Serum IgM to *Chlamydia trachomatis* in pregnancy: its usefulness for screening

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ABSTRACT

Asymptomatic infection with *Chlamydia trachomatis* represents an important health problem. A non-invasive diagnostic test to screen pregnant women is needed that is cost effective and can be used widely, especially in developing countries. In this setting, quantitation of antichlamydial IgM antibodies may offer an additional strategy for the control of *C. trachomatis* infection. The aim of this prospective study is to evaluate the quantitation of serum antichlamydial IgM antibodies, based on absorbance (*A*) values, in pregnant women for the prediction of *C. trachomatis* infection. Serum samples from a cohort of 148 pregnant women (first to third trimesters; age range: 18-35 years) presenting to the antenatal department at Safdarjang Hospital were tested for IgM antibodies specific to *C. trachomatis* by an enzyme-linked immunosorbent assay (ELISA) kit (Novum Diagnostics, Germany). Co-infection with other STD pathogens was ruled out. In this cohort, 85 (57.4%) pregnant women were found to be positive for IgM antibodies to *C. trachomatis*. Based on the cut-off value of the ELISA test (calculated as 0.558), pregnant women with an *A* value between 0.558 and 0.999 and those with a value >1.000 were categorised as low positive (LP, $n=41$) and high positive (HP, $n=44$), respectively. The differences in mean *A* values for the LP versus negative groups (0.7504 versus 0.2249, $P<0.05$) and the HP versus negative groups (1.5353 versus 0.2249, $P<0.05$) were statistically significant. Maximum seroprevalence (44.4%, $P<0.05$) was found among those in the HP group in the first trimester of pregnancy. Multigravidae (34.4%, $P<0.5$) and multiparous (34.9%, $P<0.5$) pregnant women in the HP group were at an increased risk of chlamydial infection. As overall results indicated that pregnant women in the HP group were at higher risk, we stress the importance of large-scale screening of pregnant women for *C. trachomatis* infection, particularly in developing countries where sophisticated techniques for collection/diagnosis are as yet unavailable.

KEY WORDS: Chlamydia trachomatis. IgM. Pregnancy.

Introduction

Chlamydia trachomatis infection during pregnancy may cause a variety of perinatal complications and several studies report that up to 35% of pregnant women harbour *C. trachomatis* in the endocervix.[1-3] In the largest prospective study of chlamydial cervical infection during pregnancy, which controlled for the presence of *Mycoplasma hominis* and other possible risk factors, *C. trachomatis* showed significant association with preterm birth and intra-uterine growth retardation.[4] Pregnant women with *C. trachomatis* infection are 10-fold more likely to suffer an adverse outcome (stillbirth and neonatal death),[5] and gestation periods are shown to be significantly shorter in pregnant women infected with *C. trachomatis*.[6]

It is suggested that diagnosis and treatment of pregnant women (and their sexual partners) infected with *C. trachomatis* will prevent these adverse outcomes, as well as post-partum and perinatal disease.[3] In this regard, the Centers for Disease Control (CDC) recommends screening and treatment of chlamydial infection during pregnancy to prevent the adverse effects during the pregnancy, the transmission of infection among infants, and the maternal post-natal complications.[7]

As asymptomatic infection represents an important reservoir for *Chlamydia* spp.,[8] treating patients who have symptoms (and their partners) may not be effective in reducing the prevalence of *C. trachomatis* infection.[9] Pregnant women with significant immunoglobulin M (IgM) antibody titres against *C. trachomatis* are at higher risk of preterm delivery and premature rupture of the membranes.[10,11] Therefore, active screening using quantitation of antichlamydial IgM antibodies may offer an additional strategy for the control of *C. trachomatis* infection in pregnant women, and may obviate the need for invasive procedures.

The aim of this study is to evaluate the usefulness in pregnant women of quantitating antichlamydial IgM antibodies, based on absorbance (*A*) values, to predict *C. trachomatis* infection.

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Material and methods

Patients

Serum samples from a cohort of 148 pregnant women (first to third trimester, age range: 18-35 years) presenting to the antenatal department at Safdarjang Hospital were tested for IgM antibodies specific to *C. trachomatis*. Prior consent was obtained from women enrolled for the study. All women included in the study were married and belonged to a range

of socio-economic groups. Each was interviewed using a standardised questionnaire for clinical and obstetric history (history of previous pregnancy, gravidity, parity and symptoms of lower genital tract infection, if any). Those who had received either systemic or vaginal antibiotic therapy in the preceeding two weeks were excluded from the study. Patients with diabetes, hypertension, prior history suggestive of genito-urinary infections or VDRL positivity were also excluded. All the women underwent a pelvic examination and were evaluated for the presence of mucopurulent discharge and friability of the cervix. Endocervical swabs were collected for microscopic examination for various bacterial and fungal infections.

Antibody detection

Detection of specific IgM antibodies to *C. trachomatis* was performed by enzyme-linked immunosorbent assay (ELISA) on all sera collected using a *C. trachomatis* IgM ELISA kit (Novum Diagnostics, Germany), following the manufacturer's guidelines. The kit utilises a solid-phase enzyme immunoassay for the qualitative and semi-quantitative determination of antichlamydial IgM antibody in human serum.

In brief, patients' sera (10 μL) were diluted with IgM sample diluent, and, together with ready-to-use controls, were pipetted into wells and incubated at 37°C so that *C. trachomatis*-specific IgM antibodies present would bind to the immobilised antigen in the well. Plates were then washed to remove unbound sample and control material, horseradish peroxidase-conjugated antihuman IgM antibody was added to each well, and the plate incubated again. Subsequently, the plate was washed, TMB substrate was added to each well, and the plate incubated. The reaction was stopped with sulphuric acid and the plate was read in an ELISA reader (Titertek, Finland) at 450 nm.

STD pathogens

C. trachomatis-positive pregnant women were investigated to rule out presumptive endocervical infections with *Trichomonas vaginalis* (wet mount), *Neisseria gonorrhoeae* (Gram's stain), *Candida* spp. (Gram's stain) and bacterial vaginosis (Gram's stain). An ELISA test was used to detect serum antibodies to human immunodeficiency virus (HIV) using an Innotest assay kit (Innogenetics, Belgium), as per the manufacturer's instructions. Results of routine VDRL tests were recorded.

Statistical analysis

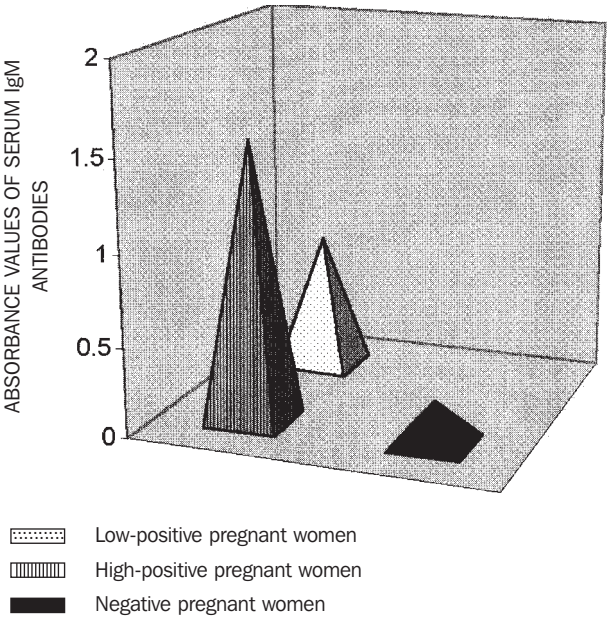
For a statistical comparison of *C. trachomatis*-positive pregnant women with *C. trachomatis*-negative pregnant women, data on obstetric history/clinical characteristics and serum antichlamydial IgM levels were analysed using the χ^2 test. Means were compared using Student's *t*-test.[12]

Results

IgM antibodies to chlamydial infection

Of the 148 pregnant women studied, 85 (57.4%) were positive for IgM antibodies to *C. trachomatis*. Mean *A* value in seropositive women was statistically significant ($P<0.05$) when compared with seronegative women (1.0817 versus 0.2249). Cut-off value was calculated as 0.558. Those patients

Fig. 1. Histogram showing quantitation of *C. trachomatis* IgM antibodies based on *A* values in sera of pregnant women.



in the *A* ranges 0.558-0.999 and >1.000 were categorised as low positive (LP, $n=41$) and high positive (HP, $n=44$), respectively. The difference in mean *A* values for both LP versus negative (0.7504 versus 0.2249) and HP versus negative (1.5353 versus 0.2249) pregnant women was found to be statistically significant ($P<0.05$, Figure 1).

STD pathogens in seropositive pregnant women

In the LP group, 25 women were tested for the presence of HIV antibodies but none proved to be positive. In the HP group ($n=44$) only one woman (2.2%) had co-infection with HIV (Figure 2). All women in both LP and HP groups tested negative for syphilis (Figure 2). Examination for other endocervical infections was performed in 23 in the LP group and all patients in the HP group. Co-infection with *Candida* spp. was found in one (4.3%) LP woman and in two (4.5%) HP women. Out of the two groups, only one (4.3%) LP woman had bacterial vaginosis and one (2.2%) HP woman showed co-infection with *T. vaginalis*. All women in both groups (LP and HP) were negative for *N. gonorrhoeae* infection (Figure 2).

Obstetric history/clinical characteristics

Prevalence of *C. trachomatis* infection in relation to age, trimester, gravidity and parity is shown in Table 1. Mean age in the LP group was low (22.7 years) and statistically significant ($P<0.05$) in comparison to that in the seronegative pregnant women (23.7 years), while the HP group had a mean age of 24.1 years, which was not significant in relation to the chlamydia-negative group (Table 1). LP women in the second trimester were found to have the highest prevalence of serum antichlamydial IgM antibodies (29.3%, $P<0.5$). In comparison, the HP women showed the highest prevalence in the first trimester (44.4%, $P<0.05$) (Table 1).

Table 1. Comparison of obstetric history in women infected with *Chlamydia trachomatis*

Obstetric history	Seropositive women (*n*=85)		Seronegative women (*n*=63)
	LP (*n*=41) No(%)	HP (*n*=44) No(%)	No(%)
Mean age (years)	22.7 *	24.1^{NS}	23.7
Trimester			
First	5 (27.8)	8 (44.4)†	5 (27.8)
Second	12 (29.3)**	16 (39.0)	13 (31.7)
Third	24 (26.9)	20 (22.5)	45 (50.6)
Gravidity			
Primigravidae (*n*=52)	16 (30.8)***	11 (21.1)	25 (48.1)
Multigravidae (*n*=96)	25 (26.0)	33 (34.4)††	38 (39.6)
Parity			
Nulliparous (*n*=65)	21 (32.3)^{NS}	15 (23.1)	29 (44.6)
Multiparous (*n*=83)	20 (24.1)	29 (34.9)†††	34 (41.0)

P< *0.05, **0.5 or ***0.01, compared with seronegative pregnant women.

P< † 0.05, †† 0.5 or ††† 0.5, compared with seronegative pregnant women.

NS: not significant.

Among those in the LP group, the primigravidae were found to be at higher risk of chlamydial infection (30.8%, *P*<0.01), while prevalence of *C. trachomatis* infection during pregnancy was highest among the multigravidae in the HP group (34.4%, *P*< 0.5) (Table 1). Nulliparous women in the LP group showed a higher seroprevalence (32.3%) but this was not significant when compared to the chlamydia-negative pregnant women. However, multiparous women in the HP group were at increased risk of chlamydial infection. (34.9%, *P*<0.5) (Table 1).

Discussion

Infection with *C. trachomatis* is a major health problem, particularly in developing countries. In India, the prevalence of endocervical *C. trachomatis* infection was reported to vary from 3.3% to 23% in various studies.[13-17] Screening for chlamydial infection is crucial in order to prevent adverse pregnancy outcome, as many pregnant women are asymptomatic[18] and the infection may persist for extended periods of time in diagnosed and untreated cases.[19] With the availability of a non-invasive *C. trachomatis*-specific screening test, a large population can be screened.

Currently, there is an unwarranted sense of futility about the prospects for diagnosis of chlamydial infection in women due to the expense and limited availability of facilities for the isolation of *C. trachomatis*.[20] Although non-culture tests are available that do not require strict handling of specimens, are easier to perform and less expensive than culture tests, they do have limitations.

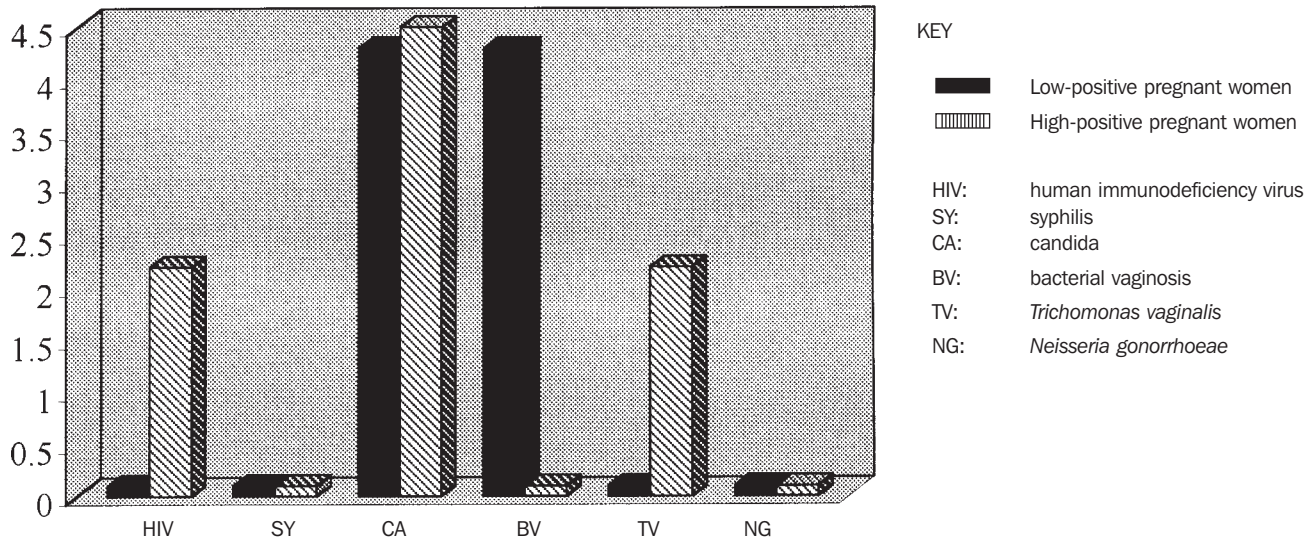
The significance of IgM antichlamydial antibodies in pregnancy is not clearly understood. Berman *et al*.[21] considered them to indicate a recent or invasive infection. Among women with cervical *C. trachomatis* infection, Harrison *et al*.[22] showed that those with serum IgM-positive infection constituted the high-risk group with regard to low birthweight and premature rupture of membranes. In a study from Lucknow, India, Jain *et al*.[23] reported a 35.9% prevalence of antichlamydial IgM antibodies in asymptomatic women in the third trimester of pregnancy. Furthermore, in a study of a cohort of 140 women with uncomplicated urogenital *C. trachomatis* infection who attended a clinic for sexually transmitted diseases in Rotterdam, IgG and/or IgM antibodies were reported in 83.3% and 48.0% of culture-positive and -negative cases, respectively.[24]

The present study revealed an overall high prevalence (57.4%) of *C. trachomatis* IgM antibodies in a cohort of 148 asymptomatic pregnant women. This could be due to the fact that IgM assays are extremely variable in their ability to detect IgM (from 1-2 months to 18-24 months) post infection. Earlier studies indicate that IgM antibodies to *C. trachomatis* may persist for years and that antibody titre indicates the severity of disease.[25] There is evidence that the presence of high-titre IgM (i.e. >1 in 128) and/or IgG (i.e. >1 in 2048) antibodies to *C. trachomatis* strongly suggests acute infection.[25] However, in the present study, we were unable to determine antibody titres due to financial constraints.

We arbitrarily categorised seropositive women in the study by quantitation of *A* values for IgM antichlamydial antibodies into LP (*A*: 0.558-0.999) and HP (*A*>1.000) cases. The values for serum antichlamydial IgM antibodies in these two groups were statistically significant in comparison with seronegative women.

As the diagnostic value of a single high-titre antichlamydial IgG antibody, or any other stable high-titre antibody, is reported to be uncertain,[26] the detection of serum IgM antibodies to *C. trachomatis* during pregnancy and their quantitation based on *A* values will permit more laboratories to diagnose perinatal chlamydial infection and may be useful in screening for the infection. Furthermore, quantitation based on *A* values for antichlamydial IgM antibodies detected by ELISA may be more suitable for widespread screening programmes, particularly in an Indian setting in which screening of pregnant women for *C. trachomatis* is not done on a routine basis. Subsequently, treatment may be given to those pregnant women with HP

Fig. 2. Histogram showing co-infection of *C. trachomatis*-positive pregnant women with other endocervical STD pathogens.



values for *C. trachomatis* IgM antibodies after further confirmation by culture or DFA test.

In the present study, one woman in the HP group was found to be positive for HIV, and *Candida* spp. and *T. vaginalis* infections were more often associated with those in the HP group. Although increased incidence of certain vaginal microorganisms has been reported during pregnancy,[27,28] diagnosis in our study was achieved on endocervical samples, and this could have led to the low prevalence. Infection with STDs is a cause for concern because of the increased risk of acquiring HIV.[29] In a prospective study by Hardy *et al*.,[30] increases in preterm birth and low birthweight were found only in the presence of co-infection with *T. vaginalis*. Possible additive or synergistic effects of cervical/vaginal microorganisms or bacterial vaginosis have been demonstrated by others.[31]

First-trimester multigravidae and multiparous pregnant women in the HP group appeared to be at the highest risk of chlamydial infection, as has been reported in various other studies;[6,32] however, this increased prevalence remains unexplained. CDC recommends screening of women for *C. trachomatis* infection in the first trimester of pregnancy to prevent transmission of the infection and reduce adverse outcomes during pregnancy.[7] In the LP group, second-trimester primigravidae and nulliparous pregnant women showed the highest prevalence of antichlamydial IgM antibodies. As overall results are indicative of an early infection, those pregnant women in the HP group would appear to be at an increased risk.

The implications of these observations are important and cannot be ignored. Although there are a few conflicting reports from the West regarding the diagnostic efficacy of IgM antichlamydial antibodies in pregnant women and an association with adverse obstetric outcome, we recommend them for an initial widespread screening of pregnant women because of cost-effectiveness, especially in developing countries where sophisticated techniques are as yet unavailable. Subsequently, these women may be given treatment to prevent transmission of *C. trachomatis* to infants during birth. Therefore, larger population-based studies should be done in pregnancy to validate the efficacy of such testing for the prediction of *C. trachomatis* infection, as pregnant women with high *A* values for *C. trachomatis* IgM antibodies appeared to be at higher risk in our hospital-based study. □

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