PREVALENCE OF SPECIFIC ANTIBODIES TO *Chlamydia trachomatis* AMONG WOMEN ON ROUTINE GYNECOLOGICAL VISIT TO JIMMA UNIVERSITY SPECIALIZED HOSPITAL, JIMMA, SOUTH – WEST ETHIOPIA

ZEWDINEH SAHLEMARIAM GEBREMESKEL

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ABSTRACT

Key words: Chlamydia trachomatis, IgG/IgM seroprevalence, ELISA, antibody index

Genital Chlamydia trachomatis infection is a key global issue facing women's reproductive health. The worldwide prevalence of Chlamydia trachomatis genital infection (CTGI) is estimated to be 700 million, with highest prevalences in sub-Saharan Africa.

To determine the prevalence of antibodies to genital Chlamydia trachomatis infection and to assess related socio-demographic and reproductive factors, 184 women routinely visiting the gynecology out-patient department of Jimma University Specialized Hospital (JUSH) were investigated during March 2005 – February 2006. Specific IgG and IgM antibodies to Chlamydia trachomatis were detected using an Enzyme Linked Immunosorbent Assay (ELISA) system on 184 sera collected from these women. Socio-demographic information as well as gynecologic/reproductive information was collected by interviewing. Physical examination was also done for the patients. Complete data were obtained for 184 women. The majority of the women (47.3%) were in the 20-29 year age group. The minimum age of the study participants was 12 years. The prevalence of antibodies showing exposure to genital Chlamydia trachomatis was: IgG 45.6% and IgM 5.4%. Seven women (3.8%) had positive serology for both IgG and IgM. Higher prevalence of IgG antibodies was found in women in the 20-29 year and 30-39 year age groups which was 21.7% and 12.5%, respectively, although the difference in the different age groups was not significant ($p>0.05$). Thirty-four percent of the study subjects had a history of one or more abortion, of whom 17.4% had positive IgG serology. The minimum and mean ages of the women at first sexual intercourse were 12 years and 16 years, respectively. Physical examination revealed no symptoms of reproductive tract infection (RTI), sexually transmitted infection (STI), urinary tract infection (UTI) or other gynecologic abnormalities in 19% of the women. Evidence of Chlamydial infection was found in half of the women who were asymptomatic. The majority of the women (81%) had either one or multiple symptoms on clinical evaluation.

History of previous episodes of pelvic inflammatory disease (PID) was documented in 14 women, of whom 6 showed positive IgG serology. The most frequent complaints at presentation were: lower abdominal pain/tenderness (39%), vaginal discharge (32%) and

vaginal bleeding (18.5%). Positive serology to Chlamydia trachomatis IgG antibodies was identified in 14.1% and 16.8% of all women with vaginal discharge and lower abdominal pain, respectively. Thirty-five percent of the study subjects claimed to have had 2 or more sexual partners. Forty-nine percent of the women had their first sexual intercourse at an age of 12 – 16 years, of whom 23.4.8% had positive IgG serology.

This study has highlighted high prevalence of antibodies to genital Chlamydia trachomatis and high frequency of behavioral and reproductive risk factors among the study participants. Health care providers should be aware of the high prevalence of Chlamydial infection in women. Similar population-based studies of chlamydia prevalence should be conducted in different communities to elucidate the actual burden of the problem in Ethiopia and design intervention strategies.

10. INTRODUCTION AND LITERATURE REVIEW

Chlamydiae are obligate intracellular bacteria that grow in eukaryotic cells and cause a wide spectrum of human disease (CDC, 2001). Species were grouped according to their biologic and biochemical properties and a greater than 95% homology in their 16s ribosomal RNA sequences (Weisberg *et al*., 1986).

Chlamydiae have a unique biphasic lifecycle with dimorphic forms that are functionally and morphologically distinct. An extracellular form, the elementary body (EB), is infectious but metabolically inactive. Once endocytosed, the EB differentiates into a larger pleomorphic form called the reticulate body (RB), which replicates by binary fission. The precise mechanism by which EBs attach and gain entry into the host cell is unknown. One study suggested that chlamydiae employ a molecular mimic of heparin sulfate to attach to glycosaminoglycan (GAG) receptors on eukaryotic cell surfaces (Stephens, 1994). GAG appears to form a trimolecular complex with the host cell since EB infectivity is inhibited by the addition of heparin or heparin sulfate to culture, and pretreatment of EBs with heparin sulfate lyase abolishes EB infectivity. The mechanism of endocytic uptake remains unclear. Once inside the host cell, chlamydiae reside in a membrane – bound vacuole that can evade phagolysosomal fusion. The endosome is transported to the distal region of the Golgi apparatus and incorporates host – derived sphingolipids into the inclusion membrane (Hackstadt *et al*., 1995; Hackstadt *et al*, 1996). Thus it appears that Chlamydiae are able to intercept host vesicular traffic bound for the plasma membrane to sequester lipids and possibly other host substances synthesized in the Golgi. Subversion of host vesicular traffic may represent a dual advantage for Chlamydia in obtaining materials from the host for its metabolism as well as in modifying the inclusion membrane to evade lysosomal fusion and immune detection (CDC, 2001).

The recognition of genital chlamydial infection as an important public health problem was made first by the recognition of its role in acute clinical syndromes, as well as in

serious reproductive and ocular complications, and secondly by our awareness of its prevalence when diagnostic tests became widely accessible (CDC, 2001).

Chlamydia trachomatis is a bacterial infection of global public health significance. It is associated with trachoma (serovars A, B, B_1 and C), lymphogranuloma venereum (serovars L_1, L_2 and L_3) and genital infection (serovars D to K) (Moss, 2001). Urogenital *Chlamydia trachomatis* infection can cause pelvic inflammatory disease (PID) and represents a major public health problem to the reproductive health of women in developing and industrialized countries (CDC, 2001; Moss, 2001). The world health organization estimates that 89 million new cases of genital *Chlamydia trachomatis* infection occur each year and the majority of these infections are geographically found in developing countries reflecting provision and access to health care, health seeking behavior and the global population distribution. Highest prevalence is in Sub – Saharan Africa, lowest in East Asia and the Pacific (WHO, 1995).

Table 1. Estimated number of new cases, prevalence and incidence of genital *Chlamydia trachomatis* infection between ages 15 and 49, by sex and United Nations global region: 1995 (WHO, 1995).

Region	**New cases (million)**		**Prevalence (%)**		**Incidence (per 1000)**	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**
North America	1.64	2.34	0.8	2.7	21.46	30.73
Western Europe	2.30	3.20	0.8	2.7	21.46	30.73
Australia	0.12	0.17	0.8	2.7	21.46	30.73
Latin America and Caribbean	5.01	5.12	2.5	4.0	40.03	40.77
Sub – Saharan Africa	6.96	8.44	4.8	7.1	55.04	65.95
North Africa and Middle East	1.67	1.28	1.2	1.7	19.93	16.29
Eastern Europe and Central Asia	2.15	2.92	1.7	3.7	27.29	37.09
East Asia and Pacific	2.70	2.63	0.4	0.7	6.53	6.75
South and South East Asia	20.20	20.28	3.7	4.9	41.65	44.32
Overall	42.75	46.38	-	-	-	-

In the last two decades, genital chlamydial infection has been identified as a major public health problem because of the recognition that chlamydial infection is associated with disease syndromes such as non – gonococcal urethritis, mucopurulent cervicitis, PID, ectopic pregnancy and tubal infertility (Washington *et al*., 1987; CDC, 2001).

Genital infections due to *C. trachomatis* present unique problems for public health control programs because 50% - 70% of infections in women (and perhaps men) are clinically silent (CDC, 2001; Moss, 2001). Unrecognized and untreated, the bacteria may remain infectious in the host for months and be readily transmitted to sex partners. Furthermore, most reported infections occur in the 15 to 24 – year - old age group. Young women with cervical chlamydial infection are at a risk for PID, which can lead to long – term reproductive sequelae such as chronic pelvic pain, ectopic pregnancy, and tubal infertility. Babies born to infected mothers are also at risk for conjunctivitis and pneumonia (CDC, 2001; Moss, 2001). There is often co-infection with bacterial vaginosis - associated organisms and *N. gonorrhoeae* may also be present (Peeling, 1995).

Chlamydia trachomatis infection of the female genital tract can vary from an asymptomatic self-limiting infection to a severe debilitating illness with serious long term complications both of the reproductive tract itself and also as a more disseminated disease. Whilst in the asymptomatic phase, ongoing damage to the fallopian tubes may be occurring and the diagnosis may only be made many years later, when complications are detected (Rogstad, 2001).

A woman's exposure to Chlamydia is usually a result of sexual intercourse. The site of initial infection is most often the cervix; the urethra and the rectum may also be infected (Paavonen *et al*., 1982; Bradley *et al*., 1985).

Chlamydia causes symptoms in a minority of infected women. When symptoms occur, they include vaginal discharge and dysuria. The ascension of lower, genitourinary infection to the endometrium and fallopian tubes may cause lower abdominal pain and

menstrual abnormalities. Untreated infection among women often persists for months. During this period, complications may develop, and many of these women transmit their infection to others (CDC, 1993).

Chlamydia trachomatis enters into columnar or transitional epithelial cells of the genital tract, rectum and peritoneum. The reticulate body of the Chlamydia replicates and, when new elementary bodies are assembled, they leave the cell, with resultant cell death. In the fallopian tube, there is subepithelial inflammation, epithelial ulceration and scarring. Damage is mediated by cell death and exacerbated by a delayed hypersensitivity reaction to chlamydial 60kDa heat shock proteins (HSP-60) (Rogstad, 2001).

Complications of *Chlamydia trachomatis* Infection in the Female

Acute Urethral Syndrome

This develops as a result of urethral infection, although the majority of women with chlamydial infection of this site are asymptomatic. The diagnosis should be considered in women presenting with symptoms of cystitis, particularly where the dysuria has been present for more than one week. Examination may reveal meatal discharge, erythema or swelling but is usually normal. A clue to a urethral rather than bladder cause for the symptoms is suggested by lack of suprapubic tenderness and absence of haematuria (Rogstad, 2001).

Cervicitis

Women with cervicitis can be asymptomatic or complain of vaginal discharge, which may be yellow or green. If there is coexistant bacterial vaginosis, then they may also complain of odor and postcoital bleeding occurs in some. The cervix may appear completely normal despite being infected with Chlamydia. However, at least a third may show evidence of infection with either a hypertrophic ectopy of the endocervix, as manifested by cervical edeama, congestion and bleeding (19%), or have a mucopurulent discharge (37%) (Harirson, 1985).

Endometritis

Nearly half of patients with chlamydial cervictis will also have endometritis. This can be asymptomatic or the patient may complain of menorrhagia, metrorrhagia or postcoital bleeding (Rogstad, 2001).

Pelvic Inflammatory Disease (PID)

Pelvic inflammatory disease (PID) occurs when there is upper genital tract infection and can be used to refer to salpingitis alone or include endometritis. It can be acute, sub acute, chronic or silent. Up to 80% of cases of PID in the developed world are due to sexually transmitted infection, and European studies suggest *Chlamydia trachomatis* is the cause of at least 60% of cases. Accurate diagnosis is difficult without the use of invasive techniques such as laparoscopy therefore data on frequency are difficult to interpret. However, it appears that between 10 and 40% of women infected with *Chlamydia trachomatis* develop PID (Stum *et al*., 1984). The incidence is greatest in 15-19 year olds, and the relative risk of acquiring PID in women is also highest in this group. The combined oral contraceptive pill confers some protection against ascending infection, and if PID does occur it is usually less severe. The risk of *Chlamydia trachomatis* causing PID is increased by the presence of an intrauterine contraceptive device (IUCD), particularly at initial insertion or when it is changed. The risk of *Chlamydia trachomatis* causing salpingitis is also increased by manipulations of the cervix during termination of pregnancy or other gynecological procedures (Rogstad, 2001).

PID presents as acute lower abdominal or pelvic pain. There may be deep dyspareunia, vaginal bleeding and discharge as well as pyrexia. Clinical examination can reveal lower abdominal tenderness; adnexal tenderness and cervical excitation (pain on cervical movement). Bilateral masses may be felt, particularly if there is tubo-ovarian abscess formation (Leugur *et al.,* 2000).

Table 2. Complications of *Chlamydia trachomatis* in women (Rogstad, 2001).

Site		Symptoms, signs and complications
Urethra	urethritis	frequency/dysuria
Cervix	cervicitis	mucopurulent vaginal discharge
Uterus	endometritis	vaginal discharge irregular bleeding post coital bleeding
Fallopian tubes	salpingitis	pelvic inflammatory disease ectopic pregnancy tube infertility chronic pelvic pain
Liver	perihepatitis	right upper quadrant pain
Bartholins gland	bartholinitis	swelling and pain of vulva
Conjunctiva	conjunctivitis	discharge from eye
Systemic	Reiter's syndrome	arthritis (large joints) iritis keratodermia blenorrhagica
Pregnancy/ Neonate	pneumonitis, conjunctivitis, post-partum endometritis	

Complications of Chlamydial PID

As PID worsens, tubo-ovarian abcesses can form and peritonitis develops, as well as Fitz-Hugh-Curtis syndrome. Long term complications of PID include ectopic pregnancy, tubal infertility and chronic pelvic pain. The risk of sequaele increases disproportionately with subsequent infections. For ectopic pregnancy or tubal infertility the odds ratio increases from 6 after one episode of PID to 17 after two episodes (Westrom, 1994).

In women who have had PID the risk of ectopic pregnancy increased by 7-10 times, and 43% of cases of ectopic pregnancy may be due to Chlamydia, either recognized or unrecognized (Communicable Diseases Report, 2000).

Chlamydia trachomatis infections are a major cause of tubal factor subfertility. However, the precise pathogenesis of *C. trachomatis* infections remains to be elucidated. Not all women who have undergone a *C. trachomatis* infection will develop tubal pathology. Host factors, virulence of the microorganism and environmental factors determine the course and morbidity of *C. trachomatis* infections. Depending on these factors and their interaction, *C. trachomatis* infections will either be cleared or persist. A clearance of 44.7% has been reported in asymptomatic and untreated women after a 1 year follow-up (Morre *et al*., 2002). In women who clear a *C. trachomatis* infection adequately, the risk of tubal damage may be low, since the host has been exposed to the microorganism for a short period. Persistent exposure to the microorganism may result in a chronic inflammatory response and may increase the risk of tubal damage, as has been previously suggested (Grayston *et al*., 1985; Patton *et al*., 1994).

Therapeutic Management of *C. trachomatis* Genital Infections

The twin goals of microbiological clearance and clinical cure are obvious aims in any symptomatic patient with chlamydial infection. However, many people infected with chlamydia have no symptoms, and their partners may or may not be infected. It is this practice of treating asymptomatic cases or contacts of infection that reinforces the need for safe and effective therapy (UK Clinical Effectiveness Guidelines, 1999).

An understanding of the unique reproductive cycle of chlamydiae gives the theoretical basis for therapy. The elementary bodies (EBs) transform to reticulate bodies (RBs); RBs divide and finally differentiate back to EBs, which are released by host cell lysis. The metabolic activity within cells means that antibiotics must achieve sufficient intracellular or tissue levels to be effective. The whole cycle takes about 40 hours in culture systems; this slow life cycle implies that a long course (over 5 days) of therapy is required or high tissue levels from a single dose therapy must be maintained over such a period. Recommendations for the treatment of genital chlamydial infections have been published (Toomey and Barnes, 1990; CDC, 1998). Two new antimicrobials approved by the FDA for the treatment of chlamydia, ofloxacin and azithromycin, offer the clinician additional therapeutic choices. A substantial advantage of azithromycin, in comparison with all other therapies, is that a single dose is effective; this antimicrobial may prove most useful in situations in which compliance with a 7 – day regimen of another antimicrobial can not be ensured. In view of the high efficacy of tetracycline and doxycycline, cost should also be considered when selecting a treatment regimen. A 7 – day treatment regimen of erythromycin is recommended for chlamydial infection during pregnancy (CDC, 1998).

The diagnosis of genital Chlamydial infection relies entirely on laboratory tests. Methods of diagnosis based on isolation of the organism, detecting its infectious EBs using monoclonal antibodies or detecting its components by Enzyme immunoassay (EIA) are sensitive during the acute phase of the infection. These conventional antigen detection tests, however, are of low sensitivity in cases of chronic or repeated Chlamydial infections (Treharne *et al*., 1977; Moss, 1987; Moss, 200). Consequently, application and further development of serological tests based on the detection and quantitation of group – and species – specific antibodies in sera may be regarded as a valid and urgent priority particularly with regard to recurrent, latent, sub-acute and chronic disease (Bas *et al*., 2001). The fact that late disseminated chlamydial genital infection in women is chronic, crippling and largely incurable provides another reason for improved serological diagnosis. Positive serology may also help identify male urethral, prostatic and lymph gland or splenic carriers (Moss, 2001).

A variety of serological tests including complement fixation, agglutination, hemagglutination, immunodiffusion, hemolysis in gel and radioimmune precipitation tests have been used in the past. These tests detect group – specific antibodies and have low sensitivity and specificity for detecting Chlamydia antibodies. The methods commonly used today are complement fixation test, indirect fluorescent test (IFT), EIA and microimmunofluorescence (MIF) tests. In IFT or EIA, infected cells with one *C. trachomatis* serotype, purified EBs of a *C. trachomatis* serotype or a pool of *C. trachomatis* D to K (CTDK) are used as a single antigen. These tests detect group – specific antibodies and can not differentiate between antibody responses to CTDK, *C. pneumoniae* and *C. psittaci*. In contrast, the MIF test detects and separates antibodies to *C. trachomatis* from those to non – genital Chlamydia species including *C. pneumoniae* and *C. psittaci* (Moss, 2001).

The potential value of using serological tests for Chlamydia was argued as early as 1987. One conclusion was that the addition of species – specific Chlamydia serology to the routine investigation of women presenting with PID undoubtedly aids the identification of those infected with *C. trachomatis* (Moss *et al*., 1993; Thomas *et al*., 2000).

The presence of type – specific antibodies to CTDK suggests that the patient has been exposed to the organism at some time. The natural history of *C. trachomatis* genital infection would support the concept that a number of these patients will have a current infection. A judicious application of antimicrobial therapy for these patients may have potential benefits (Moss, 2001).

There is a wide acceptance that improved antigen detection alone is not wholly satisfactory. When species – specific Chlamydia serology was combined with modern antigen detection systems, a marked increase in the sensitivity of diagnosis was achieved (Moss, 2001).

Thomas *et al* (2000) used the MIF technique to assess the significance of positive serology in fallopian tube disease and reported a marked association between level of antibody titer and the likelihood of tubal damage. The investigators further argued that by using *C. trachomatis* antibody testing more widely it may be possible to reduce the number of laparoscopies performed and that MIF Chlamydia serology should become an integral part of infertility clinics.

A study by Moss TR *et al* (1993) in Doncaster, UK in 1986 which reviewed 71 women who had a clinical diagnosis of acute PID revealed that 40.8% of these women had evidence of chlamydial infection using a combination of McCoy cell culture/antigen detection and MIF serology. Further, 45 male partners of these women were investigated for genital tract infection using conventional methods and more than 2 out of 3 were found to have recoverable Chlamydia in their anterior urethra. Of particular interest in this study was that Chlamydia was isolated from some men whose female partners were culture negative but D – K IgG positive. These men were frequently asymptomatic carriers.

The role of serology was subjected to further study by the same group between May 1983 and May 1990. In this study, over 7000 cases attending the genitourinary clinic in Doncaster were assessed serologically using the modified MIF test. The study showed that antibodies to *C. pneumoniae* and *C. psittaci* accounted for up to 50% of all Chlamydia IgG positive cases (Moss, 1993).

The sensitivities and specificities of different immunoassays using recombinant antigens or synthetic peptides for the serodiagnosis of *C. trachomatis* infections were evaluated by several investigators. In a recent study by Bas *et al* (2001) that compared the diagnostic value of outer membrane protein 2 (OMP 2) with that of other antigens, it was reported that serological assays using major outer membrane protein (MOMP) and polypeptide encoded by open reading frame 3 of the plasmid (pgp3) as antigen are able to discriminate among various Chlamydia species with reported sensitivities and specificities of 89% and 84% and 79% and 89%, respectively. The investigators

suggested that these two specific serological assays may serve to avoid both overestimating and underestimating the prevalence of *C. trachomatis* infection.

In a similar study in 1998 by Mygind *et al*, (1998) analysis of the humoral immune response to Chlamydia OMP 2 and lipopolysaccharide (LPS) revealed sensitivities and specificities of 89% and 57% and 82% and 70% for IgG reactivity to OMP 2 and LPS, respectively.

In a study that compared three commercially available peptide – based ELISA assays for the serological detection of *C. trachomatis* infections to MIF assay, serum samples from 149 women, aged 20 to 30 years, were tested. Cervical scrapings from these women were previously screened for asymptomatic *C. trachomatis* infection by PCR. Forty – three women were PCR-positive for *C. trachomatis* and 106 were negative. Thirty-six of 78 samples positive by at least one test were positive by all four tests, whereas 13 were positive by all 3 peptide assays but not by MIF, and 10 were positive only by the MIF assay. The sensitivity and specificity of two of the peptide-based assays were 84.7% and 98.7%, respectively compared to 79.2 and 83.1%, respectively, for the in-house MIF. Based on the results the authors concluded that the peptide- based ELISA assays might be good alternatives to the MIF assay for the detection of *C. trachomatis* antibodies (Morre *et al.,* 2002).

In a similar study Bax et al evaluated the performance of two peptide based Enzyme immunoassays (EIAs), pELISA (Medac, Wedel, Germany) and Chlamydia EIA (Biologische, Analysensystem GmbH, Lich, Germany), against MIF in detecting *C .trachomatis* antibodies in different groups of obstetrical, gynecological and subfertile patients. It was shown in the study that the pELISA has the highest specificity when the two assays were compared with MIF and a negative predictive value comparable to that of Chlamydia-EIA in the subfertility group (Bax, *et al*., 2003).

Serological assays have been used to detect anti- chlamydial antibodies in the fertility work-up (Thomas *et al*., 2000), predicting tubal pathology (Mol *et al*., 1997; Johnson *et al*., 2000). Their value for fertility evaluation remains the subject of debate. There is wide variation between various tests in the correlation of anti-chlamydial antibodies with current *C. trachomatis* infections or tubal pathology. The species – specific MIF is considered to be the "gold standard" for the serological diagnosis of *C. trachomatis* infections (Wang *et al*., 1977). Microimmunofluorescence is laborious, and reading of the assay is subjective, and therefore it is not suitable for daily a routine. EIAs provide objective reading and allow the handling of more samples at the same time (Bax *et al*., 2003).

In a study that determined IgG, IgA, and IgM antibody titers in sera of 80 infertile women and 100 controls by a single antigen (L-2) immunoperoxidase assay, a significantly higher prevalence of *C. trachomatis* IgA antibodies was found in infertile women with both abnormal and normal hysterosalpingogram (HSG) than in controls (77% and 14% vs 3%, respectively). The prevalence of *C. trachomatis* IgG antibody was significantly higher in infertile women with abnormal HSG as compared with infertile patients with normal HSG and controls (87% vs20% and 10%, respectively). No *C. trachomatis* IgM antibodies were found in any of the infertile or control groups. It was suggested that detection of serum *C. trachomatis* IgA antibodies could possibly serve as a marker for early recognition of persistent *C. trachomatis* infection (Sarov *et al*., 1986).
Patients with genital gonococcal or chlamydial infections are also at increased risk for HIV (Schachter, 1999). Although the risk for HIV may be lower in patients with Chlamydia infection than those with genital ulcer disease, the higher prevalence of chlamydial infection in some populations means that the population-attributable risk for HIV may be substantially higher for Chlamydia. One study showed that strengthening STD control through education, access to diagnosis and treatment reduced the incidence of HIV by 42% in study communities in Tanzania over 2 years (Oriel, 1986).

In a study of 63 women admitted to the gynecology ward of Linkoping University Hospital in Sweden for the presence of antibodies to *C. trachomatis*, 48% of infertile

women, 78% of women with ectopic pregnancy and 44% of women with PID had higher titer IgG/IgA/IgM antibodies to *C. trachomatis*. Among 55 healthy pregnant women used as controls, 13% had this high antibody titer. The results indicated the role of *C. trachomatis* in the development of sequelae to lower genital tract infections in women (Kihlstrom *et al*., 1990).

In a study that evaluated whether serological markers, assumed to be associated with persistent *C .trachomatis* infections could identify subfertile women at risk of tubal pathology, *C. trachomatis* IgA, CHSP60 – IgG and complement reactive protein (CRP), all serological markers of persistent infections were shown to be significantly more prevalent in women with tubal pathology as compared to those without tubal pathology (Den Hartog *et al*., 2005).

Risk factor studies can identify population subgroups at increased risk of genital *C. trachomatis* infection, and be used to initiate timely, effective intervention and help formulate health education strategies. Incidence is influenced by a complex interaction of demographic and behavioral factors. Aspects of sexual behavior, such as age at first sexual intercourse, number of lifetime sexual partners, frequency of partner change and unsafe sex are key determinants of STI transmission. Studies on specific populations have shown that factors such as being unmarried, the use of non - barrier contraceptives (or no contraception), a higher frequency of partner change, having concurrent partners and lower socioeconomic status are associated with higher chlamydial incidence. Young people are behaviorally vulnerable to STI acquisition as they generally have higher numbers of sexual partners and a higher frequency of partner change than older age groups. These factors are reflected in the high chlamydial incidence seen in the 16 to 24 year age groups, peaking in younger females than males (WHO, 1995; CDC, 2001; Moss, 2001).

A population-based serologic study of 19-25 year old women in northern Sweden that assessed the influence of sexual and social factors on the risk of *C. trachomatis* infections, found that the prevalence of C. trachomatis infection was 2.7% and the

seroprevalence was 24.7%. Compared to women with no history of therapeutic abortion, those having a therapeutic abortion had an unadjusted 3.15 increased risk of seropositivity, which was reduced to 2.40 after adjusting for other risk factors (Jonsson, 1995).

A Danish study which followed women in a double-blind randomized study of the effect of erythromycin on post-abortion infections found that untreated women with *C. trachomatis* infections at the time of first-trimester abortion had a cumulative risk of 72% of developing early and/or late PID after 24 months, compared to only 8% if they were treated with erythromycin prior to abortion (Sorensen, 1994).

In a study in Mexico City of 945 reproductive age subjects who were classified according to their risk for STI, a *C. trachomatis* prevalence of 11.4% by IgG and 4.4% by IgA in females; 25% IgG and 5.7% IgA among commercial sex workers; 8.4% IgG and 1.4% IgA among women with infertility due to tubal damage; and 3.6% IgG and 9.1% IgA among young primigravid (Cravioto *et al*., 2003).

Chlamydia trachomatis infection in pregnant women can lead to serious maternal, neonatal, antenatal, or post natal complications. Antepartum chlamydial infection appears to play an important part in amnionitis post partum endometritis and post abortal salpingitis (Adimora, 1994). Further, it was found that the frequency of fetal complications was higher and the birth weight lower in children whose mothers had chlamydial infection during pregnancy as compared to those whose mothers were uninfected (Gencay *et al*., 1995).

The reported prevalence of *C. trachomatis* infection among pregnant women has ranged widely from 2 to 30% in various studies (Adimora, 1994). Gencay *et al* found IgM seropositivity to *C. trachomatis* in 13.64% of cases and 5.47% of controls by ELISA technique. Rastogi *et al* (2002), in a study in antenatal cases in India, found a high seropositivity to IgM antibodies in 29.4% and 27.7%, respectively.

Swahney *et al* (2003), in a study in antenatal cases in Calcutta, India found positive results to IgM in 20.83% and borderline positive in another 12.5% of cases. Only 10% of the controls were positive for IgA antibodies and none for IgM or IgG antibodies. Moreover, 4.17% were positive and 8.33% borderline positive for IgG antibodies with an overall positivity of 45.83% in the study. These studies have highlighted the importance of performing *C. trachomatis* IgG/IgM ELISA in antenatal cases to treat the infected and prevent any maternal or neonatal complications.

Control programs emphasizing early diagnosis, targeted screening, partner notification and effective treatment have led to a gradual decline in the incidence of genital chlamydial infection in countries where these programs have been implemented (Howell *et al*., 1998). In women, screening of chlamydial infection at the time of Papanicolaou tests, prenatal visits, or attendance at family planning or pregnancy counseling clinics have been effective. In asymptomatic men, who are less likely to access care, asymptomatic infection is not adequately addressed by current public health programs (Moss, 2001).

A longitudinal seroepidemiological study by Anttila *et al* in 2001 provided evidence of an association between specific serotypes of *C. trachomatis* and cervical squamous cell carcinoma (Anttila *et al*., 2001).

As has already been stated elsewhere in the literature, the majority of genital *C. trachomatis* infections are found in the developing world with the highest prevalences in Sub – Saharan Africa (WHO, 1995). However, these estimates are biased due to the quality and quantity of the available surveillance data. Many countries do not have national surveillance data and where prevalence studies have been undertaken these have generally been derived from high risk groups such as STD clinic attendants. Nevertheless, although biased, these estimates illustrate the global importance of genital *C. trachomatis* (Moss, 2001).

A large number of risk factor studies have also been published from various countries, but invariably these have been undertaken in specific clinical settings and are insufficient to allow detailed analysis. The relationship between sexual behavior and STI/Chlamydia prevalence has been little studied in general population samples (Moss et al., 1986; Moss, 2001).

A serological and demographic/reproductive survey by Kusano Y *et al* among 1718 pregnant women in Nagasaki, Japan showed a *C. trachomatis* seroprevalence of 20.8% and a strong association between seropositivity and such factors as premarital pregnancy, non use of condoms, short level of duration of education, and more frequent induced abortion (Kusano *et al*., 2000).

A review of publications on the prevalence rates of genital *C. trachomatis* infections in Ethiopian subjects generally reveals a wide range of prevalence rates reflecting differences in the selection of study subjects and laboratory methods used in these studies.

A study by Blatz *et al* (2001) among 533 Obstetric and Gynecology outpatients in Gondar to detect chlamydial LPS antibodies by a genus – specific ELISA revealed that 90% of the women who were positive for cervical *C. trachomatis* antigen were positive for IgG while 49% had IgA and 28% IgM antibodies. MIF assay of the 436 positive sera showed species – specific antibodies in 21% (*C. trachomatis*), in 69% (*C. pneumoniae*) and in 49% (both species).

A seroepidemiological study by Duncan *et al* (1995) among 1846 women attending family planning clinics in Addis Ababa showed that 62% of the study subjects had anti – *C. trachomatis* antibodies in their sera as detected by the MIF assay.

In a study by Assefa *et al* (1996) among Obstetric and Gynecology outpatients of Gondar hospital, *C. trachomatis* antigens were detected by a commercial ELISA kit in 41 of 639 cervical swab samples.

In summary, the complexities of genitourinary chlamydial disease surely merit a greater effort to clarify the role of serology in diagnosis. Apart from studies of antigen screening for urogenital chlamydial disease, the wider application of serology could enhance our awareness of the prevalence of exposure to this organism in selected populations.

2. Study Objectives

2.1 General Objective

The aim of the present study was to determine the prevalence of *C. trachomatis* – specific antibodies among women visiting the obstetric and gynecology clinic of Jimma University specialized hospital and to elucidate the reproductive and socio – demographic factors associated with genital *C. trachomatis* infection such as age, marital status, number of life time partners and use of condoms.

2.2 Specific Objectives

- To determine the prevalence of IgG and IgM antibodies specific for *C. trachomatis*;
- To evaluate association of selected risk factors with *Chlamydia trachomatis* seropositivity;
- To evaluate the association of pelvic inflammatory disease and other symptoms with Chlamydia seropositivity.

3. Materials and Methods

3.1 **Study Design**

A cross-sectional study was conducted to determine the seroprevalence of antibodies to genital *C. trachomatis* and to assess associated socio-demographic and reproductive features among the study subjects.

3.2 **Study population, area and period**

The number of women attending the gynecology out-patient department of Jimma University Specialized Hospital (JUSH) during the study period (March 2005 – July, 2006) to be enrolled in the study was determined using the formula:

$$n = \frac{Z_{1-\alpha}^2 \, p \, (1- p)}{d^2}$$

Where z is the critical value under the standard normal distribution.

Considering a previous estimated seroprevalence (p) in Ethiopian women of 18%, a level of significance (α) of 0.05, and a degree of freedom (d) of 5%, a sample size (n) of 226 women was proposed to be studied.
But due to various limitations, 81% of the calculated sample size, i.e., 184 women, was covered in this study.

The women were enrolled in this study after giving a written informed consent (annex I) which was read and explained to each participant or a guardian in the language that they were conversant with. Consecutive women visiting the department were entered in this study on the basis of first come first to register without other criteria for pre-selection.

3.3 **Data Collection**

Patient socio-economic and reproductive data was collected by means of a structured questionnaire (annex II) administered in private by the investigator in Amharic with translation into other languages if required.

Clinical data (annex III) were obtained by two physicians who performed a thorough and careful physical examination with particular attention to gynecological presentations of examinees.

Specimen collection, storage and examination

Of the 200 patients for whom data on socio-demographic characteristics, reproductive features and clinical presentation was obtained, *Chlamydia trachomatis* IgG/IgM ELISA determination was done for 184 due to patient refusal to give blood samples (six of them), unsuitability of specimens (two hemolysed/lipemic sera) and resource limitations. Complete data for 184 participants was therefore analyzed.

Venous blood sample (3ml) was drawn from each patient employing standard venipuncture procedure under aseptic conditions. Specimens were collected into glass tubes without anticoagulant and properly labeled for subsequent processing. Proper precaution was taken at every stage of the collection process to avoid hemolysis as hemolysed samples result in erroneous ELISA readings. Specimens were transported soon after collection during each day to the laboratory of the School of Medical Laboratory at Jimma University where the sera were separated by centrifugation at 3000rpm for 5 minutes, transferred into clean Eppendorf tubes and stored at -20^0C until the date of testing.

***Chalmydia trachomatis* IgG and IgM ELISA**

The assay for detection and quantitation of IgG and IgM antibodies to urogenital *C. trachomatis* in patient sera was carried out at the SMLT, JU in February 2006 using *C. trachomatis* IgG (Immundiagnostik, Hamburg, Germany) and IgM (CARBIOTECH Inc., USA) ELISA systems. All frozen sera and kit reagents were brought to room temperature (20 – 25^0C) prior to the assay. Test protocols provided by manufacturers were strictly followed for optimal results.

Test Principle

Diluted patient serum was added to wells coated with purified *C. trachomatis* antigen (serum diluent for IgM assay contains sorbent to remove rheumatoid factor and human IgG interference). Either specific IgG or IgM antibody, if present, binds to the antigen. All unbound materials were washed away and an enzyme conjugate was added to bind to the antigen-antibody complex, if present. Excess enzyme conjugate was washed off and substrate was added. The plate was incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated, measured as optical density at 450nm, is proportional to the amount of IgG/IgM specific antibody in the sample. Readings were made using a dual wavelength with interference filter of 600 – 650nm.

Test Procedure

A 1: 20 dilution of test samples was prepared by adding 10µl of well – mixed sample to 200µl of sample diluent. Serum diluent for IgM assay contains sorbent to remove rheumatoid factor and human IgG interference. Negative control, positive control and calibrator were provided ready to use.

100µl of diluted sera, calibrator and controls were dispensed into appropriate wells. For the reagent blank, 100µl sample diluent was dispensed into 1A well position on the strip. The holder was tapped to remove air – bubbles from the liquid and the wells were mixed well. The strips were incubated for 20 minutes at room temperature. Liquid was then removed from all wells and wells were washed three times with 300 - 350µl of 1X wash buffer. The strips were blotted on absorbent paper. 100µl of enzyme conjugate was then dispensed to each well and the wells were incubated for 20 minutes at room temperature. The wells were again washed three times with 300 -350µl of 1X wash buffer and blotted on absorbent paper. 100µl of tris-metabisulfite (TMB) substrate was dispensed and the wells were incubated for 10 minutes at room temperature. 100µl of stop solution (1N HCl) was added to all wells. The optical densities of the wells were read at 450nm with in 15 minutes employing a dual wavelength with reference filter of 400 – 600nm using Multiskan Version 6.0 microplate reader (Labsystems, Helsinki, Finland).

Automation of Test Procedure

Genesis - Lite Version 3.05, windows – based microplate software, supplied and specified by Thermo Labsystems (Labsystems, Helsinki, Finland) and Thermo Life Sciences (Basingstoke, UK) was employed to operate Multiskan RC Version 6.0 microplate reader (Labsystems, Helsinki, Finland). Readings were processed via a serial RS- 232C interface.

Washing steps in the assay protocol were performed using ELx50^{TM} automated strip washer (BIO-TECH Instruments Inc., Vermont, USA).

Quality Control

As specified by the manufacturers, each test run was considered valid provided the following criteria were met:

1. The optical density (O.D.) of the calibrator (provided in kit) was greater than 0.250;
2. The antibody index (A.I.) for negative control (provided in kit) was less than 0.9;
3. The A.I. for positive control (provided in kit) was greater than 1.2.

Accordingly, the calibrator O.D., negative control antibody index, and positive control antibody index for two runs in the IgG ELISA were 3.064, 2.783; 0.19, 0.15; and 2.54, 2.49, respectively. All the established criteria were met and therefore the test runs were valid. Similarly, the values for two runs in the IgM ELISA were 2.004, 2.270; 0.02, 0.02; and 3.15, 2.81, respectively. Here also, both the IgM test runs were valid as they fulfilled the established criteria.

In addition to validation of the test runs, the automated strip washer was programmed to run a self test and priming program prior to execution of washing steps in each run, as washing steps are critical in the ELISA assay procedure.

Calculation of Results

1. Cut-off value was calculated as: Calibrator O.D. X Calibrator Factor*

* 0.40 for IgG and 0.35 for IgM.

2. The antibody index of each determination was calculated by dividing the O.D. value of each sample by the cut-off value. Patient values were recorded on a format (annex IV).

Interpretation of Calculated Antibody Indices

The following guide, established by the manufacturer, was utilized to interpret test results:

1. < 0.9: No detectable IgG/IgM antibody to *C. trachomatis* by ELISA.
2. 0.9 – 1.1: Borderline positive for IgG/IgM antibody to *C. trachomatis* by ELISA.
3. >1.1: Detectable IgG/IgM antibody to *C. trachomatis* by ELISA.

3.4. Data Analysis and Interpretation

Data entry, processing, tabulation and analysis were performed using SPSS version 10.0 software. The Chi – square statistic was used to test for associations. A level of significance (α) of 0.05 was chosen.

4. Ethical Considerations

The study participants were briefed on the purposes of the study and those who volunteered were recruited. A written informed consent signed by each subject or a guardian was obtained (annex I). Confidentiality of information was maintained.

5. Results

Out of 200 patients for whom data on socio-demographic characteristics, reproductive features, and clinical presentation was obtained, *C. trachomatis* IgG/IgM ELISA determination was done for 184 patients due to patient refusal to give blood samples, unsuitability of specimens and resource limitation. Complete data for these 184 study participants was, therefore, analyzed.

The socio-demographic characteristic of the study subjects is shown in Table 3.

Forty – seven percent of the study participants were between the ages of 20 -29 years. The minimum age of the women was 12 years. Sixty-five percent of the women came from Jimma and the surrounding towns, while the rest (35%) had their residence in rural areas. A quarter of the women (25%) had no education, 9% were literate and one-third (31.5%) had elementary education. A great majority of the women (83.7%) were married while the rest were unmarried (11%) or divorced or widowed (5%). Seventy percent of the women had a monthly income of less than 300 Birr, and 31% had a monthly income of 300 birr or more.

Table 3. Comparison of *C. trachomatis* IgG ELISA results by selected socio-demographic factors, Jimma, South- West Ethiopia.

Factors	IgG ELISA Results			
	Positive	Negative	Borderline	Total
	No. (%)	No. (%)	No. (%)	No. (%)
Age				
10-19	14 (7.6)	9 (4.9)	5 (2.7)	28 (15.2)
20-29	40 (21.7)	34 (18.5)	13 (7.1)	87 (47.3)

30-39	23 (12.5)	21 (11.4)	9 (4.9)	53 (28.8)
40-49	5 (2.7)	6 (3.3)	1 (0.5)	12 (6.5)
50-59	1 (0.5)	1 (0.5)	1 (0.5)	3 (1.6)
$60\text{-}69^{+}$	1 (0.5)	-	-	1 (0.5)
Residence				
Urban	54 (29.3)	46 (25)	20 (10.9)	120 (65.2)
Rural	30 (16.3)	25 (13.6)	9 (4.9)	64 (34.8)
Education				
Illiterate	22 (12.0)	21 (11.4)	2 (1.1)	45 (24.5)
Read & Write	9 (4.9)	4 (2.2)	3 (1.6)	16 (8.7)
Elementary	26 (14.1)	21 (11.4)	12 (6.5)	58 (31.5)
Junior High	12 (6.5)	10 (5.4)	7 (3.8)	29 (15.8)
Senior High	14 (7.6)	12 (6.5)	7 (3.8)	33 (17.9)
12^{+}	1 (0.5)	2 (1.1)	-	3 (1.6)
Marital Status				
Single	9 (4.9)	5 (2.7)	7 (3.8)	21 (11.4)
Married	70 (38)	63 (34.3)	21 (11.4)	154 (83.7)
Divorced	5 (2.7)	3 (1.6)	1 (0.5)	9 (4.9)
Income (Birr/month)				
‹100	29 (15.8)	23 (12.5)	4 (2.2)	56 (30.4)
200-299	25 (13.6)	30 (16.3)	16 (8.7)	71 (38.6)
300^{+}	30 (16.3)	18 (9.8)	9 (4.9)	57 (31.0)

The reproductive features of women presenting to the gynecology clinic of Jimma University Specialized Hospital are presented in Table 4.

Approximately half of the women (52%) claimed to use one or a combination of contraceptives, while 48% denied use of any type of contraceptive. Oral contraceptive pills and injectables were the most frequently used contraceptives among the women (40% and 35%, respectively). The rate of condom use among the women was only 10%. Thirty-four percent of the women had a history of one or more abortion and 9% gave a

history of premarital pregnancy. The majority of the women interviewed (63%) had only one sexual partner, while 35% had 2 or more lifetime sexual partners. The minimum and mean ages of the women at first sexual intercourse were 12 years and 16 years respectively. Approximately half of the women (49%) had their first sexual intercourse at an early age of 12-16 years while the remaining half had their first sexual debut at the age of 17 years or above.

Table 4. Reproductive features vs. IgG serology of the study participants, Jimma, South-West Ethiopia.

Factor	IgG ELISA Results			
	Positive No. (%)	Negative No. (%)	Borderline No. (%)	Total No. (%)
Use of contraceptives				
Pills	28 (15.2)	33 (17.9)	13 (7.1)	74 (40.2)

Injectables	30 (16.3)	25 (13.6)	10 (5.4)	65 (35.3)
Implant	2 (1.1)	-	-	2 (1.0)
IUCDs	1 (0.5)	1(0.5)	1(0.5)	3 (1.6)
Condom	7 (3.8)	4 (2.2)	7 (3.8)	18 (9.8)
History of abortion				
Yes	32 (17.4)	23 (12.5)	8 (4.3)	63 (34.2)
No	52 (28.3)	48 (26.1)	21(11.4)	121 (65.8)
History of premarital pregnancy				
Yes	5 (2.7)	10 (5.4)	1 (0.5)	16 (8.7)
No	79 (42.9)	61 (36.3)	28 (15.2)	168 (91.3)
Number of lifetime sexual partners				
None	2 (1.1)	1 (0.5)	1 (0.5)	4 (2.2)
One	55 (29.9)	48 (26.1)	13 (7.1)	116 (63.0)
2-3	24 (13.0))	21 (11.4)	16 (8.7)	61 (33.2)
>3	3 (1.6)	1 (0.5)	-	3 (1.6)
Age at first sexual intercourse				
No	2 (1.1)	1 (1.1)	1 (0.5)	4 (2.0)
12-16yrs	43 (23.4)	36 (19.6)	11 (6.0)	90 (49.0)
>16yrs	39 (21.2)	34 (18.5)	17 (9.3)	90 (49.0)

* A patient may use one or a combination of contraceptives. It may be therefore, misleading to calculate total numbers or percentages.

Clinical presentations of the study subjects are shown in Table 5.

Overall, in 19% of the women, physical examination revealed no symptoms of reproductive tract infection (RTI), sexually transmitted infection (STI), urinary tract infection (UTI) or other gynecologic abnormalities. Evidence of chlamydial infection was found in half of the women who were asymptomatic. The majority of the women (81%) were found to have any or multiple symptoms on clinical evaluation.The most frequent complaints of the presenting patients were lower abdominal pain/tenderness (39%) and

vaginal discharge (32%). Abnormal vaginal bleeding was found in 18.5% of the women. Only 14 patients (7.6%) gave a history of previous episodes of PID or chronic pelvic pain. Symptoms of UTI (dysuria, urgency/frequency, incontinence) were documented in a minority of cases. Further, 6% of these women complained of vaginal/vulvar itching and 4.3% had amenorrhea.

Table 5. Gynecological conditions (symptoms) of study participants, Jimma, South-West Ethiopia.

Clinical findings/ Symptoms	Number**	%**
History of PID	14	7.6
Lower abdominal pain/tenderness	72	39.0

Adenexal tenderness	23	12.5
Vaginal discharge	59	32.0
Vaginal bleeding	34	18.5
Cervical excitation	11	6.0
Mass	13	7.0
Pyrexia	12	6.5
Urinary symptoms		
Dysuria	8	4.3
Frequency/urgency	10	5.4
Incontinence	6	3.3
Hematuria	1	-
Other symptoms		
Amenorrhea	8	4.3
Dysmenorrhea	4	2.3
Post-coital bleeding	1	-
Dyspareunia	1	-
Vaginal/vulval itching	11	6
Back pain	4	2.3
Menstrual irregularity	2	1
Failure to conceive	3	1.6

** A patient may present with more than one symptom, therefore it may be misleading to sum up the number of patients with a particular type of symptom and calculate percentages.

Table 6 shows results of *C. trachomatis* IgG/IgM ELISA on sera collected from the study subjects.

Examination of sera collected from the participating women by an ELISA technique for antibodies specific for *C. trachomatis* revealed an overall seropositivity of 45.6% for IgG and 5.4% for IgM antibodies. Only 7 women (3.8%) had positive reactivity to both IgG and IgM antibodies. Borderline IgG and IgM reactivity (defined as calculated antibody

index between 0.9 and 1.1 units) was found in 16% and 3% of the sera, respectively (Table 6).

Table 6. Seroprevalence of genital *C. trachomatis* among gynecology clinic attendees, Jimma, South- West Ethiopia.

n=184	Number	%
IgG seroprevalence		
Positive	84	45.6
Borderline positive	29	15.8
Negative	71	38.6
IgM seroprevalence		
Positive	10	5.4
Borderline positive	6	3.3
Negative	168	91.3

Analysis of socio-demographic data vis-à-vis ELISA serology (Table 3) showed higher prevalence of IgG antibodies in women aged between 20 to 29 years (21.7%) and 30 to 39 years (12.5%) although the difference was not significant as compared to the other age groups ($p>0.05$). The difference in seropositivity to IgG antibodies between urban and rural residents was not significant either (29.3% versus 16.3%, respectively; $p>0.05$).

Prevalence of IgG antibodies to *C. trachomatis* was higher among women who had no education (12%) and those with elementary education (14.1%), although the difference was not significant ($p>0.05$). The rate of positivity was significantly higher in married women (38%) than in single (4.9%) or divorced (2.7%) women. Comparatively lower prevalence of IgG antibodies (13.6%) was found in women with a monthly income of 200 – 299 birr per month than the other two income categories.

As shown in Table 4, 17.4% of all women with a history of abortion had positive IgG serology and only 2.7% of all women who had a history of premarital pregnancy were positive for IgG antibodies. Thirteen percent of all women who had 2-3 sexual partners showed positive IgG serology and 23% of all women who had their first sexual encounter at an early age of 12 – 16 years were positive for IgG antibodies.

Three percent of all women with a history of PID had positive reactivity to *C. trachomatis* IgG antibody. Positive serology to *C. trachomatis* IgG antibodies was identified in 14.1% and 16.8% of all women with vaginal discharge and lower abdominal pain, respectively. Four percent of all women with cervical motion tenderness were positive for IgG antibodies. Serology was positive in 8.2% of women with vaginal bleeding. Six percent of all women with symptoms of UTI (dysuria, frequency/urgency) were found to have positive IgG serology. Four percent of the women with vaginal/vulvar itching were also positive for IgG antibodies

Table 7. Comparison of IgG ELISA results by symptoms of gynecology clinic attendants, Jimma, South-West Ethiopia.

Symptoms**	IgG ELISA Results		
	Positive No. (%)	Negative No. (%)	Borderline No. (%)
History of PID	6 (3.3)	5 (2.7)	3 (1.6)

Lower abdominal pain/tenderness	31(16.8)	27 (14.7)	14 (7.6)
Vaginal discharge	26 (14.1)	22 (12.0)	11 (6.0)
Vaginal bleeding	15 (8.2)	15 (8.2)	4 (2.3)
Cervical excitation	7 (3.8)	3 (1.6)	1(0.5)
Mass	7 (3.8)	4 (2.3)	2 (1.1)
Pyrexia	6 (3.3)	4 (2.3)	2 (1.1)
Urinary symptoms			
Dysuria	6 (3.3)	1 (0.5)	1 (0.5)
Frequency/urgency	5 (2.7)	3 (1.6)	2 (1.1)
Incontinence	2 (1.1)	3 (1.6)	1(0.5)
Hematuria	-	1 (0.5)	-
Other symptoms			
Amenorrhea	5 (2.7)	2 (1.1)	1(0.5)
Dysmenorrhea	3 (1.1)	-	1 (0.5)
Post-coital bleeding	-	1 (0.5)	-
Dyspareunia	1 (0.5)	-	-
Vaginal/vulvar itching	7 (3.8)	2 (1.1)	2 (1.1)
Back pain	3 (75.0)	-	1(0.5)
Menstrual irregularity	2 (1.1)	-	-
Failure to conceive	2 (1.1)	-	1(0.5)

** A patient may present with more than one symptom, therefore it may be misleading to sum up the number of patients with a particular type of symptom and calculate percentages.

6. Discussion

The main features of *C. trachomatis* infection are threefold: infection is often asymptomatic, sequelae may be severe and if untreated, infection can persist for more than a year (Brunham, 1990). The most serious sequelae of infection occurs in women where infection with *C. trachomatis* may lead to PID, ectopic pregnancy and infertility. These sequelae may have important lifetime consequences and are extremely costly to treat (World Bank, 1993).

The role of serology in the diagnosis of *C. trachomatis* infection continues to be a contentious issue (Robinson, 1991). The accuracy of interpretation is believed to be enhanced by considering the IgG/IgM serostatus within the clinical context (Moss and Darougar, 1993). When species-specific Chlamydia serology was combined with more modern antigen detection systems, a marked increase in the sensitivity of diagnosis was achieved. This combined approach facilitates the identification and treatment of more cases of genital chlamydial disease in populations which minimizes the very serious risk of female patients being subjected to multiple re-exposures to the pathogen from both exogenous and endogenous sources (Ward, 1999).

Chlamydial infection has been consistently high among adolescents; in some studies, up to 30 to 40 percent of sexually active adolescent females studied have been infected (Tommey *et al*, 1990).

The majority of the women in this study were adolescents and young adults aged between 20 – 29 years (47%) and 30 – 39 years (29%). These were sexually active females most at risk of chlamydial infection. Sixty-five percent of the women were from urban areas and a great majority (84%) were married. The high seroprevalence of *C. trachomatis* among women aged between 20-29years (21.7%) and 30-39 years (12.5%) indicated that age is the socio-demographic factor most strongly associated with chlamydial infection (CDC, 1993).

Although marriage is believed to provide stable closed sexual relationship between partners and a way of decreasing the acquisition of sexually transmitted infections, 38% of married women in this study were found to be positive for Chlamydia antibodies. The majority of chlamydial infection in women is asymptomatic and persistent and these women might have acquired the infection before they were married. Further, the low rate of condom use among the study participants illustrates the tendency to rely on female – controlled contraceptives with establishment of long term relationships among married couples. Also women who rely on oral contraceptives for protecting against pregnancy are less likely to use condoms compared to other women (IOM, 1997).

The finding in this study of high prevalence of chlamydial antibodies in women with little or no education may indicate lack of awareness among these women of STI/chamaydial transmission. In a study of pregnant women in Japan, Kusano *et al* (2000) revealed that short duration of education, among other factors was significantly associated with seropositivity.

Poverty and other socioeconomic factors also contribute to the risk of exposure to Chlamydial infections and other STDs (IOM, 1997). Low socioeconomic groups have limited or access to health services and do not afford to pay for their treatment. The finding in this study that a combined 29.4% of the study subjects with a monthly income of 100 – 300 birr had positive IgG serology illustrates the role of poverty in STD transmission.

The finding in this study of serological evidence of chlamydial infection in 46% of cases is in agreement with the 54% reported by Duncan *et al* (1995) among antenatal care attendees in Addis Ababa. The prevalence in this study was higher than the 21% reported by Blatz *et al* (2001) in women presenting to the obstetric and gynecology clinic of Gondar Hospital, who previously were found to be positive for chlamydial antigen in cervical swab samples.

In the present study, IgM antibody to *C. trachomatis* was detected only in 5.4% of the cases. This figure is much lower than the 13.9% reported by Blatz *et al* (2001) but corresponds to the finding by Moss *et al* (1993) in which only 2.6% of the women in the study were found to have positive IgM serology. This low prevalence of IgM antibodies may be due to the inclusion of larger numbers of cases with chronic infections.

The risk factors studied in the present work can identify population subgroups at increased risk of genital *C. trachomatis* infections. In addition to socio-demographic factors, behavioral/reproductive risk factors were also assessed in this study.

Women with a history of gynecological events, such as IUD insertion or induced abortion are more likely to be infected with Chlamydia (Hillis *et al*., 1993). The finding in this study that 17.4% of all women with a history of one or more induced abortion had positive chalmydia IgG serology was in agreement with the finding of Jonsson (Jonsson, 1995) in which the seroprevalence of *C. trachomatis* was found to be 24.7% in 19-25 year old Swedish women and compared to women with no history of therapeutic abortion, those ever having a therapeutic abortion had an unadjusted 3.15 increased risk of seropositivity, which was reduced to 2.40 after adjusting for other risk factors. Sorenssen (1994) showed a higher cumulative risk of developing early and/or late PID in women with *C. trachomatis* infection at the time of first trimester pregnancy. The insertion of instruments appears to be a key factor in the rapid spread of chlamydial infection.

Aspects of sexual behavior such as age at first intercourse and number of life time sexual partners are key determinants of STI transmission (Moss, 2001). The finding in this study that 13% of the women with 2-3 life time partners had positive IgG serology and an additional 8.7% had borderline IgG positivity as evidence of chlamydial infection is comparable with the finding of Nessa *et al* (2004) in which a chlamydia prevalence of 43% among Hotel-Based Sex Workers who practiced unsafe sex with multiple clients was observed.

The finding in this study that a sizable proportion of the women used to have multiple sexual partners, low rate of condom use and the fact that most chlamydial infections in women are chronic or persistent is reflected in the high prevalence of chlamydial antibodies among the women who currently are married.

This study showed that 49% of the women had their first sexual intercourse at the age of 12-16 years of whom 23.4% showed positive IgG serology. These findings substantiated the established association between sex at an early age and risk of acquisition of chlamydial infection.

It appears that between 10 and 40% of women infected with *C. trachomatis* develop PID (Stum *et al*., 1984). The finding in this study of 7.6% PID prevalence is lower than that reported by Duncan *et al* (1995) among antenatal care attendees which was 23%. The difference in frequency in the two studies may be due to the difficulty of accurate diagnosis of PID on clinical grounds without the use of invasive techniques like laparoscopy (Rogstad, 2001).

The frequency of vaginal discharge in women in this study (32%) is quite higher than the 4% reported by Duncan *et al* (1995) among antenatal care attendees. The difference could be explained by difference in the study subjects in the two studies. The present finding is comparable to the 25% rate by Svensson *et al* (1981).

In women with chronic abdominal pain, there is evidence of a past infection with *C. trachomatis* (Wolner-Hanssen *et al*., 1983). The finding in this study that 17% of all women with lower abdominal pain had positive chlamydia serology is consistent with this observation. Duncan *et al* (1995) found a lower rate (9%) among antenatal care attendees.

Use of oral contraceptives (OC) may modulate the course of PID and therefore also the rate of sequelae of chlamydial salpingitis (Svensson *et al*., 1984; Wolner-Hanssen *et al*., 1985). Although OC use has been associated with a 50% decrease in PID in reported studies, it is unclear whether OC use prevents ascending infection or protects against symptomatic infection (Wolner-Hanssen *et al*., 1990). There was high rate of OC use and few clinically diagnosed cases of PID in this study.

In summary, the present study showed high prevalence of IgG antibodies and low IgM seropositivity in women presenting to the gynecology clinic of a university hospital. This study also revealed high rate of non-specific symptoms of STI and genital infection among the study subjects despite the low prevalence of IgM antibodies which generally indicate active infection. The assumption that IgG generally represents past infection had been refuted by some studies. In a follow-up study of contacts of cases of genital

chlamydial infection, Moss and Darougar (1999) showed that in nearly half of the contacts with IgG at a level of ≥ 1/16, chlamydial antigen was also detected in their genital tract, indicating that at least in about half of the IgG-positive contacts, the presence of IgG was a true indicator of a current *C. trachomatis* genital infection (CTGI). Also, in approximately two-third of contacts with IgG levels of 1/64 to 1/256, chlamydial antigen was also present in their genital tract. These findings suggest that in patients with symptoms of non-specific genital infections who have not been treated, the presence of IgG may indicate a current CTGI.

7. Conclusion and Recommendations for Future Direction

The findings of the present study have demonstrated high prevalence of antibodies to *Chlamydia trachomatis* and evidence of high rates of behavioral and reproductive risk factors among the study population. The few published studies on chlamydia prevalence in Ethiopia have generally been confined to high risk groups such as women attending family planning clinics and STD clinic attendees and have been based on small numbers. Detected prevalences are therefore relative and can not be extrapolated to the wider population. Until the time when comprehensive antigen screening studies and culture could be undertaken in samples drawn from the general population, chlamydia serology

should be widely applied to selected populations through out the country to enhance our awareness of the prevalence of exposure to *C. trachomatis*. Health care workers must be aware of the high prevalence of chlamydia especially among women and recognize chlamydial illness, arrange for screening and treatment of asymptomatic patients and their partners and counsel sexually active patients about the risk of STIs. Programs designed to reduce the risk of STIs/HIV by means of behavioral changes should also emphasize the high risk of chlamydial infection and its sequelae.

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9. Appendices

ANNEX I

CONSENT FORM – FOR PARTICIPATION AS A VOLUNTEER IN THE RESEARCH UNDERTAKING

(TO BE TRANSLATED IN TO THE PATIENT'S LANGUAGE)

I, the principal investigator of this study, am a post – graduate student from the Faculty of Medicine, Addis Ababa University. I am here to study the magnitude of a

sexually transmitted disease called urogenital *C. trachomatis* infection that is implicated to be one major causes of a disease among women called Pelvic Inflammatory Disease (PID). The disease PID, if undiagnosed and untreated for a long time, may lead to infertility as well as other reproductive complications.

I am requesting you to participate in the study which would require your response to an interview on some related issues, a physical examination by a physician and a laboratory examination. The laboratory examination requires collection of 3 – 4 ml of blood from a vein in your forearm employing standard procedure and sterile disposable syringe and needle. The procedure is a routine medical practice that would pose no ill effects on your health. Confidentiality of your name, address, the information that you provide during the interview and the results of the laboratory investigation would be strictly maintained.

The laboratory findings would be judiciously used in conjunction with the clinical findings to initiate appropriate treatment for your medical problem. The study findings would also be used to design and implement control strategies in the study area in the future.

Upon completion of the study a report would be compiled and presented to the Faculty. The report would focus on the results and anonymity of your identity would be guaranteed.

_____

I, the undersigned, confirm that the objective of the study has been explained to me in the language that I am conversant and that I have given my consent to participate in the study.

Name of patient (or guardian) _____

Signature _____

Date _____

ANNEX II

QUESTIONNAIRE ON DEMOGRAPHIC AND REPRODUCTIVE FEATURES OF STUDY SUBJECTS

Patient Name _____ Address _____

I. ***SOCIODEMOGRAPHIC FACTORS***

• Age (years)

10 – 19 ____

20 – 29 ____

30 – 39 ____

40 – 49 ____

50 – 59 ____

60 – 69 ____

• Residence: Urban ____Rural ____

• Marital status: Single ____ Married ____ Divorced ____

• Education

Unable to read and write ____

Read and write ____

Elementary ____

Junior high ____

Senior high ____

12^{+} _____

• Income: < 100 Birr ____ 100 – 299 Birr ____ 300^{+} ____

II. ***REPRODUCTIVE FACTORS***

• Age at first intercourse ____

• Number of life time partners____

• Frequency of partner change ____

• Use of contraceptives

Pills ____ IUCDs ____ Condom _____ Implant____ Injectables ____

• History of termination of pregnancy: Yes___ No ___

If yes, frequency (number) ____

• History of premarital pregnancy: Yes ___ No ___

ANNEX III

CLINICAL PRESENTATIONS (SIGNS AND SYMPTOMS) OF STUDY SUBJECTS

Patient Name _____ Address _____

- **Previous history of PID ____**
- **Lower abdominal tenderness ____**
- **Adnexal tenderness ____**
- **Cervical excitation ____**
- **Bilateral masses ____**
- **Vaginal discharge ____**
- **Vaginal bleeding ____**
- **Pyrexia ____**

Other signs and symptoms: _____

Signature (clinician): _____

Date: _____

ANNEX IV

ELISA of *C. trachomatis* – SPECIFIC ANTIBODIES ON SERA COLLECTED FROM WOMEN ATTENDING THE OBSTETRIC AND GYNAECOLOGY CLINIC OF JUSH

RESULT RECORD FORMAT

Patient name: _____
Patient number: _____

Antibody index:

IgG: _____

IgM: _____

Signature (principal investigator): _____

Date: _____