DNA Not detected	No abnormality detected

19.	35 yrs	1121170	Primary Infertility	11 yrs	0.18	Negative	Chlamydia DNA Not detected	Hypothyroidism
20.	28 yrs	890055	Primary Infertility	5 yrs	0.40	Negative	Chlamydia DNA Not detected	B/L Polycystic ovarian syndrome
21.	28 yrs	1121937	Primary Infertility	2 yrs	0.24	Negative	Chlamydia DNA Not detected	Chocolate cyst
22.	24 yrs	1100831	Primary Infertility	3 yrs	0.70	Negative	Chlamydia DNA Not detected	No abnormality detected
23.	22 yrs	1018814	Primary Infertility	4 yrs	0.35	Negative	Chlamydia DNA Not detected	Azoorpermia
24.	28 yrs	1062912	Primary Inferility	1 yrs	0.27	Negative	Chlamydia DNA Not detected	Oligospermia
25.	23 yrs	1123592	Primary Infertility	11 yrs	0.41	Negative	Chlamydia DNA Not detected	Oligospermia
26.	20 yrs	1052915	Primary Infertility	2 yrs	0.15	Negative	Chlamydia DNA Not detected	Azoospermia
27.	28 yrs	957028	Primary Infertility	7 yrs	0.30	Negative	Chlamydia DNA Not detected	Oligospermia & B/L Cornual Block
28.	33 yrs	1128203	Primary Infertility	9 yrs	0.059	Negative	Chlamydia DNA Not detected	Oligospermia

29.	36 yrs	1123846	Primary Infertility	3 yrs	0.14	Negative	Chlamydia DNA Not detected	No abnormality detected
30.	34 yrs	981504	Primary Infertility	3 ½ yrs	0.03	Negative	Chlamydia DNA Not detected	Oligoasthenozoospermia
31.	21 yrs	1072974	Primary Infertility	1 ½ yrs	0.13	Negative	Chlamydia DNA Not detected	No abnormality detected
32.	30 yrs	1134583	Primary Infertility	11 yrs	0.3	Negative	Chlamydia DNA Not detected	Oligospermia
33.	32 yrs	1112208	Primary Infertility	1 yrs	0.4	Negative	Chlamydia DNA Not detected	Fibroids
34.	22 yrs	1132313	Primary Infertility	4 yrs	0.7	Negative	Chlamydia DNA Not detected	Beaded tubes
35.	32 yrs	1126004	Primary Infertility	12 yrs	0.4	Negative	Chlamydia DNA Not detected	Azoospermia
36.	27 yrs	1152792	Primary Infertility	8 yrs	0.5	Negative	Chlamydia DNA Not detected	Oligospermia
37.	22 yrs	1153337	Primary Infertility	3 yrs	0.7	Negative	Chlamydia DNA Not detected	No abnormality detected
38.	22 yrs	918239	Primary Infertility	3 yrs	0.5	Negative	Chlamydia DNA Not detected	TB PCR Detected in endometrial curettage

39.	26 yrs	1165170	Primary Infertility	3 yrs	0.6	Negative	Chlamydia DNA Not detected	No abnormality detected
40.	21 yrs	1119480	Primary Infertility	7 yrs	0.2	Negative	Chlamydia DNA Not detected	Oligospermia
41.	24 yrs	1032406	Secondary Infertility	5 yrs	0.2	Negative	Chlamydia DNA Not detected	Oligospermia
42.	32 yrs	1143302	Primary Infertility	18 yrs	0.2	Negative	Chlamydia DNA Not detected	No abnormality detected
43.	26 yrs	1166380	Secondary Infertility	2 yrs	1.4	Positive	Chlamydia DNA detected	Chlamydia trachomatis Infection
44.	28 yrs	702366	Primary Infertility	10 yrs	0.3	Negative	Chlamydia DNA Not detected	Oligospermia
45.	25 yrs	11219	Primary Infertility	9 yrs	0.2	Negative	Chlamydia DNA Not detected	Azoospermia
46.	24 yrs	1196077	Secondary Infertility	2 yrs	0.4	Negative	Chlamydia DNA Not detected	Oligospermia
47.	28 yrs	1174818	Primary Infertility	18 yrs	0.3	Negative	Chlamydia DNA Not detected	Azoospermia
48.	28 yrs	1086428	Primary Infertility	1 ½ yrs	0.2	Negative	Chlamydia DNA Not detected	Azoospermia

49.	22 yrs	111585	Secondary Infertility	1 yrs	0.01	Negative	Chlamydia DNA Not detected	No abnormality detected
50.	25 yrs	Private	Primary Infertility	4 yrs	0.3	Negative	Chlamydia DNA Not detected	No abnormality detected
51.	30 yrs	Private	Primary Infertility	12 yrs	0.3	Negative	Chlamydia DNA Not detected	No abnormality detected
52.	31 yrs	Private	Primary Infertility	13 yrs	0.3	Negative	Chlamydia DNA Not detected	No abnormality detected
53.	38 yrs	Private	Primary Infertility	14 yrs	0.4	Negative	Chlamydia DNA Not detected	Azoospermia
54.	28 yrs	Private	Primary Infertility	4 ½ yrs	0.7	Negative	Chlamydia DNA detected	Chlamydia trachomatis infection
55.	32 yrs	1151130	Primary Infertility	8 yrs	0.3	Negative	Chlamydia DNA Not detected	PCOD/Oligospermia
56.	25 yrs	117094	Primary Infertility	5 yrs	0.2	Negative	Chlamydia DNA Not detected	Azoospermia
57.	26 yrs	Private	Primary Infertility	11 yrs	0.28	Negative	Chlamydia DNA Not detected	No abnormality detected
58.	30 yrs	1199126	Primary Infertility	3 yrs	0.2	Negative	Chlamydia DNA Not detected	No abnormality detected

59.	30 yrs	Private	Primary Infertility	5 yrs	0.6	Negative	Chlamydia DNA Not detected	No abnormality detected
60.	30 yrs	1201410	Secondary Infertility	5 yrs	0.5	Negative	Chlamydia DNA Not detected	No abnormality detected
61.	38 yrs	1200651	Secondary Infertility	13 yrs	0.1	Negative	Chlamydia DNA Not detected	Multiple fibroids / B/L tubal block
62.	31 yrs	1091155	Primary Infertility	8 yrs	0.4	Negative	Chlamydia DNA Not detected	Oligospermia
63.	33 yrs	1184832	Primary Infertility	6 ½ yrs	0.1	Negative	Chlamydia DNA Not detected	No abnormality detected
64.	24 yrs	Private	Primary Infertility	3 yrs	0.1	Negative	Chlamydia DNA detected	No abnormality detected
65.	35 yrs	1206744	Secondary Infirmity	15 yrs	0.1	Negative	Chlamydia DNA Not detected	No abnormality detected
66.	31 yrs	Private	Primary Infertility	8 yrs	0.2	Negative	Chlamydia DNA Not detected	No abnormality detected
67.	23 yrs	Private	Secondary Infirmity	1yr	0.23	Negative	Chlamydia DNA Not detected	No abnormality detected
68.	36 yrs	Private	Secondary Infirmity	8 yrs	0.15	Negative	Chlamydia DNA Not detected	Tubal block

69.	28 yrs	1174816	Primary Infertility	12 yrs	0.02	Negative	Chlamydia DNA Not detected	Azoospermia
70.	35 yrs	Private	Primary Infertility	10 yrs	0.3	Negative	Chlamydia DNA Not detected	Azoospermia
71.	29 yrs	Private	Primary Infertility	4 yrs	0.3	Negative	Chlamydia DNA Not detected	No abnormality detected
72.	22 yrs	1211115	Primary Infertility	5yrs	0.2	Negative	Chlamydia DNA Not detected	Azoospermia
73.	30 yrs	Private	Primary Infertility	10 yrs	0.04	Negative	Chlamydia DNA Not detected	Azoospermia
74.	28 yrs	1173382	Primary Infertility	13 yrs	0.2	Negative	Chlamydia DNA detected	B/L Cornual Block
75.	27 yrs	929022	Primary Infertility	2 ½ yrs	0.2	Negative	Chlamydia DNA Not detected	Azoospermia
76.	28 yrs	Private	Primary Infertility	8 yrs	0.12	Negative	Chlamydia DNA Not detected	No abnormality detected
77.	25 yrs	1173306	Primary Infertility	6 yrs	0.03	Negative	Chlamydia DNA Not detected	Oligospermia & Polyp
78.	25 yrs	588080	Primary Infertility	6 yrs	0.65	Negative	Chlamydia DNA Not detected	No abnormality detected

79.	23 yrs	Private	Primary Infertility	5 yrs	0.23	Negative	Chlamydia DNA Not detected	No abnormality detected
80.	25 yrs	Private	Primary Infertility	5 yrs	0.48	Negative	Chlamydia DNA Not detected	Azoospermia
81.	26 yrs	Private	Primary Infertility	4 yrs	0.4	Negative	Chlamydia DNA Not detected	Azoospermia
82.	27 yrs	Private	Primary Infertility	13 yrs	0.33	Negative	Chlamydia DNA Not detected	No abnormality detected
83.	23 yrs	Private	Primary Infertility	4 yrs	0.15	Negative	Chlamydia DNA Not detected	No abnormality detected
84.	22 yrs	1214811	Primary Infertility	5 yrs	0.3	Negative	Chlamydia DNA detected	No abnormality detected
85.	29 yrs	Private	Secondary Infertility	1 yrs	0.62	Negative	Chlamydia DNA Not detected	No abnormality detected
86.	26 yrs	1154204	Primary Infertility	1 ½ yrs	0.51	Negative	Chlamydia DNA Not detected	Necrospermia
87.	27 yrs	1216530	Secondary Infertility	8 yrs	0.38	Negative	Chlamydia DNA Not detected	Oligorpermia
88.	34 yrs	Private	Primary Infertility	14 yrs	0.38	Negative	Chlamydia DNA Not detected	Uterine Septum

89.	27 yrs	Private	Primary Infertility	4 yrs	0.31	Negative	Chlamydia DNA Not detected	No abnormality detected
90.	38 yrs	Private	Primary Infertility	18 yrs	0.2	Negative	Chlamydia DNA Not detected	No abnormality detected
91.	28 yrs	Private	Secondary Infertility	6 yrs	0.09	Negative	Chlamydia DNA Not detected	Tubal Block
92.	33 yrs	Private	Primary Infertility	4 ½ yrs	0.27	Negative	Chlamydia DNA Not detected	Oligospermia
93.	25 yrs	Private	Primary Infertility	1 ½ yrs	0.35	Negative	Chlamydia DNA Not detected	Azoospermia
94.	30 yrs	Private	Primary Infertility	12 yrs	0.44	Negative	Chlamydia DNA detected	B/L Ampullary block
95.	28 yrs	1144705	Primary Infertility	7 yrs	0.3	Negative	Chlamydia DNA Not detected	No abnormality detected
96.	25 yrs	Private	Primary Infertility	5 yrs	0.29	Negative	Chlamydia DNA Not detected	Oligospermia
97.	22 yrs	1171028	Primary Infertility	5 yrs	0.31	Negative	Chlamydia DNA Not detected	PCOD / Oligospermia
98.	28 yrs	Private	Primary Infertility	5 yrs	0.19	Negative	Chlamydia DNA Not detected	No abnormality detected

99.	25 yrs	636084	Primary Infertility	10 yrs	0.08	Negative	Chlamydia DNA Not detected	PCOS
100.	30 yrs	Private	Primary Infertility	2 ½ yrs	0.31	Negative	Chlamydia DNA Not detected	Hydrosalphinx
101.	38 yrs	Private	Primary Infertility	13 yrs	0.22	Negative	Chlamydia DNA Not detected	PCOS
102.	31 yrs	1185692	Primary Infertility	9 yrs	0.58	Negative	Chlamydia DNA Not detected	No abnormality detected
103.	22 yrs	118563	Primary Infertility	6 ½ yrs	0.38	Negative	Chlamydia DNA Not detected	No abnormality detected
104.	22 yrs	1231053	Primary Infertility	3 yrs	0.01	Negative	Chlamydia DNA detected	PCOD / Oligospermia
105.	38 yrs	1228096	Secondary Infertility	1 yrs	0.25	Negative	Chlamydia DNA Not detected	No abnormality detected
106.	22 yrs	Private	Primary Infertility	4 yrs	0.62	Negative	Chlamydia DNA Not detected	No abnormality detected
107.	30 yrs	Private	Primary Infertility	7 yrs	0.49	Negative	Chlamydia DNA Not detected	Endometrial TB (PCR+)
108.	24 yrs	Private	Primary Infertility	5 yrs	0.3	Negative	Chlamydia DNA Not detected	Ovarian cyst

109.	22 yrs	1236096	Primary Infertility	5 yrs	0.4	Negative	Chlamydia DNA Not detected	Oligospermia
110.	31 yrs	Private	Primary Infertility	2 yrs	0.32	Negative	Chlamydia DNA Not detected	No abnormality detected
111.	30 yrs	Private	Secondary Infertility	8 yrs	0.17	Negative	Chlamydia DNA Not detected	No abnormality detected
112.	29 yrs	1239380	Primary Infertility	4 ½ yrs	0.04	Negative	Chlamydia DNA Not detected	Tubal Block at fimbrial end
113.	32 yrs	Private	Secondary Infertility	4 yrs	0.25	Negative	Chlamydia DNA Not detected	No abnormality detected
114.	24 yrs	905580	Primary Infertility	1 yrs	0.26	Negative	Chlamydia DNA detected	No abnormality detected
115.	29 yrs	1239381	Secondary Infertility	3 yrs	0.35	Negative	Chlamydia DNA Not detected	Oligospermia