"THE PREVALENCE OF CHLAMYDIA TRACHOMATIS BY ELISA IN INFERTILE WOMEN"

By

REG.NO.BJ0108006

DISSERTATION

Submitted to the

KLE University, Belgaum, Karnataka

In partial fulfillment

Of the requirements for the degree of

MASTER OF SURGERY

IN

OBSTETRICS AND GYNAECOLOGY

DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY,

JAWAHARLAL NEHRU MEDICAL COLLEGE,

BELGAUM – 10, KARNATAKA

MAY - 2011

KLE UNIVERSITY, BELGAUM, KARNATAKA

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LIST OF ABBREVIATIONS USED

ATP	Adenosine triphosphate
CF	Complement fixation
c-hsp 60	Chlamydia trachomatis heat shock protein-60
DNA	Deoxy ribonucleic acid
EIA	Enzyme immunoassay
ELISA	Enzyme linked immunosorbent assay
HSG	Hysterosalpingography
HSP60	Heat shock protein 60
ICMR	Indian Council of Medical Research
IFN-g	Interferon gamma
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IVF-ET	Invitro fertilisation-Embryo transfer
KD	Kilodalton
KLES	Karnataka Lingauyat Educational Society
LCR	Ligase Chain reaction
LPS	Lipopolysaccharide
MIF	Microimmunofluorescence
MOMP	Major Outer membrane protein
MRC	Medical Research Centre
PCR	Polymerase Chain reaction

PID	pelvic inflammatory disease
RNA	Ribonucleic acid
ROC	Receiver operator characteristic
rRNA	Ribosomal ribonucleic acid
RU	Relative units
STD	Sexually transmitted disease
TFI	Tubal factor infertility
TMA	Transcription Mediated Amplification
TNF-a	Tumour necrosis factor alpha
WHO	World Health Organization

ABSTRACT

"The Prevalence of Chlamydia trachomatis by ELISA in infertile women"

Background: Chlamydia trachomatis has currently emerged as a most common sexually transmitted pathogen. The undetected and untreated infection with Chlamydia trachomatis may lead to infertility. Infertility due to Chlamydia trachomatis infection is a preventable one, if detected early. Data pertaining to infertility attributed to Chlamydia trachomatis is very limited in India, thus preventing any policy from being formulated regarding screening of patients with infertility. Hence this study was undertaken to estimate the prevalence of Chlamydia trachomatis infection among infertile women.

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Results: Of the 257 subjects 19 were positive for Chlamydia trachomatis infection. The prevalence of Chlamydia trachomatis infection in infertile women is found to be 7.3%.There was no significant association between the type of infertility and Chlamydia

trachomatis IgG ELISA positivity in our study. All the positive cases detected in the study were asymptomatic.

Conclusion: The frequency of Chlamydia trachomatis infection in infertile women is 1 in 15, which is high. Early diagnosis and identification of Chlamydia trachomatis by a low cost test such as IgG ELISA would prevent further harmful and damaging sequelae of Chlamydia trachomatis infection.

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Under the Guidance of

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation/thesis entitled **"THE PREVALENCE OF CHLAMYDIA TRACHOMATIS BY ELISA IN INFERTILE WOMEN"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. GEETA DURDI** **MD**, Asso. Professor, Department of Obstetrics & Gynaecology, and co- the guidance of **Dr. S.C METGUD** **MD**, Professor, Department of Microbiology, Jawaharlal Nehru Medical College, Nehru Nagar, Belgaum – 590010.

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INTRODUCTION

Chlamydia trachomatis has currently emerged as a most common sexually transmitted pathogen.[1] Recent reports suggest that genital Chlamydia trachomatis may be more important than gonococcal infection to lead to PID[2].Chlamydial infections produce less severe symptoms than other sexually transmitted diseases. These deceptively mild symptoms allow the infection to go unnoticed with minimal patient awareness until secondary or tertiary symptoms develop. The sequelae of undetected and untreated infections like acute salpingitis and pelvic inflammatory disease lead not only to significant morbidity, but far more importantly to infertility. Infertility due to Chlamydia trachomatis infection is a preventable one, if detected early.[3]

In PID cases due to Chlamydia trachomatis, high levels of persistent circulating IgG antibodies are produced. High levels of Chlamydial IgG antibodies are associated with tubal damage and increased risk of tubal factor infertility.[4] It suggests that these women have suffered more frequent, more prolonged or more severe infection than others. Tubal occlusion following PID is one of the most common causes of infertility among women, and in more than 30 percent of women, infertility is due to tubal occlusion. Among women with Chlamydial lower genital tract infection, 20 percent will develop PID, 4 percent chronic pelvic pain, 3 percent infertility and 3 percent adverse pregnancy outcome.[5] The intense and chronic infection elicited and maintained by re-infection or persistent infection with Chlamydia

trachomatis leads to thedamaging sequelae such as infertility, and it can cause severe tubal immunopathology in spite of the absence of overt symptoms.PID and its chronic sequelae are associated with Chlamydial IgG antibody formation.[2]

A correlation between serum antibody titres and the presence of tubal factor subfertility has been established.[4] Chlamydia trachomatis antibody testing could provide a clinically useful screening test for predicting tubal factor infertility in women.[2] The two most routinely used methods of assessment for tubal disease are hyterosalpingography and laparoscopy. Laparoscopy is considered the gold standard for the assessment of tubal factor infertility. However it is an invasive and expensive procedure making it unsuitable for screening purposes. Therefore Chlamydia trachomatis antibody testing should be done before laparoscopy or HSG. Introduction of a serological test allowing specific diagnosis of tubal factor infertility due to Chlamydia trachomatis into clinical practice would confirm data, exclude other possible causes of infertility, and lead to prescription of appropriate antibiotic treatment. Early identification of women with high risk of developing PID may help to prevent long term sequel and may improve treatment efficacy during the early stage of infection. In the absence of requisite infrastructure and skills for culture and direct fluorescent assay, ELISA can play a significant role in screening for Chlamydia trachomatis in infertile women.[3] Data pertaining to infertility attributed to Chlamydia trachomatis is very limited in India, thus preventing any policy from being formulated regarding screening of

patients with infertility. Hence this study was undertaken to estimate the prevalence of Chlamydia trachomatis infection among infertile women

AIMS AND OBJECTIVES

Primary Objective: To study the prevalence of Chlamydia trachomatis infection in infertile women.

REVIEW OF LITERATURE

Chlamydiae are non-motile obligate intracellular bacteria. Their unique developmental cycle differentiates them from all other microorganisms. They replicate within the cytoplasm of the host cells, forming characteristic intracellular inclusions that can be seen by light microscopy. They differ from viruses by possessing both DNA and RNA and have cell walls quite similar in structure to gram negative bacteria. They are susceptible to many broad spectrum antibiotics, possess a number of enzymes, and have a restricted metabolic capacity. None of these metabolic reactions result in the production of energy. Hence they have been considered energy parasites that use the ATP produced by the host cells for their own requirements. Chlamydiae are presently placed in their own order, the chlamydiales, family Chlamydiaceae, with one genus, Chlamydia. There are four species: Chlamydia trachomatis, Chlamydia psittaci, Chlamydia pecorum, and Chlamydia pneumonia.[6]

Chlamydia trachomatis includes the organisms causing trachoma, inclusion conjunctivitis, lymphogranuloma venereum and genital tract diseases. Chlamydia trachomatis also causes pneumonia in infants and immunocompromised hosts, and it is associated with oligoarthritis (Reiter's syndrome). Chlamydia trachomatis is almost exclusively a human pathogen and is the most common sexually transmitted bacterial agent. Sexual transmission of Chlamydia trachomatis occurs through serovars D through K. It causes cervicitis, urethritis, endometritis, salpingitis and perihepatitis.[6] Chlamydia trachomatis strains are sensitive to the action of sulfonamides and produce a glycogen like material within the inclusion vacuole that stains with iodine.

Chlamydia trachomatis attaches to a heparan sulfate- like molecule on the surface of susceptible host cells.[6] This molecule apparently functions as a bridge between the specific receptor on the surface of the epithelial cell and a receptor on the elementary body. Chlamydiae are ingested by susceptible host cells by a mechanism that is not yet completely defined but is similar to receptor-mediated endocytosis. The uptake process is directly influenced by the Chlamydiae, and ingestion of Chlamydiae is specifically enhanced. After attachment, the endosomal body enters the cell in an endosome, with in which the entire growth cycle is completed. The Chlamydiae prevent phagolysosomal fusion. Once the endosomal body (of 0.25-0.35 µm diameter) has entered the cell, it reorganizes into a reticulate body (0.5-1 µm diameter) that is richer in RNA. After approximately 8 hours, the reticulate body begins dividing by binary fission. After 18-24 hours of infection, reticulate bodies become endosomal bodies by a poorly understood reorganization or condensation process. These endosomal bodies are released to initiate another cycle of infection. The endosomal bodies are specifically adapted for extracellular survival and are the infectious forms of Chlamydia trachomatis.[6]

Chlamydiae possess group specific, species specific and type specific antigens. Although the organisms are antigenically complex, only a few antigens play a role in diagnosis and pathogenesis. The group complement fixation (CF) antigen is a lipopolysaccharide (LPS) with a ketodeoxy octanoic acid as the reactive moiety. The major outer membrane protein (MOMP) contains both species and subspecies specific antigens. The MOMP is responsible for most of the reactivity seen in the microimmunofluorescence test. Studies involving DNA sequencing of the MOMP gene have localized the serotyping epitopes to the variable portions of the MOMP

gene. A 60 kDa heat shock protein has a sequence homology to analogous human genes may play an important role in inducing immunopathology.[6]

Chlamydia trachomatis is a slow growing intracellular organism. Its lack of mitochondria results in its obligatory intracellular existence and also causes its growth cycle to be extremely slow.[7] The growth cycle of Chlamydia trachomatis is 48-72 hours. Therefore several weeks to months are required for the growth to reach the numbers sufficient to cause clinical symptoms. The ability of Chlamydia trachomatis to persist in the Fallopian tubes of infected women is becomingly increasingly evident. A number of investigations have documented the presence of this organism in the Fallopian tubes for months or years after initial infection. Even following apparently successful antibiotic treatment evidence of chlamydial persistence can be obtained by molecular techniques. The ability to reactivate Chlamydia trachomatis from an apparently culture-negative state has been demonstrated in humans. The mechanism leading to Chlamydia trachomatis persistence and its capacity to enter a reversible culture-negative state has not been fully delineated in vivo. However, a plausible mechanism can be accurately deduced from in vitro experiments, and an immune mechanism of Chlamydia trachomatis-induced fallopian tube occlusion has been postulated.

Chlamydia trachomatis elementary bodies in semen from an infected male are transmitted to female sexual partner. The organism ascends the female reproductive tract and infects epithelial cells in the fallopian tube. Within the cell the elementary bodies convert to reticulate bodies and start to replicate. Pathways leading to apoptosis are blocked, ensuring continued survival of the infected cell and sustained induction of localized inflammation.[8] When elementary bodies reach a certain density they are released from the epithelial cell and infect neighbouring epithelial cells. In

response to the extracellular elementary bodies, immune system activation occurs and IFN-g, TNF-a and other pro-inflammatory cytokines are released. This activates the woman's immune defences, resulting in macrophage and T lymphocyte activation and release of IFN-g, TNF-a as well as other pro-inflammatory mediators. These mediators engulf and destroy the cell-free elementary bodies while concurrently arresting the intracellular replication of the reticulate bodies.[8]

Replication of reticulate bodies, the intracellular replicating form of Chlamydia trachomatis, is inhibited within cultured epithelial cell lines by addition of products of activated lymphocytes and macrophages, interferon gamma (IFN-g) and tumour necrosis factor-alpha (TNF-a).[8] However, chlamydial viability is maintained and when the pro-inflammatory cytokine or antibiotic is washed out of the culture the aberrant forms again assume the shape of healthy reticulate bodies and replication resumes, resulting in the eventual release of the infectious extracellular chlamydial elementary bodies. Large, aberrant non-replicative reticulate body-like forms accumulate within the cells under these conditions. The immune response keeps the numbers of elementary bodies low and inhibits the intracellular replication of the reticulate bodies.[8] When the concentration of elementary bodies is reduced below certain critical level immune system activation ceases and reticulates body replication resumes. The subsequent conversion of the reticulate bodies to elementary bodies and their release from the epithelial cell induces a new cycle of immune system activation and the reversible inhibition of chlamydial growth.[8]

Thus, the immune system keeps the number of organism present in the Fallopian tubes at a low level. Hence, Chlamydia trachomatis utilizes the immune response against it to foster its persistence. Perhaps this contributes to the asymptomatic nature of the infection. The interruption of reticulate body replication

allows the organism to persist in the intracellular form and escape immune destruction. In this persistent form c-hsp60 is released, inducing localized inflammation. With each cycle of replication more tubal epithelial cells become damaged, resulting eventually in interference with Fallopian tube patency. Scar formation and tubal occlusion leads to increased susceptibility to ectopic pregnancy or infertility.

Recent investigations have identified a Chlamydia trachomatis specific 57-KD protein that is responsible for this inflammatory response. The histopathology of the inflammatory response to 57-KD protein in fallopian tube is similar to that seen in blinding trachoma. This protein has been identified as a heat shock protein. Patients with tubal factor infertility due to Chlamydia trachomatis have antibodies directed against this protein and are a common feature of upper genital tract infection due to Chlamydia trachomatis.[9] For tissue damage to occur after Chlamydia infection, reinfection or reactivation of primary has to take place, which induces a booster immune response and a subsequent delayed hypersensitivity reaction, leading to tubal pathology. This immune response may not be species specific, and may also be induced by related species like Chlamydia pneumoniae. Tubal pathology was found more in patients with both Chlamydia trachomatis and Chlamydia pneumoniae antibodies, suggesting that Chlamydia pneumoniae infections might have a synergistic effect on the development of tubal pathology.[4] Tubal pathology will not be seen in all women with Chlamydia IgG antibodies. Chronic inflammation through persistent or recurrent infection may elicit a delayed hypersensitivity reaction which would result in tubal scarring. Heat shock protein 60 (HSP60) may play a crucial in chronic inflammation. HSPs are intracellular proteins. They have two major functions: they act as intracellular housekeeping proteins and they play a role in response to cellular

stress. The stress elicited activation of heat shock protein is frequently found in infections. HSPs are major and predominant microbial proteins and can induce a strong immune response. Cells chronically infected with Chlamydia trachomatis continue to produce Chlamydia HSP60 at high levels.[4] The strong homology between microbial and human HSPs may incite an autoimmune inflammatory reaction in the host. The prolonged exposure of the immune system to this protein, and concomitantly to human HSP60, may induce immunopathological responses culminating in tubal damage.

Alternating cycles of elementary body infection with destruction of new epithelial cells and intracellular persistence with c-hsp60 release eventually result in scar formation and loss of fallopian tube patency.[8] Antibiotic treatment similarly results in inhibition of reticulate body replication. The dilemma currently facing clinicians who deal with infertility as well as centres practising IVF-ET is how to assess patients with tubal factor infertility, who have never had symptomatic evidence of a Chlamydia trachomatis infection, for the possible presence of this organism in their fallopian tubes. Reactivation of a persistent chlamydial infection by treatments utilized to foster a pregnancy could interfere with a successful outcome.[8]

Salpingitis may produce tubal scarring, infertility, and ectopic pregnancy. Most Chlamydial infections in women are clinically in apparent, and yet such infections are often associated with tubal damage. Its slow growth does not induce a rapid or violent inflammatory response. This explains the slow and insidious nature of symptoms of acute chlamydia trachomatis infections. In symptomatic cases, cervicitis presents with mucopurulent or purulent discharge that is apparent on speculum examination. Pelvic inflammatory disease may present with pelvic pain, fever and vaginal discharge of mucopus consistence.[10] Grossly purulent cervical mucus

(mucopus) is one of the sign of Chlamydia trachomatis infection. It reflects a high concentration of polymorphonuclear leucocytes in the mucus. It has been stated that 85 percent of women with ≥ 10 polymorphs per 1200 x field of gram stained cervical mucus, had chlamydia trachomatis infection.[9] The chance of isolation of chlamydia trachomatis increases with increasing concentration of polymorphs in the cervical mucus. Examination would reveal lower abdominal tenderness or tenderness in the adnexa. Lower abdominal pain for more than 4 days was the most sensitive (76%), but the least specific predictor of Chlamydial salpingitis. Abdominal pain for 4 to 7 days was a more specific (89%), but less sensitive (33%), predictor of Chlamydia trachomatis.[9] Chlamydial endometritis may present with abnormal bleeding. Abnormal bleeding not related to oral contraceptive use may be a manifestation of Chlamydial endometritis. Non oral contraceptive users with heavier menstrual bleeding tend to be infected with Chlamydia trachomatis. This group of women would also present with metrorrhagia. It has been stated that, in women with Chlamydia infection, presence of adnexal mass had a 75 percent positive predictive value for acute salpingitis, and absence of a pelvic mass had a 28 percent negative predictive value for acute salpingitis.[9] Only 20 percent of women with Chlamydial salpingitis would have fever at initial examination. It has a 75percent positive predictive value and 28 percent negative predictive value for chlamydia trachomatis infection. Over the last decade, Chlamydia has received considerable attention as an etiological agent in acute PID. The two major sequelae of PID due to Chlamydia trachomatis are infertility and ectopic pregnancy. Studies in Scandinavian countries were the first to demonstrate the important role of Chlamydia trachomatis in the etiology of acute PID.[17]

Tubal factor infertility is due to functional impairment of the fallopian tube involving adhesions, scarring and occlusion, which is confirmed by laparoscopy. The major problem in the control of genital tract Chlamydia infections is that as many as 70-80 percent of women and 50 percent of men who are infected do not experience symptoms. This results in a large reservoir of undiagnosed, infected individuals who can transmit the infection to sexual partners. Chlamydia was reported to be isolated from the tubes, adhesions, or peritoneum of woman with tubal factor infertility and no clinical signs of PID.[9] 57 to 86 percent of women with tubal factor infertility would have antichlamydial antibodies. The relative risk of tubal factor infertility associated with past exposure to Chlamydia trachomatis (IgG titre ≥ 1:64) has been estimated to be as high as 7.8 percent. The level of chlamydial antibodies has also been directly related to the severity of tubal damage and pelvic adhesions seen on diagnostic laparoscopy. Only 10-15 percent of women with antichlamydial antibodies would have past clinical symptoms or a confirmed diagnosis of PID, thus evolving the concept of silent or atypical PID associated with Chlamydia trachomatis infection leading to tubal factor infertility.[9]

In about half of the asymptomatic women with lower genital tract infection, the microorganism will be cleared spontaneously. In the remaining group the infection persists over many years. In about 10 percent of individuals who have persistent infection, the infection will ascend to the upper genital tract and induce pelvic inflammatory disease.[4] Asymptomatic women with endocervical chlamydial infection, ascending spread of microorganisms from the cervix may cause upper genital tract infections. Women with endocervical chlamydial infection are considered at risk for ascending infections during uterine instrumentation, HSG and

laparoscopy with hydrotubation or chromopertubation. Screening and treatment with prophylactic antibiotics before these procedures would prevent ascending infections.[4]

Chlamydia trachomatis stimulates both humoral and cell-mediated immune system, leading to antibody formation and T-cell activation. After primary Chlamydia infection, immunity is considered to be only partially protective because the microorganism is sequestered within intracellular sites and remain unrecognized by the immune system.[4] This may predispose to its persistence in the genital tract and to recurrent infections. The immune response is serotype specific, which allows for re-infection with other chlamydial serovars, or with the same serovar after antigenic mutations in the major outer membrane protein. Recurrent Chlamydial infections are common, the recurrence rate being 30-50% in adolescents. The tissue injury appears to be induced by cell-mediated immune response, including a delayed hypersensitivity response. It has been estimated to occur in 10-15 percent of couples. Tuboperitoneal factors which include post-infectious tubal damage, tubal obstruction, and pelvic adhesions are the main cause of subfertility in 10-30 percent of cases.[4] In developing countries the proportion of tubal factor subfertility among subfertile couples is up to 85 percent.

Tuboperitoneal disease can be diagnosed by hysterosalpingography (HSG) or laparoscopy. Laparoscopy is considered the best available test for diagnosing tubal factor subfertility and is the accepted reference test in the evaluation of diagnostic performance of other tests. But, as the procedure is invasive, requires expertise and general anesthesia is required, laparoscopy is unsuitable for screening purposes on a large scale. As compared to laparoscopy, the sensitivity and specificity of HSG in diagnosing tubal patency is 63 percent and 85 percent, respectively. For diagnosing adhesions, the sensitivity of HSG is only 46 percent. Clinical pelvic infection

following HSG has been reported in up to 4 percent of cases, and in 10 percent of women with tubal disease. The poor predictive value for tuboperitoneal disease and the high prevalence of febrile morbidity question HSG as the best screening test in high-risk patients for tubal factor subfertility. It was noted that the majority of tubal pathology cases are due to Chlamydia infections and the development of late sequelae is associated with Chlamydia IgG antibody formation. Chlamydia antibody testing in serum has been introduced as a screening method for tubal factor subfertility.

Diagnostic tests:

The various diagnostic tests available for the diagnosis of Chlamydia trachomatis are Cell culture, Enzyme immunoassay (EIA), ELISA and PCR.

I. Culture:

Culture analysis of the endocervical swab sample is considered is considered the diagnostic gold standard to detect cervical Chlamydia trachomatis infection. Isolation of Chlamydia trachomatis is possible through monkey kidney cells, HeLa L, and Mc Coy cells. Mc Coy cells and He La cells are most commonly used. Cell culture is the recommended procedure for primary isolation of Chlamydia trachomatis. The most common technique involves inoculation of clinical specimens with cycloheximide treated McCoy or other appropriate cells. The basic principle involves centrifugation of the inoculums onto the cell monolayer, incubation of monolayer for 48-72 hours, and staining. Use of fluorescein-conjugated monoclonal antibodies represents the most sensitive method for detecting Chlamydia trachomatis inclusions. It also allows earlier detection of inclusions in cell culture.[6]

Shell vial method consists of McCoy cells plated onto 13mm coverslips contained in 15mm diameter disposable glass vials. The cell confluence is selected to give a light confluent monolayer after 24 to 48h of incubation at 37°C. The clinical

specimens are shaken with glass beads before being used for inoculation. The standard inoculation procedure involves removing medium from the cell monolayer and replacing it with the inoculums in a volume of 0.1 to 1 ml. The specimen is then centrifuged onto the cell monolayer at approximately 3000 x g at room temperature for 1 hour. The vials are held at 35°C for 2 hours, before the cells are washed or the medium is changed to medium containing 1 to 2 µg of cycloheximide per ml. The cells are incubated at 35°C for 48-72 hours, and one coverslip is examined for inclusions by immunofluorescence, iodine staining or Giemsa staining. Slide reading can be facilitated by examining the Giemsa stained coverslip under dark-field microscope.[6]

Chlamydia trachomatis strains can be further serotyped by using type specific and subspecies specific batteries of monoclonal antibodies. The most specific method is Microwell typing system, in which inclusions are stained with pools of type specific and subspecies specific monoclonal antibodies. Other methods of serotyping include the use of restriction endonuclease pattern of PCR amplified DNA and direct sequencing of variable domains in the Chlamydial MOMP.

Factors like sample collection, transportation time, storage of sample and toxicity of the swab, can decrease the sensitivity. It is considered to be only 70 – 80% sensitive. Its sensitivity is estimated to be in the range of 70-95% even in the experienced laboratories. It is very laborious and requires 2 – 7 days for results. These problems have made the studies of Chlamydia prevalence by culture, difficult and unreliable.

II. Direct Cytological Examination:

Chlamydia trachomatis infections of the cervix can be diagnosed by demonstrating intacytoplasmic inclusions on cytological examination. This can be done by Giemsa staining. More sensitive methods include Fluorescence conjugated monoclonal antibody techniques.

a. Giemsa staining: The smear is air dried and fixed with absolute methanol for at least 5 min and dried again. It is then covered with diluted, freshly prepared Giemsa stain for 1 hour. The slide is rapidly rinsed with 95 percent ethanol to remove excess dye and to enhance differentiation and then dried and examined microscopically. Endosomal bodies stain reddish purple.[6]

b. Direct fluorescent antibody technique: This test is based on detecting endosomal bodies in smears. Anti-MOMP monoclonal antibodies are used for detection of Chlamydia trachomatis, as they are species specific and superior quality of immunofluorescence due to even distribution of MOMP. The sensitivity and specificity of this technique are 75-85% and 98-99% respectively. [6]

III. Enzyme immunoassay (EIA):

In this test monoclonal or polyclonal antibodies are used to detect LPS (Lipopolysaccharide), which is more soluble than the MOMP. Most EIAs take several hours to perform, and thus are suitable for batch processing, thus allowing to test many specimens. EIA are less sensitive than optimal culture systems. The specificity of these tests is in the order of 97 percent. They are not amenable to screen low prevalence populations, because of the low predictive value of a positive result in such groups. Confirmatory tests have been developed to address this problem.

Blocking confirmatory tests have been validated for cervical and urethral infections. These include repeat assay of the positive samples with monoclonal antibody directed against the Chlamydia-specific epitope on the LPS, and direct fluorescent antibody based on MOMP detection. [6]

IV. Nucleic acid probes:

Many nucleic acid probes have been developed and evaluated by research laboratories. One commercially available probe test is PACE 2, which utilizes DNA-RNA hybridization to increase sensitivity by detecting Chlamydial RNA.

V. Amplified Nucleic acid tests:

Three nucleic acid amplification tests are currently available. They are Polymerase Chain Reaction (PCR), Ligase Chain Reaction (LCR), and Transcription Mediated Amplification (TMA). The PCR and LCR tests identify nucleotide sequences on cryptic plasmid, which is present in multiple copies on each Chlamydia trachomatis endosomal body. The TMA test is directed against rRNA. These tests should be able to detect less than one endosomal body in purified suspensions. [13] Sensitivity of these tests with clinical specimens is lower because of sampling problems and inhibition of amplification reactions by factors in the clinical specimens. In a study done by Loeffelholz et al, comparing culture and PCR, the sensitivity and specificity of culture were 85.6% and 100% respectively, where as that of PCR was 97% and 99.7%.[11] In another study done by Bass et al the sensitivity and specificity of culture was found to be 86% and 100%, and that of PCR was found to be 96.5 and 100%.[12] In a study done by Ossewaarde et al, taking PCR as gold standard, the sensitivity and specificity of culture was found to be 72.3% and 98.8% and that of PCR was 100% and 98.3%.[13] Though studies reported that PCR has a

higher sensitivity compared to culture, PCR is costly and cannot be recommended as a primary screening procedure for Chlamydia trachomatis. Studies published on endocervical Chlamydia colonization in subfertile patients using DNA amplification tests such as the polymerase chain reaction (PCR) and ligase chain reaction (LCR) have reported prevalences of 1.3-1.9%.[4] This low prevalence makes screening and treatment of subfertile women for endocervical infections, not cost-effective.

VI. SEROLOGY

a. Microimmunofluorescence: The most common used method for detecting Chlamydia antibodies in serum is microimmunofluorescence (MIF). The preparation of multiple antigens made the test technically difficult. The number of antigens were reduced by pooling antigens of epidemiologically related serotypes (A-C, D-K, L1-L3), or by using one broadly reacting serotype. Usually L2 is used as a broadly reacting antigen, because sera from patients with genital infections tend to cross-react with L2. Although MIF is considered as the standard for serodiagnosis in Chlamydia infections, its use has several limitations. Using a broadly reacting antigen would cause unintended cross-reactions. MIF is technically demanding, time consuming, and requires experienced readers for reliable results. The sensitivity of MIF varies between varies between 30 and 88 percent, and specificity varies between 45 and 100 percent.[3] Chlamydia antibody testing is most accurate in predicting distal tubal pathology, instead of unspecified tuboperitoneal abnormalities or proximal tubal occlusion. According to receiver operator characteristic (ROC) curves, in which the best combination of sensitivity and specificity is depicted, Land and co-workers found the most suitable statistical cut-off titre of Chlamydia antibody testing is 16. A high cut-off value may be chosen, if the positive patients have to be subjected for laparoscopy, or if there is limited access to laparoscopy. In older patients, a low cut-

off level may be chosen to prevent delay in diagnosing tubal pathology. The cross-reactivity between Chlamydia trachomatis and Chlamydia pneumoniae is a major concern, as it leads to false positive MIF results.[4]

b. ELISA (Enzyme linked immunosorbent assay): The micro-immunofluorescence method is a sensitive test for measuring antichlamydial antibodies. There is no standard MIF assay, and different assay conditions in different laboratories lead to interlaboratory variability in results. In the need for rapid, technically less demanding and reliable serological tests for the detection of Chlamydia antibodies, ELISA has been developed. The most commonly used antigens in ELISA tests for the detection of Chlamydia IgG antibodies are synthetic peptides derived from variable domains of MOMP of L2. In patients with Chlamydia positive endocervical swabs, it has been shown that ELISA performs as well as MIF in detecting Chlamydia trachomatis IgG antibodies. ELISA can play a significant role in screening in low resource settings.[3] Studies have shown correlation between serum Chlamydial antibody titres and tubal factor infertility.

ELISA consists of two reactions. In the first reaction, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) bound to the antigens. Second incubation is carried out using an enzyme labeled anti-human IgG (enzyme conjugate), which promotes a colour reaction. Serological surveillance showed that Chlamydia trachomatis is associated with 23 to 62% of cases of acute PID. Serum IgG antibodies are common among women with laparoscopically verified salpingitis. Infertile women who have scarred tubes and women with mucopurulent cervicitis commonly have Chlamydial antibodies. Manik et al and keyhani et al studies suggested that ELISA can play a significant role in screening of Chlamydia trachomatis in infertile women. In a review

by Land JA, it was stated that PID and its chronic sequelae are associated with Chlamydial IgG antibody formation and a correlation between tubal factor infertility and serum antibody titres has been established.[4] In a study done by Sharma et al in 2003, it was reported that Chlamydia specific IgG antibody was significantly higher (70%) in women with tubal factor infertility, verified by HSG and laparoscopy, than in healthy fertile women (35%) and women with causes other than tubal factor infertility (55%). [14]

Antibody responses to HSP60 may signal chronic persistent or repeated infection, and may be indicative of chronic inflammatory tissue reaction. Antibodies to HSP60 were found in 70 percent of women with occluded tubes and < 20 percent of subfertile women with patent tubes. Chlamydia HSP testing has a sensitivity of 70% and specificity of 83% in predicting tubal occlusion. The sensitivity and specificity would increase to 85% in patients positive for C.trachomatis IgG by MIF. HSP testing might discern patients who have had chronic infections and in whom late sequelae have developed.

In a case controlled study done by Malik et al the prevalence of Chlamydia trachomatis was found to be 28.1% in infertile women with normal mantoux test, normal x-ray chest, no specific findings in endometrial biopsy, husbands having normal semenogram.[3] 38 percent women positive for Chlamydia trachomatis were found to have tubal factor infertility by hysterosalpingography. 27 percent of women with primary infertility and 30.6 percent of women with secondary infertility were positive for Chlamydia trachomatis. The association of Chlamydia trachomatis infection and female infertility was found to be significant ($P<0.01$). 36.2 percent of the asymptomatic cases were found to be positive for Chlamydia trachomatis, and the infection is found to be significantly associated with asymptomatic presentation. In

symptomatic patients, bleeding per vaginum and vaginal discharge are found to be significantly associated with Chlamydia trachomatis infection. Pelvic inflammatory disease was not significantly associated with Chlamydia trachomatis infection in this study.

In a commentary by Krishna ray, it was stated that 92 million new cases of Chlamydia trachomatis occur all over the world, and 43 million are from south-east asia.[5] 23.2 percent of female sex workers and married contacts attending an STD clinic in Mumbai, India, were positive for Chlamydia trachomatis. 28.7 percent of women in an urban slum in Delhi were positive for Chlamydia trachomatis by direct fluorescent antibody test for detection of elementary bodies in cervical smear or Polymerase chain reaction (PCR), in that study. Another study involving a population of urban and rural adult population in Tamilnadu was quoted, where the prevalence was 3.9%.[2] 20 percent of women with Chlamydial lower genital tract infection will develop PID, 4 percent will develop chronic pelvic pain, 3 percent will develop infertility and 2 percent will have adverse pregnancy outcome. The risk of TFI after a single episode of PID is 10% with each episode doubling the risk.[2]

In a case controlled study done by Keyhani et al, comprising of 64 infertile women, the prevalence of Chlamydia trachomatis by IgG ELISA was found to be 7.8%.[2] 13.5 percent of cases of tubal factor infertility and 45.5 percent of cases with both tubal factor infertility and history of PID were found to be positive for Chlamydia trachomatis. 80% of seropositive cases were on the 20-24 year age group. In a prospective cohort study done by Gijsen et al, it was reported that 18% of patients showed a decline in IgG antibody titer by two steps by microimmunofluorescence (MIF).[15] In the cases that showed decline in the titre by MIF, the cut-off values by ELISA did not change. The study concluded that a decline

in the IgG antibody is not the significant cause of false negative Chlamydia antibody test results. In a cross-sectional study done by Vinita et al, the prevalence of Chlamydia trachomatis infection by first void urine sample PCR was 9%.[16]

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MATERIAL & METHODS

Place of the study: The present study was done at Assisted reproduction centre at KLES Dr.Prabhakar Kore Hospital, Belgaum.

Study design: Cross-sectional study

Study period: The study was done from January 2009 to September 2010.

Sample size: The study sample consisted of 257 infertile women, attending assisted reproduction centre at KLES Dr.Prabhakar hospital, Belgaum, from January 2009 to September 2010.

Calculation of Sample size (n):By taking 28% prevalence from Malik et al study and relative error of 20 percent, by applying

Formula $n= 4pq/d^2$, the sample size was calculated to be 257.[3]

P = prevalence

q = 100-P

d= Relative error

Inclusion Criteria: All infertile women attending ARC, KLES Dr.Prabhakar Kore Hospital, Belgaum from January 2009

Exclusion criteria: Patients who were not consenting to give blood sample were excluded from the study.

Protocol:

A. Written and informed consent

B. Detailed history regarding

1. Age of the patient
2. Husband's age
3. Marital history – This included married life, duration of infertility, type of infertility
4. Obstetric history – This included parity, living issues, abortions, ectopic pregnancy, and duration from last delivery or last abortion.
5. Menstrual history – This included frequency, duration, quantity and consistency/any special characteristics of menstrual flow, and association with pain.
6. Symptoms of vaginal discharge, back pain and history of treatment with tetracyclines

C. Detailed account of the Transvaginal sonography undergone by the patient

1. Probe tenderness
2. Tubo-ovarian mass
3. Hydrosalpinx
4. Significant free fluid in pouch of douglas

Infertility was defined as inability to conceive for more than a year despite regular unprotected intercourse. Primary infertility was defined as those cases in which conception has never occurred, where as the term secondary infertility was defined as inability to conceive after a previous successful conception.

Investigations: All cases participating the study are subjected to Chlamydia trachomatis IgG ELISA. Ethical clearance for this study was obtained from the institutional ethics committee in January 2009.

Specimen collection for ELISA & storage: The skin over the cubital fossa or the site for withdrawing blood was cleaned by spirit swab and under aseptic precautions 2ml of peripheral venous blood was drawn into a syringe and transferred into a plain vacutainer. The samples were centrifuged and the serum was pipetted out and stored in plastic vials in a refrigerator. The samples were accumulated till 24 samples are collected and then processed for ELISA. Patient samples to be investigated were stored at +2 to +8°C for a maximum period of 14 days. Diluted samples were incubated within one working day.

ELISA equipment:The ELISA test kit contains

1. Microtiter strips each with 8 break-off reagent wells coated with Chlamydia trachomatis antigens (Purified MOMP antigen from the BGM infected with serotype K)
2. Calibrator 200 RU/ml (IgG, human)
3. Calibrator 20 RU/ml (IgG, human)
4. Calibrator 2 RU/ml (IgG, human)
5. Positive control(IgG, human)
6. Negative control(IgG, human), enzyme conjugate-peroxidase labeled anti-human IgG, sample buffer
7. Wash buffer
8. Chromogen/substrate solution
9. Stop solution (0.5M sulphuric acid).

The format was 96 x 01. A semi quantitative assay was done in our study.

Procedure of ELISA: All reagents were brought to room temperature (+18°C to 20°C) around 30 min before use. Protective wrapping of the microplate was opened after it has reached room temperature. Calibrators, controls and enzyme conjugate were mixed thoroughly before use.Wash buffer is a 10x concentrate. If crystallization occurred in the concentrated buffer, it was warmed to 37°C and mixed well before diluting. The quantity required was removed from the bottle using a clean pipette and diluted with deionizer or distilled water (1 part reagent plus 9 parts distilled water). Patient samples were diluted 1:101 in sample buffer.

Calibrator was incubated along with positive and negative controls and patient samples. 100µl calibrators, positive and negative controls or diluted patient samples were transferred into the individual microplate wells according to the pipetting protocol and incubated for 30min at room temperature (+18°C to +25°C). Samples were washed manually by emptying the wells and washing 3 times using 300µl of working strength wash buffer for each wash. They were also washed automatically, by washing 3 times with 400µl working strength wash buffer. The buffer was left for 30 to 60s in each well, per washing cycle. The wells were then emptied, and all the liquid was thoroughly disposed off from microplate by tapping it on absorbent paper with the openings facing downwards.

100µl of enzyme conjugate (peroxidase labeled anti-human IgG) solution was pipetted into each of the microplate wells and incubated for 30min at room temperature.Washing of the wells was done as described above. 100µl of chromogen/substrate solution was pipetted into each of the microplate wells and

incubated for 15min at room temperature. The incubation was stopped by pipetting 100µl of stop solution in the same order and at the same speed as the chromogen/substrate solution was introduced. Photometric assessment of the colour intensity was made at a wave length of 450nm and at a reference wavelength of 620-650nm within 30min of adding the stop solution.

	1	2	3	4
A	K2	P 6	P 14	P 22
B		P 7	P 15	P 23
C		P 8	P 16	P 24
D	P 1	P 9	P 17	
E	P 2	P 10	P 18	
F	P 3	P 11	P 19	
G	P 4	P 12	P 20	
H	P 5	P 13	P 21	

Table 1. Pipetting protocol

The colour intensity obtained from the colorimeter was interpreted by semiquantitative analysis by the formula, extinction value of the control/ extinction of calibrator 2.

Interpretation:

A ratio of ratio < 0.8 = Negative ≥0.8 - < 1.1 = Borderline ≥ 1.1 = Positive.

Analysis:

- The prevalence is calculated by the formula, Number of IgG ELISA positive cases/ sample size x 100
- Chi- square test is applied to know the association of the type of infertility and chlamydia trachomatis infection.

Fig 1. Spectrophotometer for ELISA

Fig 2. Antigen coated wells for Chlamydia trachomatis IgG ELISA

Fig 3. Serum samples before incubation

Fig 4.Serum Samples after incubation

RESULTS

The study was conducted at KLES Hospital & MRC Belgaum from 2009 to 2010. The total number of subjects enrolled in the study were 257. Chlamydia IgG ELISA was done in all the subjects enrolled in the study.The mean age of the women enrolled in the study was 28.87 ± 5.09 yrs. Of the 257 women enrolled in the study, 6 cases were in the age group of 15-20 years, 70 cases were in the age group of 20-25 years, 92 cases were in the age group of 25-30 years, 58 cases were in the age group of 30-35 years, 30 cases were in the age group of 35-40 years, and one case was in the age group of 40-45 years.

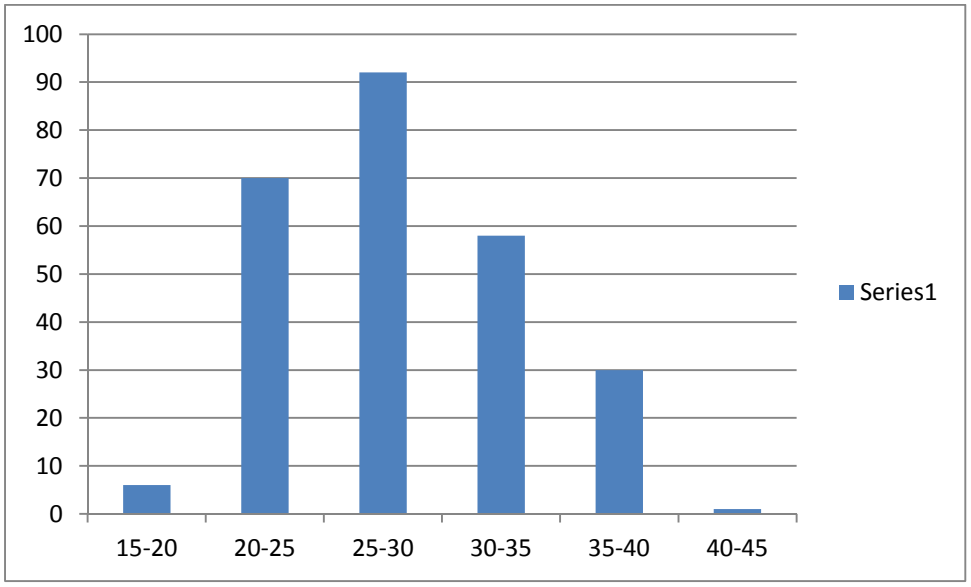

Chart 1. The age group distribution of women participated in the study

Of the 257 women, 19 had vaginal discharge, 3 had pain abdomen, 10 had back pain and 3 gave history of tetracycline therapy. Transvaginal sonography was done in all the cases, and there was no evidence of probe tenderness, tuboovarian mass and hydrosalpinx in any of the subjects.

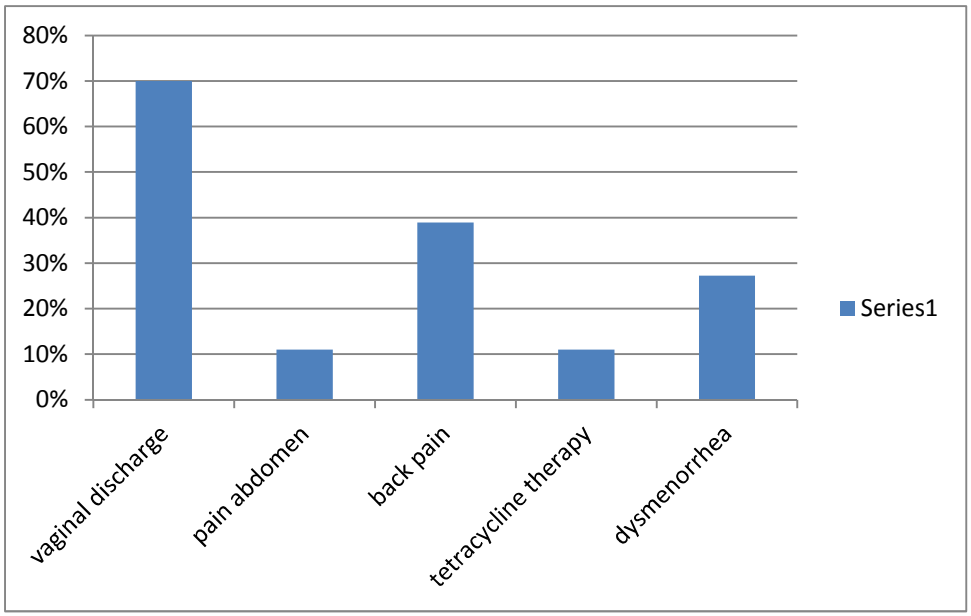

Chart 2.Clinical symptoms of PID in the study sample

S.NO.	Clinical symptom	No. of subjects(n=257)	Percentage
1.	Vaginal discharge	19	70%
2.	Pain abdomen	3	11%
2.	Back pain	10	38.91%
3.	Tetracycline therapy	3	11%
4.	Dysmenorrhea	7	27.23%

Table 1. Distribution of clinical symptoms of PID in the study sample

Of the 257 women enrolled in the study, 199 had primary infertility and 58 women had secondary infertility .

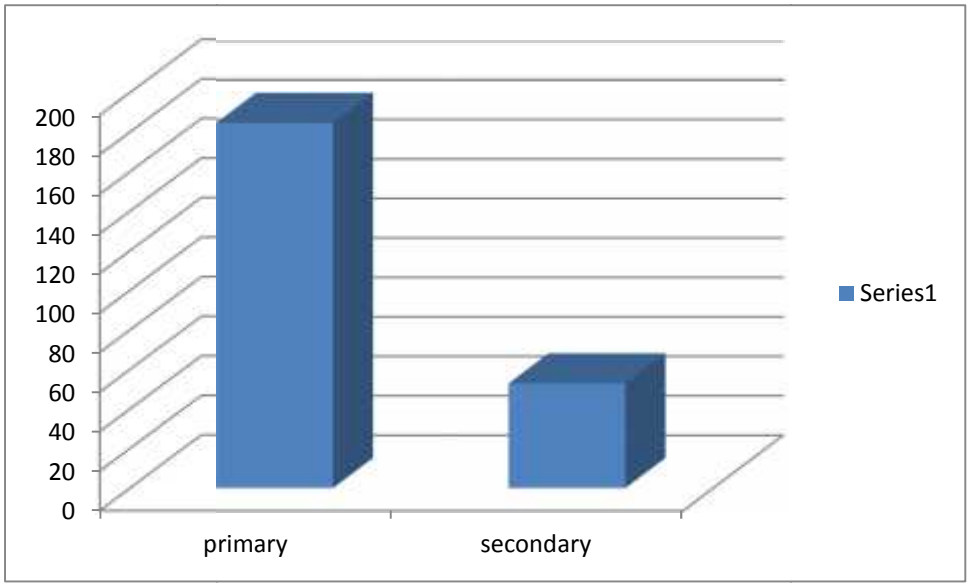

Chart 3. The type of infertility in the women enrolled in the study.

Among the 257 infertile women subjected to IgG ELISA for Chlamydia trachomatis,19 women were positive. The prevalence of Chlamydia trachomatis by IgG ELISA positivity was 7.3 percent.

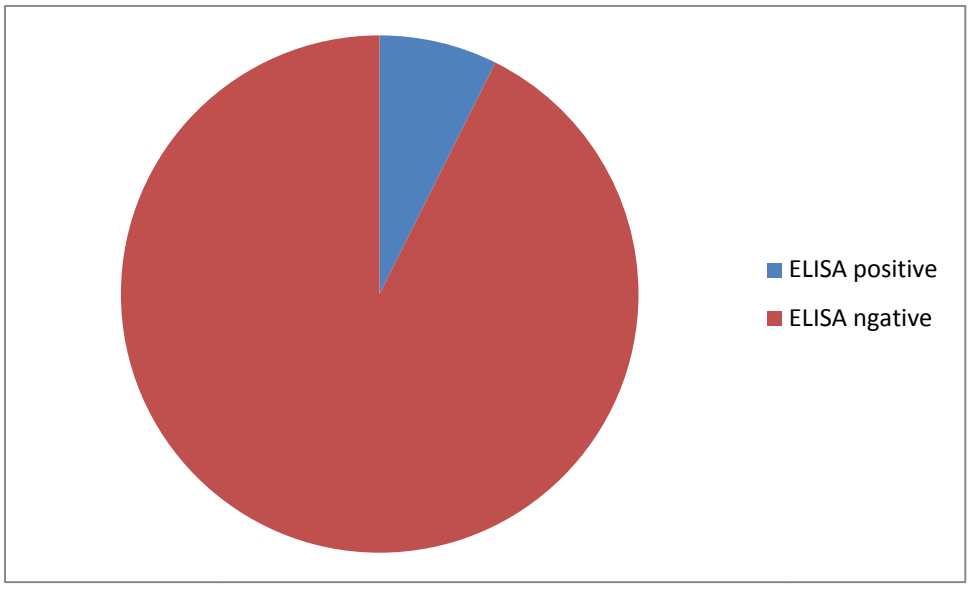

Chart 4. The prevalence of Chlamydia trachomatis in the study

Of the 19 positive women 14 had primary infertility and 5 had secondary infertility

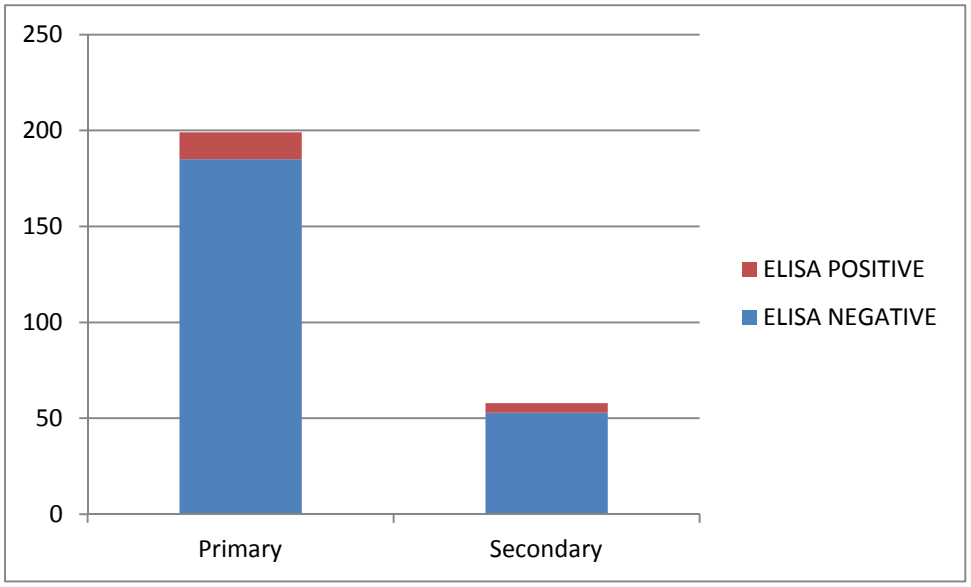

Chart 5.The type of infertility in Chlamydia IgG ELISA positive women

The strength of association of type of infertility and Chlamydia trachomatis IgG ELISA positivity was tested by applying Chi-Square test. The Chi-Square value was 0.014 and P value was 0.903. Therefore there was no significant association between the type of infertility and Chlamydia trachomatis IgG ELISA positivity in our study.

Five of the positive cases were in the 20-25 years age group, nine in the 26-30 years age group, three in 31-35 years age group and two in 36-40 years age group.

All the positive cases detected in the study were asymptomatic.

Clinical profile	Total no. infected
Symptomatic(30)	0
Asymptomatic(236)	19

Table 2.Chlamydia IgG ELISA positivity in women with and without clinical symptoms of PID

DISCUSSION

Infertility is becoming an emerging health problem in many countries of the world, including India. Chlamydia trachomatis is a well known cause of PID. It has been reported that 3 percent of patients with Chlamydia trachomatis infection will develop infertility. It can cause more severe tubal immunopathology than other agents in spite of the absence of overt symptoms.

In our study, the prevalence of Chlamydia trachomatis was 7.3%. Majority of the positive cases were in 26-30 years age group. The detection limit of the ELISA test was defined as three times the standard deviation of an analyte free sample and is the smallest detectable antibody titre. The detection limit of anti-chlamydia trachomatis IgG ELISA which was used in our study is 1RU/ml. The quality of the antigen used ensures high specificity of the ELISA. This ELISA kit has no cross reactions. This ELISA test has no interference with hemolytic, lipemic or icteric samples for concentrations of up to 10mg/ml for hemoglobin, 20mg/ml for triglyceride and 0.4mg/ml for bilirubin The sensitivity and specificity of anti-Chlamydial assay using MOMP antigen is 61% and 84% respectively, with a positive predictive value of 65% and negative predictive value of 84%.[17] The sensitivity of EUROIMMUN IgG ELISA kit used in the study is 78.2 percent and specificity is 97.1 percent.[18]

In India, the prevalence of this infection in women attending STD clinics and high risk groups were studied, but studies on the prevalence in infertile women are limited. As infertile couple belongs to a specialized subset of population and the treatment of infertility involves high cost with unpredictable outcome, a study estimating the overall burden of this infection in infertile women would enlighten the importance of evaluation for this infection in the infertile women.

In a study done in Aligarh, India, in 2006, the prevalence of Chlamydia trachomatis was found to be 28 percent in infertile women.[3] The high prevalence in that study was due to the exclusion of Mantoux positive women, women having abnormal chest X-ray, couples having male factor infertility and those having abnormal endometrial biopsy. In this study, the association of Chlamydia trachomatis and infertility was found. But the sample size of the study group was 55, which was very low to estimate the prevalence. The study group had not included all groups of infertile women, but only those with specified characteristics. In our study, all the infertile women, who attended our assisted reproduction centre, were included in the study. This would reflect the accurate prevalence of the infection in infertile women.

In a case controlled study done in Tehran, the prevalence of Chlamydia trachomatis in infertility was 7.8%.[2] The size of the study sample in this study was 64, which is very low to calculate the prevalence. In our study the sample size was 257 infertile women. This

would increase the power of our study and the reliability of the results obtained in the study. 13.5% of women with tubal factor and 45.5% with history of PID were seropositive for Chlamydia trachomatis. The prevalence obtained in this study is consistent with our study. As the study was done in a country outside India, the prevalence obtained in their study would not be applicable to the Indian population. Hence our study provides the prevalence that is more applicable to the Indian population.

In our study we have used IgG ELISA to diagnose Chlamydia trachomatis infection. A prospective cohort study done at Netherlands, reported that a decline in the IgG antibody titre is not the significant cause of false negative Chlamydia antibody test result.[15] This would ensure the reliability of IgG ELISA used in our study.

In a study done by Loeffelholz, polymerase chain reaction of the endocervical swab was used to detect the genital chlamydia trachomatis infection.[11] As the tubal factor infertility in Chlamydia trachomatis infection is related to the immunopathology of the infection, a serologic test such as IgG ELISA would be more appropriate in the diagnosis of Chlamydia trachomatis infection in infertile women. In a case-controlled study done in Ghana, West Africa, 1.6 percent of infertile women and 2.1 percent of the control group, were positive for Chlamydia trachomatis by PCR of the first void urine specimens ($P>0.05$).[19] The IgG seroprevalence rates in the same individuals were

39.3 percent in the study group and 19.4 percent in the control group (P<0.001). Evidence exists that Chlamydia trachomatis may persist in a metabolically active state for a long period in the upper genital tract, despite PCR negative results from the cervix.[20,21] Reactivation of Chlamydia upper genital tract infection is known to occur in women in whom no Chlamydia DNA can be detected in the cervix.[22] This questions the statement that PCR negative patients are not at risk of upper genital tract infection by Chlamydia trachomatis. PCR involves high cost and is not appropriate for screening, and a cost-effective test like ELISA is more appropriate as it could be done over a larger sample and requires less technical expertise.

In a study done in Tehran, all the women positive for Chlamydia trachomatis IgG ELISA, were asymptomatic.[2] The reasons for the asymptomatic nature of infection being absence of mitochondria causing extremely slow growth cycle, inhibition of apoptotic pathways in the infected cells and inhibition of replication of reticulate bodies by proinflammatory cytokines, are well known.[7,8] In our study, none of the women positive for Chlamydia trachomatis IgG ELISA, had shown the signs of lower genital tract disease and/or pelvic inflammatory disease. These findings are consistent with the literature and the previous study.

In a study done at a teaching hospital in Orissa, the prevalence of Chlamydia trachomatis infection was estimated by the PCR of cervical swab in women attending outpatient obstetrics & gynecology department.[23] The prevalence obtained in the study was 7.04%. This prevalence was consistent with our study. The effective sample size in their study was 71. In our study, the sample size was 257, which was comparatively higher. In their study, the sample had included married women attending the outpatient department and doesn't provide the prevalence in infertile women, which is an important sequel of genital Chlamydia trachomatis infection. In our study, the sample included only infertile women and hence our study is more of problem oriented.

In a multicentre trial done by ICMR, the overall prevalence of Chlamydia trachomatis in infertile women was found to be 3.3%.[24] The data obtained in this study is more appropriate for comparison with our study, as the study was conducted in India, and sample size was very high. The study was conducted in 5 states and Chlamydiazyme-EIA of endocervical cervical swab was used to diagnose Chlamydia trachomatis infection. The total infertile population evaluated was 1938. The prevalence in the individual states ranged from 0.6% - 6.3%. The highest prevalence of 6.3 percent was found in Pondicherry, which is consistent with the prevalence obtained in our study.

In the study done at Tehran, which obtained a similar prevalence as our study, it was found that 13.5 percent of women with tubal factor infertility were seropositive for Chlamydia trachomatis.[2] Hence screening for Chlamydia trachomatis in all the cases of infertility at the initial visit is cost-effective, helps in identification of cases and prediction of tubal factor infertility.

CONCLUSION

- In the present study, the prevalence of Chlamydia trachomatis by IgG ELISA was found to be 7.3 percent. This would mean that irrespective of the presence of other causative factor of the infertility, the frequency of Chlamydia trachomatis infection in infertile women is 1 in 15, which is high.
- In our study there was no significant association with the type of infertility and Chlamydia trachomatis infection. Hence, Chlamydia trachomatis infection should be preferentially evaluated in both primary and secondary infertility.
- Evaluation for Chlamydia trachomatis infection should not be based on clinical symptoms, as asymptomatic infection is more likely in Chlamydial infection.
- Early diagnosis and identification of Chlamydia trachomatis by a low cost test such as IgG ELISA would prevent further harmful and damaging sequelae of Chlamydia trachomatis infection.

SUMMARY

Chlamydia trachomatis is the commonest sexually transmitted disease implicated to cause infertility. About 3 percent of individuals infected by this organism develop infertility. The infection caused by Chlamydia trachomatis is asymptomatic in nature, and would result in tubal damage and scarring without symptoms. The objective of the present study was to find out the prevalence of Chlamydia trachomatis infection in infertile women.

In this cross sectional study, 257 infertile women attending infertility clinic at KLES Dr. Prabhakar Kore Hospital & MRC, Belgaum, were enrolled. All the women were subjected to Chlamydia trachomatis IgG ELISA using purified MOMP antigen after taking relevant history. The prevalence of Chlamydia trachomatis obtained in our study by IgG ELISA using purified MOMP antigen was 7.3 percent. All the women infected by Chlamydia trachomatis were asymptomatic.

There was no correlation between the type of infertility and Chlamydia trachomatis infection, in our study. Early diagnosis and identification of Chlamydia trachomatis by a low cost test such as IgG ELISA would prevent further harmful and damaging sequelae of Chlamydia trachomatis infection.

REFERENCES

1. Joygee AG, Thyagarajan SP, Venkatesan C, Ganapathy M. Need for specific & routine strategy for the diagnosis of genital chlamydial infection among patients with sexually transmitted diseases in India. Indian J Med Res.2003; 118: 152-157.

2. Keyhani AH, Nazer M, Mirsalehian A. Evaluation of serum chlamydial IgG antibody in women with tubal factor infertility. Research J Medicine & Med. Sci.2006; 1(4): 150-153.

3. Malik A, Jain S, Hakim S, Shukla I, Rizvi M. Chlamydia Trachomatis infection and female infertility. Indian J Med Res. 2006; 123: 770-775.

4. Land JA, Evers JL. Chlamydia infection and subfertility. Best Pract Res Clin Obstet Gynaecol. 2002; 16: 901-912.

5. Krishna Ray. Chlamydia trachomatis and infertility. Indian J Med Res. 2006; 123: 730-734.

6. Schachter J, Stamm WE. Chlamydia. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC and Yolken RH, editors. Manual of clinical microbiology. Washington DC: American society of microbiology; 1999.p. 795-806.

7. Martens MG. Pelvic inflammatory disease. In: Jock JA, Jones III HW, editors. Te Linde's Operative gynecology. Philadelphia: Lippincott Williams & Wilkins; 2008.p.660-686

8. Witkin SS. Immunological aspects of genital Chlamydia infections. Best Pract Res Clin Obstet Gynaecol. 2002; 16: 865-874.

9. Hanssen PW. Diagnosis of pelvic inflammatory disease. Landers DV, Sweet RL, editors. Pelvic inflammatory disease. NY: Springer-Verlag New York, Inc; 1997. p. 30-58.

10. Blanchard TJ, Mabey DCW. Chlamydial infections. In: Emmerson AM, Hawkey PM, Gillespie SH, editors. Principles and practice of clinical bacteriology. Chichester: John Wiley and sons Ltd.; 1997.p. 723-743.

11. Loeffelholz MJ, Lewinski A, Silver SR et al. Detection of Chlamydia treachomatis in endocervical specimens by polymerase chain reaction. J. Clin. Microbiol. 1992; 30(11): 2847-2851.

12. Bass CA, Jungkind DL, Silverman NS et al. Clinical evaluation of a new polymerase chain reaction assay for detection of chlamydia trachomatis in endocervical specimens. J. Clin. Microbiol. 1993; 31: 2648-2653.

13. Ossewaarde JM et al. Development and clinical evaluation of polymerase chain reaction test for detection of Chlamydia trachomatis. J.Clin.Microbiol. 1992; 2122-2128.

14. Sharma K, Aggarwal A, Arora U. Seroprevalence of Chlamydia trachomatis in women with bad obstetric history and infertility. Indian J Med Sci. 2002; 56: 216-217.

15. Gijsen AP, Land JA, Goossens VJ, Slobe ME, Bruggeman CA. Chlamydia antibody testing in screening for tubal factor subfertility: the significance of IgG antibody decline over time. Human Reproduction. 2002; 17(3): 699-703.

16. Vinita D et al. Prevalence of Chlamydia trachomatis by first void urine polymerase chain reaction test in women attending outpatient clinic. J Obstet Gynecol India.2006; 6: 511-513.

17. Bas S, Muzzin P, Vischer TL. Chlamydia trachomatis serology: Diagnostic value of outer membrane 2 compared with that of other antigens. J. Clin. Microbiol. 2001. 39(11) 4082-4085.

18. Euroimmun. Anti-Chlamydia trachomatis ELISA (IgG) test instruction. Lübeck: Medizinische Labordiagnostika AG; 2004.

19. Siemer J, Theile O, Larbi Y et al. Chlamydia trachomatis infection as arisk factor for infertility among women in Ghana, West Africa. Am. J. Trop. Med. Hyg. 2008. 78(2): 323-327.

20. Dieterle S, Rummel C, Bader LW et al. Presence of major outer membrane protein of Chlamydia trachomatis in patients with chronic salpingitis and salpingitis isthmica nodosa with tubal occlusion. Fertility and Sterility. 1998; 70: 774-776.

21. Gerard HC, Branigan PJ, Balsara GR et al. Viability of Chlamydia trachomatis in fallopian tubes of patients with ectopic pregnancy. Fertility and Sterility. 1998; 70: 945-948.

22. Land JA, Gijsen AP, Evers JLH et al. Chlamydia trachomatis in subfertile women undergoing uterine instrumentation: screen or treat? Human Reproduction.2002; 17: 525-527.

23. Dwibedi B, Pramanik JM, Sahu P et al. Prevalence of genital Chlamydial infection in females attending an outpatient obstetrics & gynecology department in Orissa. Indian J Dermatol Venereol Leprol. 2009.75(6): 614-616.

24. Chandok, N., Datey, S., Gaur, L.N. and Saxena, N.C. Prevalence of Chlamydia trachomatis in women attending different clinics at tertiary level hospitals. J Obstet Gynaecol India 53 (2003) 463.

CONSENT FOR PARTICIPATION IN RESEARCH

Sr. no: _____

Patients name : Mrs. _____

Mrs. _____, we here by request you to participate in the study titled "THE PREVALENCE OF *CHLAMYDIA TRACHOMATIS* BY ELISA IN INFERTILE WOMEN." This study is conducted by Dr. XXXX, Postgraduate student in Obstetrics & Gynecology, under the guidance of Dr. XXXX, Professor, Dept. of OB& GYN, 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.

RESEARCH PURPOSE

As Chlamydia trachomatis is one of the most common sexually transmitted disease, identification of a test of higher sensitivity by this clinical study, may help in early diagnosis of infection and prevention of adverse sequelae.

PROCEDURE INVOLVED:

2ml of blood will be drawn from a peripheral vein for Chlamydia trachomatis IgG antibody testing by ELISA.

RISKS AND BENEFITS

There is no extra risk. The benefit will be that, if you are detected to be positive, the treatment against the infection can be started early.

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.

RESEARCH RELATED INJURY

There is no research related injury, in this study.

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.

WITHDRAWL FROM THE STUDY

You may withdraw from the study, freely, at your will, any time during the study.

CONTACT INFORMATION

If you have any doubts/questions about the study, please contact Dr.XXXX, PG, Department of Obstetrics & Gynecology, JNMC Belgaum. For further clarifications, you may contact the chief investigator, Dr. XXXX, ARC, Department of Obstetrics & Gynecology, J.N.M.C.Belgaum. For details and clarifications regarding participants' rights, you may contact the chairman of ethical committee, Dr. XXXX, Principal, JNMC. Belgaum. Ph no: 0831 2473777

CONSENT STATEMENT

I undersigned _____ have been explained in my vernacular language, about the study, and my participation in this study is voluntary. I may withdraw from the study at any time. Also I have been given enough time to clear my doubts and was explained of my rights as a study participant.

Participants Name _____ Signature _____

Witness Name _____ Signature _____

Experimenters Name _____ Signature _____

Date :

Place :

CONSENT FOR PARTICIPATION IN RESEARCH

Sr. no: _____

Patients name : Mrs. _____

Mrs. _____, we here by request you to participate in the study titled "THE PREVALENCE OF *CHLAMYDIA TRACHOMATIS* BY ELISA IN INFERTILE WOMEN." This study is conducted by Dr.N.Siddhartha, Postgraduate student in Obstetrics & Gynecology, under the guidance of Dr. S. S. Patted, Professor, Dept. of OB& GYN, 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.

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RESEARCH PURPOSE

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1. In emergency to protect your rights and welfare.
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CONSENT STATEMENT

I undersigned _____ have been explained in my vernacular language, about the study, and my participation in this study is voluntary. I may withdraw from the study at any time. Also I have been given enough time to clear my doubts and was explained of my rights as a study participant.

Participants Name _____ Signature _____

Witness Name _____ Signature _____

Experimenters Name _____ Signature _____

Date :

Place :

PROFORMA

"THE PREVALENCE OF *CHLAMYDIA TRACHOMATIS* BY ELISA IN INFERTILE WOMEN"

Sr. no_____ **Date_____**

Patients name_____ Age_____

Husband's name_____ Age_____

Address_____

_____

Obstetric history:

Married life: _____

Duration of infertility: _____

Primary: _____

Secondary: _____

Parity:_____ Living : _____ Abortions: ____ Ectopic Pregnancy: _____

Last Delivery: _____ Last Abortion: _____

Menstrual History:

Frequency: Regular ☐ Irregular ☐

Duration of flow : _____

Amount of blood loss: Scanty ☐ Moderate ☐

Excessive blood loss (passage of clots) ☐

Associated with pain: Yes ☐ No ☐

Presence of foul smell: Yes ☐ No ☐

Symptoms suggestive of Chlamydia trachomatis	**Yes**	**No**
Vaginal discharge (of mucopus consistency)	☐	☐
Back pain	☐	☐
History of treatment with tetracyclines	☐	☐

Duration of treatment _____ (in months)

Ultrasound findings	**Yes**	**No**
Probe tenderness :	☐	☐
E/O Tubo-ovarian mass :	☐	☐
E/O Hydrosalpinx :	☐	☐
E/O Fluid in POD :	☐	☐

MASTER CHART

Name of the patient	age(years)	type	Duration of infertility(years)	Vaginal discharge	Pain abdomen	Back ache	Dysmenorrhea	H/O tetracycline treatment	Duration(years)	TVS	Chlamydia trachomatis ELISA
Afreen Shamshar	23	secondary	4	present	absent	present	absent	absent	-	normal	negative
ahswini miskini	26	primary	6	absent	absent	absent	absent	absent	-	normal	negative
Akshata uppin	19	primary	0	absent	absent	absent	absent	absent	-	normal	negative
ambica patil	27	secondary	3	absent	absent	absent	absent	absent	-	normal	negative
Anamica Bharathi	28	primary	5	absent	absent	absent	absent	absent	-	normal	negative
anita bhima	22	primary	6	absent	absent	absent	absent	absent	-	normal	positive
anita nayak	30	secondary	3	absent	absent	absent	absent	absent	-	normal	negative
anjum bangappa	23	primary	7	absent	absent	absent	absent	absent	-	normal	negative
anusha premachand	24	primary	6	absent	absent	absent	absent	absent	-	normal	negative
arati patil	23	primary	3	absent	absent	absent	absent	absent	-	normal	negative
archana anupkumar	32	primary	5	absent	absent	absent	absent	absent	-	normal	negative
aruna patil	26	primary	2	absent	absent	absent	absent	absent	-	normal	negative
asha tagadi	28	primary	0.5	absent	absent	absent	absent	absent	-	normal	negative
asharani tutikar	29	primary	2	absent	absent	absent	absent	absent	-	normal	negative
Bharati kavadalli	37	primary	5	absent	absent	absent	absent	absent	-	normal	negative
Bharati lingappa	35	primary	10	absent	absent	absent	absent	absent	-	normal	negative
bibi jahara	32	secondary	5	present	absent	absent	absent	absent	-	normal	negative
Chaitra tergaonkar	29	primary	2.5	absent	absent	absent	absent	absent	-	normal	negative
Chandrakala patil	36	primary	8	absent	absent	absent	absent	absent	-	normal	negative
Chandrakala pujar	38	secondary	5	absent	absent	absent	absent	absent	-	normal	negative
Channabasamma sidnall	38	secondary	6	absent	absent	absent	absent	present	5 days	normal	negative
Channawwa Shrishail	35	primary	14	present	absent	present	absent	absent	-	normal	negative
deepa marathi	21	primary	3	absent	absent	absent	absent	absent	-	normal	positive
Deepa palkar	24	primary	2	absent	absent	absent	absent	absent	-	normal	negative
deepa sangati	25	primary	5	absent	absent	absent	absent	absent	-	normal	negative
deepa shettar	28	primary	3	absent	absent	absent	absent	absent	-	normal	negative
Deepa suryavanshi	27	primary	1.5	absent	absent	absent	absent	absent	-	normal	negative
deepa yadahalli	27	secondary	8	absent	absent	absent	absent	absent	-	normal	negative
Durga megeri	34	primary	6	absent	absent	absent	absent	absent	-	normal	negative
fatima nadaf	25	primary	9	present	absent	absent	absent	absent	-	normal	negative
Gangawwa benni	22	primary	3	absent	absent	absent	absent	absent	-	normal	negative
Gangawwa dundappa hd	38	secondary	17	absent	absent	present	present	absent	-	normal	negative
Gangawwa Hongal	38	secondary	18	absent	absent	absent	present	absent	-	normal	negative
gangawwa sankannavar	29	primary	12	absent	absent	absent	absent	absent	-	normal	negative
gayatri goudar	28	secondary	1	absent	absent	absent	absent	absent	-	normal	negative
Gayatri Kakade	31	primary	5.5	absent	absent	absent	absent	absent	-	normal	negative
geeta hiremath	25	primary	4	absent	present	present	absent	absent	-	normal	negative
geeta hosur	21	primary	4	absent	absent	absent	absent	absent	-	normal	negative
Geetanjali nikam	32	primary	11	absent	absent	absent	absent	absent	-	normal	negative
Girija badiger	35	primary	6	absent	absent	absent	absent	absent	-	normal	positive
Girija matapathi	34	primary	13	absent	absent	absent	absent	absent	-	normal	negative
gouramma venkatesh	28	primary	9	absent	absent	absent	absent	absent	-	normal	negative
Gulmeet Kaur	40	secondary	10	absent	absent	absent	present	absent	-	normal	negative
Habijan ankali	31	primary	16	absent	absent	absent	absent	absent	-	normal	negative
Hanamawwa Basappa	28	primary	11	absent	absent	absent	absent	absent	-	normal	negative
Hanamawwa Basappa	28	primary	11	absent	absent	absent	absent	absent	-	normal	negative
Hanamawwa Budihal	28	primary	11	present	absent	absent	absent	absent	-	normal	negative
indumati chanbasanavva	25	secondary	5	absent	absent	absent	absent	absent	-	normal	negative
Indumati shimpi	38	primary	21	absent	absent	absent	absent	absent	-	normal	negative
jabeen mulla	28	primary	6	absent	absent	absent	absent	absent	-	normal	negative

Name of the patient	age(years)	type	Duration of infertility(years)	Vaginal discharge	Pain abdomen	Back ache	Dysmenorrhea	H/O tetracycline treatment	Duration(years)	TVS	Chlamydia trachomatis ELISA
jayasree dewadkar	24	primary	0.5	absent	absent	absent	absent	absent	-	normal	negative
Jayasree Sontakki	30	primary	14	absent	absent	absent	absent	absent	-	normal	negative
Jyothi angadi	31	primary	2	absent	absent	absent	absent	absent	-	normal	negative
Jyothi gouda	36	primary	8	absent	absent	absent	absent	absent	-	normal	negative
jyothi kadolkar	31	primary	6	absent	absent	absent	absent	absent	-	normal	negative
jyothi kamat	23	primary	4	absent	absent	absent	absent	absent	-	normal	negative
jyothi mensinkai	30	primary	2	absent	absent	absent	absent	absent	-	normal	negative
Kamala basavraj	35	primary	8	absent	absent	absent	absent	absent	-	normal	negative
Karebasamma H	28	primary	9	present	absent	absent	absent	absent	-	normal	negative
kashawwa sannatti	27	primary	12	absent	absent	present	absent	absent	-	normal	negative
kasturi bhajantri	25	primary	9	absent	absent	present	absent	absent	-	normal	negative
kaveri gatnatti	28	primary	7	absent	absent	absent	absent	absent	-	normal	negative
kaveri hossurke	30	primary	3	present	absent	absent	absent	absent	-	normal	negative
Kavita Dindur	25	primary	4	absent	absent	absent	absent	absent	-	normal	negative
kavita hunshyal	25	primary	5	present	absent	absent	absent	absent	-	normal	negative
kavya javali	26	secondary	2	absent	absent	absent	absent	absent	-	normal	positive
Krishnaveni	39	primary	14	absent	absent	absent	absent	absent	-	normal	positive
kusum huvannavar	30	primary	6	absent	absent	absent	absent	absent	-	normal	positive
Lalita mantur	35	primary	11	absent	absent	absent	absent	absent	-	normal	negative
lalitha nanjannavar	33	secondary	0	absent	absent	absent	absent	absent	-	normal	negative
lata nasi	30	primary	9	absent	absent	absent	absent	absent	-	normal	negative
Laxmi dalawai	36	primary	16	absent	absent	absent	absent	absent	-	normal	negative
Laxmi kyatannavar	20	primary	4	absent	absent	absent	absent	absent	-	normal	negative
laxmi malakpuri	21	secondary	0	absent	absent	absent	absent	absent	-	normal	negative
laxmi patil	23	primary	7	absent	absent	absent	absent	absent	-	normal	negative
laxmi veerappa	30	primary	2.5	absent	absent	absent	absent	absent	-	normal	positive
leena kalal	22	primary	1.5	absent	absent	absent	absent	absent	-	normal	negative
Madhu manjunath	30	primary	6	absent	absent	absent	absent	absent	-	normal	negative
mahadevi gosarwade	27	secondary	8	absent	absent	absent	absent	absent	-	normal	negative
Mahadevi madiwalar	39	primary	18	absent	absent	absent	absent	absent	-	normal	negative
mahadevi nayak	22	primary	2	absent	absent	absent	absent	present	3 days	normal	negative
Mahadevi suresh	36	secondary	10	absent	absent	absent	absent	absent	-	normal	negative
Mahadevi uppar	22	primary	0.5	absent	absent	absent	absent	absent	-	normal	negative
mahadevi watone	32	secondary	4	absent	absent	absent	absent	absent	-	normal	negative
mahananda tulisigeri	23	primary	6	absent	absent	absent	absent	absent	-	normal	negative
mala basappa	20	primary	4	absent	absent	absent	absent	absent	-	normal	negative
Mala hippali	20	primary	4	absent	absent	absent	absent	absent	-	normal	negative
mala suresh	32	primary	6	absent	absent	absent	absent	absent	-	normal	negative
malini patgar	29	primary	7	absent	absent	present	present	absent	-	normal	negative
Mallawwa mallappa	20	primary	3	absent	absent	absent	absent	absent	-	normal	positive
manasa shettar	30	primary	5	absent	absent	absent	absent	absent	-	normal	positive
Mangala kori	22	primary	3	absent	absent	absent	absent	absent	-	normal	negative
Mangala Shivaram	29	primary	3	absent	absent	absent	absent	absent	-	normal	negative
Manjula ajjappa	34	secondary	6.5	absent	absent	absent	absent	absent	-	normal	negative
manjula basappa	33	secondary	2.5	present	absent	absent	absent	absent	-	normal	negative
Manjula chilakuri	23	primary	1	absent	absent	absent	absent	absent	-	normal	negative
manjula garag	30	primary	11	absent	absent	absent	absent	absent	-	normal	negative
Manjula Hittalmani	28	primary	3	present	absent	absent	absent	absent	-	normal	negative
maya kadam	22	secondary	0	absent	absent	absent	absent	absent	-	normal	negative
Meenakshi mutalik desa	26	primary	2	absent	absent	absent	absent	absent	-	normal	negative
Muttawwa ramanagouda	33	primary	11	present	present	present	absent	absent	-	normal	negative

Name of the patient	age(years)	type	Duration of infertility(years)	Vaginal discharge	Pain abdomen	Back ache	Dysmenorrhea	H/O tetracycline treatment	Duration(years)	TVS	Chlamydia trachomatis ELISA
nagaratna hullar	24	primary	3	absent	absent	absent	absent	absent	-	normal	negative
nagaveni dhananjay	33	primary	2.5	absent	absent	absent	absent	absent	-	normal	negative
Nanda ramachandra	35	primary	16	absent	absent	absent	absent	absent	-	normal	negative
neela alubhavi	25	primary	5	absent	absent	absent	absent	absent	-	normal	negative
neelawwa gosabal	28	secondary	13.5	absent	absent	absent	absent	absent	-	normal	negative
Neelawwa Patil	30	secondary	6	absent	absent	absent	absent	absent	-	normal	negative
Neeta kagatikar	38	primary	12	absent	absent	absent	absent	absent	-	normal	negative
Neetha mangesh	34	primary	9	absent	absent	absent	absent	absent	-	normal	negative
netravati ganiger	28	primary	6	absent	absent	absent	absent	absent	-	normal	negative
nilambica kurushetty	29	primary	4	absent	absent	absent	absent	absent	-	normal	negative
Nirmala vandakudar	32	primary	11	absent	absent	absent	absent	absent	-	normal	negative
padama malali	28	secondary	3	present	absent	absent	absent	absent	-	normal	negative
parvati prakash	23	primary	5	present	absent	absent	absent	absent	-	normal	negative
pooja madiwal	27	primary	9	absent	absent	absent	absent	absent	-	normal	negative
poornima ganiger	29	primary	1.5	absent	absent	absent	absent	absent	-	normal	negative
prabha	32	secondary	5	absent	absent	absent	absent	absent	-	normal	negative
Prafula gajnur	37	primary	7	absent	absent	absent	absent	absent	-	normal	negative
pratima das	22	primary	4	absent	absent	absent	absent	absent	-	normal	negative
preethi sharma	29	primary	6	absent	absent	absent	absent	absent	-	normal	negative
prema chowhar	29	secondary	3	absent	absent	absent	absent	absent	-	normal	negative
Prema pujari	38	primary	17	present	present	present	present	absent	-	normal	negative
premalata patil	33	secondary	8	absent	absent	absent	absent	absent	-	normal	negative
priya swapnil	28	secondary	4.5	absent	absent	absent	absent	absent	-	normal	negative
pushpa bhandage	28	secondary	4	absent	absent	absent	absent	absent	-	normal	negative
Pushpa Bidarkatti	30	primary	9	absent	absent	absent	absent	absent	-	normal	negative
pushpa dindolkoppar	28	secondary	2	absent	absent	absent	absent	absent	-	normal	negative
Pushpa mathad	36	secondary	0	absent	absent	absent	absent	absent	-	normal	negative
radhika harale	33	primary	1	absent	absent	absent	absent	absent	-	normal	negative
rajashree doddamani	22	primary	4	absent	absent	absent	absent	absent	-	normal	negative
Rajashree hazeri	34	secondary	0	absent	absent	absent	absent	absent	-	normal	negative
Rajashree kamble	37	secondary	7	absent	absent	absent	absent	absent	-	normal	negative
rajeshwari doddamani	24	primary	3	absent	absent	absent	absent	absent	-	normal	negative
rajeshwari naik	28	primary	4	absent	absent	absent	absent	absent	-	normal	positive
rajeshwari patil	23	primary	5	absent	absent	absent	absent	absent	-	normal	negative
Rakhi Sagarsha	31	primary	7	absent	absent	present	absent	absent	-	normal	negative
rameja	28	secondary	3.5	absent	absent	absent	absent	absent	-	normal	negative
Rasheeda	29	primary	8	absent	absent	absent	absent	absent	-	normal	negative
rashmi anil	28	primary	3	absent	absent	absent	absent	absent	-	normal	negative
rashmi girish	33	secondary	11	absent	absent	absent	absent	absent	-	normal	positive
Ratna talikoti	40	primary	1	absent	absent	absent	absent	absent	-	normal	positive
Ratnawwa bhajantri	35	primary	7	absent	absent	absent	absent	absent	-	normal	negative
Raziya begum	25	primary	3	absent	absent	absent	absent	absent	-	normal	negative
Raziya momin	22	primary	2	absent	absent	absent	absent	absent	-	normal	negative
rekha ganesh	28	primary	6	absent	absent	absent	absent	absent	-	normal	negative
rekha nilappagoudar	23	primary	5	absent	absent	absent	absent	absent	-	normal	negative
rekha sakri	30	primary	4	absent	absent	absent	absent	absent	-	normal	negative
rekha sanjeev moore	26	primary	4	absent	absent	absent	absent	absent	-	normal	negative
Rekha Suresh Jorapur	38	primary	11	absent	absent	absent	absent	absent	-	normal	negative
Renuka Bandivaddar	30	primary	2	absent	absent	absent	absent	absent	-	normal	negative
renuka nadavinkeri	22	primary	5.5	absent	absent	absent	absent	absent	-	normal	negative
renuka somangoudar	25	primary	3	absent	absent	absent	absent	absent	-	normal	negative

Name of the patient	age(years)	type	Duration of infertility(years)	Vaginal discharge	Pain abdomen	Back ache	Dysmenorrhea	H/O tetracycline treatment	Duration(years)	TVS	Chlamydia trachomatis ELISA
Reshma bepuri	31	secondary	4	absent	absent	absent	absent	absent	-	normal	negative
Reshma patil	22	primary	2	absent	absent	absent	absent	absent	-	normal	negative
Roopa patil	27	primary	4	absent	absent	absent	absent	absent	-	normal	negative
Roopa pattar	28	primary	5	absent	absent	absent	absent	absent	-	normal	positive
Roopa santosh kabadi	30	primary	10	absent	absent	absent	absent	absent	-	normal	negative
Rubii ajit	28	primary	1.5	absent	absent	absent	absent	absent	-	normal	negative
Rupika patade	34	primary	12	absent	absent	absent	absent	absent	-	normal	negative
Rusha lingamannavar	40	primary	12	absent	absent	absent	absent	absent	-	normal	negative
Sahana bargali	28	secondary	2	absent	absent	absent	absent	absent	-	normal	negative
Saishree	29	primary	7	absent	absent	absent	absent	absent	-	normal	negative
Salima patil	39	secondary	2	absent	absent	absent	absent	absent	-	normal	negative
Sanchita patil	22	primary	1	absent	absent	absent	absent	absent	-	normal	negative
Sangeetha revappa	24	primary	9	absent	absent	absent	absent	absent	-	normal	negative
Sangeetha shettar	33	primary	7	absent	absent	absent	absent	absent	-	normal	negative
Sankawwa gundappa	25	primary	14	absent	absent	absent	absent	absent	-	normal	negative
Sarala mallikarjunagoud	28	primary	4	absent	absent	absent	absent	absent	-	normal	negative
Sarita dash	32	primary	7	absent	absent	absent	absent	absent	-	normal	positive
Sarita patil	20	primary	1	absent	absent	absent	absent	absent	-	normal	negative
Sathyabhama krishna	37	primary	18	absent	absent	absent	absent	absent	-	normal	negative
Savita hosur	30	primary	7	absent	absent	absent	absent	absent	-	normal	negative
Savita kirankunj	24	secondary	1	absent	absent	absent	absent	absent	-	normal	negative
Savita kulkarni	28	primary	1	absent	absent	absent	absent	absent	-	normal	negative
Savita nadagoudar	33	primary	8	absent	absent	absent	absent	absent	-	normal	negative
Savita ramesh	26	primary	7	absent	absent	absent	absent	absent	-	normal	negative
Savita sanjay kanade	23	primary	3	absent	absent	absent	absent	absent	-	normal	positive
Savita sankratti	22	primary	4	absent	absent	absent	absent	absent	-	normal	negative
Savita sonnar	26	secondary	5	absent	absent	absent	absent	absent	-	normal	positive
Savitha suresh babu	22	primary	2	absent	absent	absent	absent	absent	-	normal	negative
Savitri desai	31	primary	7	absent	absent	absent	absent	absent	-	normal	negative
Seema Bali	35	primary	9	absent	absent	absent	absent	absent	-	normal	negative
Seema Bhosale	32	primary	17	absent	absent	absent	absent	absent	-	normal	negative
seema tejkumar	30	secondary	1.5	absent	absent	absent	absent	absent	-	normal	negative
shaheen nalband	29	primary	4	absent	absent	absent	absent	absent	-	normal	negative
shahin mulla	24	primary	4	absent	absent	absent	absent	absent	-	normal	negative
Shahnaaz Imtiyaz	22	primary	3	absent	absent	absent	absent	absent	-	normal	negative
Shahtaaz bhanu	25	primary	2	absent	absent	absent	absent	absent	-	normal	negative
shaila ramappa	27	primary	3	absent	absent	absent	absent	absent	-	normal	negative
Shailaja dandagi	28	primary	8	absent	absent	absent	absent	absent	-	normal	negative
Shailaja madukangouda	24	secondary	1	absent	absent	absent	present	absent	-	normal	negative
Shakuntala reddy	32	primary	11	absent	absent	absent	absent	absent	-	normal	positive
Sharada naik	27	primary	2	absent	absent	absent	absent	absent	-	normal	negative
Sharada Rudrapur	36	secondary	10	absent	absent	absent	absent	absent	-	normal	negative
Sharavati chandrasekhar	32	primary	5	absent	absent	absent	absent	absent	-	normal	negative
Shasikala naik	25	secondary	0	absent	absent	absent	absent	absent	-	normal	negative
Sheela rajiv	26	primary	11	absent	absent	absent	absent	absent	-	normal	negative
Sheetal Hattikar	24	secondary	3	absent	absent	absent	absent	absent	-	normal	negative
Shilpa tegginamani	25	primary	0.5	absent	absent	absent	absent	absent	-	normal	negative
Shivaleela umesh	22	primary	4	present	absent	absent	absent	absent	-	normal	negative
Shobha agadi	33	primary	3.5	present	absent	absent	absent	absent	-	normal	negative
Shobha hugar	30	primary	2	present	absent	absent	absent	absent	-	normal	negative
Shobha kudnavar	22	primary	2	absent	absent	absent	absent	absent	-	normal	negative

Name of the patient	age(years)	type	Duration of infertility(years)	Vaginal discharge	Pain abdomen	Back ache	Dysmenorrhea	H/O tetracycline treatment	Duration(years)	TVS	Chlamydia trachomatis ELISA
Shobha pattanshetti	28	primary	6	absent	absent	absent	absent	absent	-	normal	negative
Shobha saptsagar	35	primary	14	absent	absent	absent	absent	absent	-	normal	negative
Shreya Gaonkar	24	primary	2	absent	absent	absent	absent	absent	-	normal	negative
Shridevi navi	25	primary	4	absent	absent	absent	absent	absent	-	normal	negative
Shruti kulkarni	31	primary	8	absent	absent	absent	absent	absent	-	normal	negative
Shubha tawari	42	secondary	15	absent	absent	absent	absent	absent	-	normal	negative
Shubhangi Andhare	31	primary	12	absent	absent	absent	absent	absent	-	normal	negative
Shubhangi shivaji	31	primary	13	absent	absent	absent	absent	absent	-	normal	negative
Shweta muganur	35	secondary	11	absent	absent	absent	absent	absent	-	normal	negative
Sonali mangure	30	primary	1.5	absent	absent	absent	absent	absent	-	normal	negative
Sonali mutgekar	23	primary	0.5	absent	absent	absent	absent	absent	-	normal	negative
Sridevi gavani	24	secondary	3	absent	absent	absent	absent	absent	-	normal	negative
Sudha kamble	22	primary	2	absent	absent	absent	absent	absent	-	normal	negative
Sudha mareddi	34	primary	5	absent	absent	absent	absent	absent	-	normal	negative
Sujatha goudar	33	secondary	7	absent	absent	absent	absent	absent	-	normal	
Sumangala gangadharm	34	secondary	10	absent	absent	absent	absent	absent	-	normal	negative
Sumangala Hanji	35	primary	1	absent	absent	absent	absent	absent	-	normal	negative
Sumangala patil	32	primary	11	absent	absent	absent	absent	absent	-	normal	negative
Sumitra khajnekar	31	primary	8	absent	absent	absent	absent	absent	-	normal	negative
Sunanda kabbur	32	primary	2	absent	absent	absent	absent	absent	-	normal	positive
Sunita appasab	28	primary	4	absent	absent	absent	absent	absent	-	normal	negative
Sunita kulkarni	32	primary	8	absent	absent	absent	absent	absent	-	normal	negative
Sunitha gundal	21	primary	2	absent	absent	absent	absent	absent	-	normal	negative
Supriya sukumar	21	primary	0.5	absent	absent	absent	absent	absent	-	normal	negative
Surekha bhajandri	27	secondary	2	absent	absent	absent	absent	absent	-	normal	negative
Surekha ingale	38	primary	15	absent	absent	absent	absent	absent	-	normal	negative
Surekha sobrad	26	primary	3	absent	absent	absent	absent	absent	-	normal	negative
Sushila harish	27	primary	9.5	absent	absent	absent	absent	absent	-	normal	negative
Sushila timmarayappa	29	secondary	4	absent	absent	absent	absent	absent	-	normal	negative
Sushma hannolkar	30	primary	9	absent	absent	absent	absent	absent	-	normal	negative
Suvarna gopal	26	primary	9	absent	absent	absent	absent	absent	-	normal	negative
Suvarna jamabanvar	31	primary	0.66	present	absent	absent	absent	absent	-	normal	negative
Tina porwal	26	primary	3	absent	absent	absent	absent	absent	-	normal	negative
Uddawwa kanannavar	25	secondary	8	absent	absent	absent	absent	absent	-	normal	negative
Uma pujar	38	primary	11	absent	absent	absent	absent	absent	-	normal	negative
Uma shettar	32	primary	7	absent	absent	absent	absent	absent	-	normal	negative
Vaheeda begum	40	primary	5	absent	absent	absent	absent	absent	-	normal	negative
Vaishali gani	30	primary	1	absent	absent	absent	absent	absent	-	normal	negative
Vanita Sawant	31	primary	7	absent	absent	absent	absent	absent	-	normal	negative
Vanitha deshabandar	26	secondary	1.5	absent	absent	absent	absent	absent	-	normal	negative
vasantha natraj	25	primary	5	absent	absent	absent	absent	absent	-	normal	negative
Veena sanadi	22	primary	4	absent	absent	absent	absent	absent	-	normal	negative
vidya chavan	25	primary	3	absent	absent	absent	absent	absent	-	normal	negative
Vidya irappagol	37	secondary	2	absent	absent	absent	absent	absent	-	normal	negative
Vidya patil	22	primary	1	absent	absent	absent	absent	absent	-	normal	negative
Vidya Sudhakar Patil	22	primary	1	present	absent	absent	absent	absent	-	normal	negative
Vijayalakshmi Jevani	27	secondary	0	absent	absent	absent	absent	absent	-	normal	negative
vijaylakshmi kadadavar	29	primary	5.5	absent	absent	absent	absent	present	5 days	normal	negative
Vijaylakshmi kalloli	29	secondary	2	absent	absent	absent	absent	absent	-	normal	positive
Vijaylakshmi Savagoan	26	primary	1.5	absent	absent	absent	absent	absent	-	normal	negative
Vishwala taukari	38	primary	7	absent	absent	absent	absent	absent	-	normal	negative

Name of the patient	age(years)	type	Duration of infertility(years)	Vaginal discharge	Pain abdomen	Back ache	Dysmenorrhea	H/O tetracycline treatment	Duration(years)	TVS	Chlamydia trachomatis ELISA
Vishwala taukari	38	primary	7	absent	absent	absent	absent	absent	-	normal	negative
yellawwa jawali	28	secondary	12	absent	absent	absent	absent	absent	-	normal	negative
Zareena jamadar	25	primary	8	absent	absent	absent	present	absent	-	normal	negative