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INCIDENCE OF CHLAMYDIAL INFECTION IN WOMEN

S.Savitha[1*], S.Madhavan[2], R.Vinoth Raja[1]

[1]Department of Microbiology, Sri Sankara Arts and Science College, Asur, Kumbakonam.

[2]Department of Microbiology,Sengamala Thayaar Educational Trust Women's college,Mannargudi.

ABSTRACT

With the emergence of AIDS in the 1980s sexually transmitted disease (STDs) received increased attention. The most common agent is *Chlamydia trachomatis* (CT), *Nesseria gonorrhea* and etc. CT commonly causes non-gonococcal urethritis, epididymitis, cervicitis, salpaingitis and etc. The study was carried out from the women of 18 to 40 years of age. They were more prone to **CT** infection. In the study period 200 women patients were investigated. CT antigen was detected using Trachomatis Lps antigen test and intracellular inclusion was detected by Giemsa staining method.

Out of 200 people 21 women were found positive. The prevalence of CT infection in women was 10.5% between the age of 20 and 30 years. Among eighty six non-pregnant women seven positive cases were detected i.e., 18.1%. One hundred and fourteen pregnant patients were evaluated and fourteen were found positive i.e., 12.28%. The Incidence of CT in relation to gestation period was evaluated. There were no positive cases detected up to fifth month of the pregnancy. The maximum percentage of CT infected patients was detected after the sixth month of pregnancy. The rate of infection was higher in pregnant women between the age of 20 and 30 years.

Key words: EB-Elementary body, CT - *Chlamydia trachomatis,* LPs- Lipopolysaccharide, CF-Compliment fixation, RB- Reticulate body

1. INTRODUCTION

Chlamydia trachomatis (CT) is a coccoid bacilli, gram negative, non–motile and intracellular, living in man and animal cells, because it requires host cell Adenosine triphosphate (ATP) for their life cycle. So it is sometimes as "energy parasites"[1]. CT is the most common sexually transmitted pathogens. CT commonly causes non-gonococcal urethritis, epididymitis, cervicitis, salpingitis, inclusion conjunctivitis, infant pneumonia, trachoma, lymphogranuloma venerum[2].

*Correspondence

Lecturer,
Department of Microbiology,
Sri Sankara Arts and Science College,
Arasur, Kumbakonam, TN, India
savithatss@yahoo.com

CT has on "developmental cycle", not a "life cycle". CT is ingested by a mechanism similar to receptor-mediated endocytosis. After attachment, at specific sites on the surface of the cell, the elementary body (EB) enters the cell in an endosome. Once the EB (diameter, 0.25 to 0.35 mm) has entered the cell, it reorganizes into a reticulate particle (initial body RB) which is larger (0.5 to 1 mm) and richer in RNA. CT growing in the intracellular vacuole is called "inclusion".

CT has 18 different serovars. They are A-C, D-K and L1-L3. Of these 18 serovars D-K are associated to urogenital disease. This sexually transmitted pathogen (CT) is the most likely to be found in an obstetric population, with 2 to 20% of pregnant women infected [3].

For the prevalence of CT infections can cause significant morbidity with concomitant social and economic costs. If the untreated CT infected pregnant patient may (1) pass the organism to her child at delivery, (2) develop postpartum endometritis, salpingitis [4], (3) contribute to horizontal spread throughout the community and (4) experience possible adverse obstetric outcomes such as preterm delivery, low birth weight or premature rupture of the membranes[5].

Vertical transmission of CT generally occurs during labour and delivery with a frequency varying from 23 to 70% [6].. Prevalence of neonatal conjunctivitis, 11 to 50 %, and neonatal pneumonia, 3 to 16%, have been reported among infants exposed at birth [7, 8, 9]. Non–pregnant women may experience pelvic inflammatory disease (10- 40 %) [9] and its sequel of infertility and ectopic pregnancy [10].

The prevalence rate related to age was apparently high (19.1%) in women of age group 20-30 years when compared to that in other age groups which did not exceed 12%. This was expected because in this study majority of the women investigated belong to 20-30 years age group [11].

We observed a prevalence rate of 15% for Chlamydia trachomatis in relatively asymptomatic pregnant women. Other studies in India have reported positive rates of 15% in relatively asymptomatic young women, 9. 7% in high risk women commercial sex workers form Central Bombay and 15-60% in young women with infertility or PID and those attending STD clinics [12, 13, 14].

The CT may be provisionally identified by the appearance of the Giemsa and iodine stained smears and confirmed by immunofluorescence using a group antiserum. Subgroup A may now be subdivided for epidemiological purposes by the micro-immunofluorescence test using type-specific antisera [15].

The Chlamydia possess group (genus) – specific, species-specific, and type-specific antigens. Although they are antigenically complex, only a few antigens play a role in diagnosis. The group complement fixation (CF) antigen, shared by all members of the genus, is the lipopolysaccharide (LPS), with a ketodeoxyoctanoic acid as the reactive moiety. It may be analogous to the LPS of certain gram-negative bacteria [16].

A reduction these adverse events in women treated with erythromycin, preliminary data from the vaginal infections and prematurity study group showed no improvement in pregnancy outcome with treatment [17, 18, 19]. Amoxicillin must be taken 3 times daily for a week and it is bacteriostatic drug against Chlamydia whereas the macrolide and tetracycline class drugs have bactericidal activity [20].

This infection is most common disease in the United States. An estimated 3 to 4 million cases occur each year. This *C. trachomatis* is one of the most common and spoiling sexually transmitted disease in different countries. This study was designed to investigate the prevalence of Chlamydia infection among the women, especially pregnant women.

2. MATERIALS AND METHODS

2.1 .Selection of the patient

Women of all age suffer from Chlamydia; every stage of women's life new born to adult women was affected. The study was carried out from the age of 18 to 40 . A special preference or importance was given to the pregnant women and sexually active women of age 20-30 years. Among these age groups of women were more prone to CT infection.

The clinical materials i.e., the specimens required for the present study were obtained from the Primary Health Center (PHC) and a private clinic at Tanjore district.

2.2. Collection of specimen

The specimen of diagnostic importance was the white discharge with characteristics symptoms like burning sensation during urination, itching in the urinary tract, lower abdominal pain and pain during sexual intercourse.

A swab consists of a wood en applicator stick, a round which a small whisp of absorbent wool or cotton was wound to give a small pledget approximately 12mm in length X 2-3 mm in width. The swabs were sterilized in hot air oven by placing with in a test tube. The ends of the swab sticks should project beyond the mouth of the tube to facilitate handling.

A sterile swab dipped in sterile saline was usually preferred for vaginal swab collection. Using a sim's speculum high vaginal swabs was taken. After taking the swabs they were immediately placed back into the test tubes and carried to the laboratory for further examination. Two swabs were collected. One is vaginal swab for smear examination and another one is endocervical swab for rapid test [21] Plate-1.

2.3. Preparation of giemsa stain

0.3gm of Giemsa powder was weighed and it was mixed with 25ml of Glycerin and 25ml of Acetone free methanol. It was a stock solution. Before using it had to be diluted by adding 1ml (of stain) to 9 ml of distilled water [22].

2.4. Staining procedure

The smear was air dried, fixed with absolute methanol for at least 5 min, and dried again. It was then covered with the diluted Giemsa Stain (freshly prepared the same day) for an hour. The slide was rinsed rapidly in 95 % ethyl alcohol to remove excess dye and to enhance differentiation and was then dried and examined microscopically.

EBs stained reddish purple. It indicated the presences of CT. The initial bodies are more basophilic, staining bluish, as do most bacteria [22] Plate- 2 and 3.

2.5. *Chlamydia trachomatis - lps antigen test*

[Rapid test based on immuno chromatography].

SPECIMEN

For the best performance of any *Chlamydia trachomatis* test, proper sample collection technique is extremely important.

2.5.1. Collection technique for endocervical specimen

Sterile swab were used for the collection of specimen. Wearing gloves inserted sterile swab into the endocervical canal until most of the tip is no longer visible. Rotated the swab for 15-30 seconds withdraw it without touching any vaginal

surface. Testing was conducted immediately.

2.5.2. Extraction of sample

The extraction tube was filled with 18 drops (0.9m) of extraction solution. Then the swab was immersed in the extraction tube and swirled the swab vigorously for 10 seconds to ensure adequate mixing of swab specimen with the extraction. Then placed the extraction tube containing the swab in the test tube rack and left for ten to fifteen minutes at room temperature [extraction time]. And swirled the swab for a few seconds (2 to 3 times) during the incubation time while pressing it against the extraction tube wall. At the end of the extraction time (10-15 min) thoroughly removed the liquid from the swab by pinching the line of the extraction tube between thumb and finger and gently removed the swab from the tube.
Then the swab was discarded as per the guidelines for handling infectious agents. The swab extract was kept at room temperature for up to 30 minutes.

2.5.3. Test procedure

After removing Chlamydia test unit from its protective wrapper. It was placed on a level surface. Capped the extraction tube with the filter dropper and applied seven drops of extract to the sample window [<<A>>] of the test unit. Allowed the reaction to proceed for 10-12 minutes after addition of the extract suspension to the sample window. The test results were remained stable for more than an hour after addition of extract to the test unit. Only one pink coloured line was appeared in the control window ("c" control band); it showed the absence of chlamydial antigen. Two coloured lines were appeared in both the 'c' control band and 'b' test band; It indicated the presence of chlamydial antigen. [23, 24, 25] Plate-4 and 5.

3. RESULTS AND DISCUSSION

Cervical and vaginal smear from two hundred women were examined 21 were positive (10.5%). The majority of whom 90.5% were below the age of 30 years .None of them recorded with symptoms of genital tract infection such as pain in the lower abdomen, white discharges pruitius, dysmenorrhea, and dyspareunia. However, 48% of the patients had given history of white discharge.

Positive smear of CT were maximum in age groups 20 – 25 and 25 – 30 years shown a percentage of 20 and 16.67 respectively. Whereas the incidence was found to be low in women belonging to the age group 18 – 20, 30-35 and 35 – 40 years were shown 8.06 , 2. 94 and 3.57 percentages respectively. (Table-1)

Out of eighty six non- pregnant patients were observed only seven were CT infected patients. In the age groups 20 -25 and 25-30 years positive cases were 16.67%; whereas one patient each was prone to CT infection in the age groups 18 –20 (5.26%), 30- 35 (5.26%) and 35-40 (4.17%) years respectively.(Table-2)

A total of hundred and fourteen pregnant patients were examined. Out of this 14 patients were found CT infected. A maximum of 17.85% were found CT infection in the age group of 20 – 25 years ,but in the same time there was no positive result found in the age group 35-40 years.(Table-3)

Table-1

Incident of *C. trachomatis* in Relation to Age [female] were recorded during the study period

Age	No. of patients observed	No. of positive cases	Percentage
18-20	62	5	08.06
20-25	40	8	20.00
25-30	36	6	16.67
30-35	34	1	02.94
35-40	28	1	03.57

Table-2

Incident of *C.trachomatis* in Relation to Age [women – non pregnant] were recorded during the study period

Age	No. of patients observed	No. of positive cases	Percentage
18-20	19	1	08.06
20-25	12	2	20.00
25-30	12	2	16.67
30-35	19	1	02.94
35-40	24	1	03.57

Table-3

Incident of *C.trachomatis* in relation to Age [women – pregnant] were recorded during the study period

Age	No. of patients observed	No. of positive cases	Percentage
18-20	43	4	09.30
20-25	28	5	17.85
25-30	24	4	16.66
30-35	15	1	06.67
35-40	4	nil	0

Table-4

Incident of *C.trachomatis* in relation to Gestation Period [WOMEN – PREGNANT] were recorded during the study period

Month	No. of patients observed	No. of positive cases	Percentage
3-4	8	Nil	0
4-5	10	Nil	0
5-6	13	1	07.69
6-7	20	2	10.00
7-8	23	4	17.39
8-9	21	4	19.04
9-10	19	3	15.78

On comparing these two categories pregnant and non- pregnant patients' maximum percentage of CT positive cases were found in the age groups 20-25 and 25-30 years. It was also found pregnant women patients were more prone to CT infection. (Table-2&3)
The incidence of C.trachomatis in relation to gestation period was evaluated. There was no positive case found up to fifth month of the pregnancy. The maximum percentage of CT infected patients was found after this sixth month of pregnancy. (Table-4)

The earliest reports of its role in causing PID come from Scandinavia when six of 20 laproscopically confirmed cases of acute–salpingitis were reported to have change bio isolated from their tubes, and 19 of 53 from their cervix, while only one of 12 control patients had Chlamydia in cervical samples [26].

Nugent and Hillier in USA reported 14% of prevalence in pregnant. The difference of the rate of prevalence between our study and the other workers may be due to the difference in cultures religious and social behaviors [27]. High prevalence of disease (4.2%) in the persons with 20 years and less than 20 years of marriage life is probably due to sexual activity or more pregnancies or having oral contraceptives, all of which are risk factors of CT infections.

From the result it is concluded that, in case of presence of symptoms, the prevalence rate of CT infection is more and the chance ratio is higher 12.5% against 1.9%. Samji et al. reported 11.8% of the CT infection in the pregnant women with the symptoms of urinary tract infection [28].

Considering the results the researches and the awareness of the presence of CT infections in pregnant women, the midwife's and obstetricians, can identify this infection in pregnant women referring to them and can help in treatment and controlling the complications arised due to this infection.

It was found that the CT infection in women was maximum between the age of twenty and thirty years.

Plate-1 Vaginal Swab

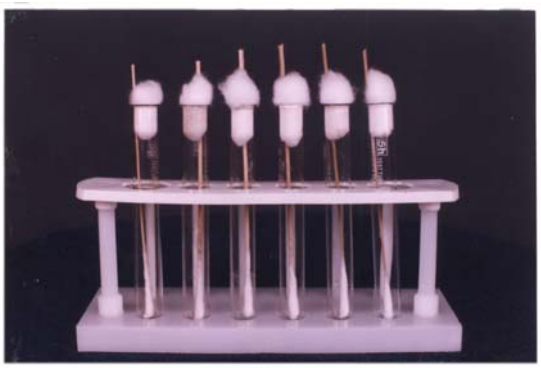


Plate -2 Giesma staining Chlamidia positive

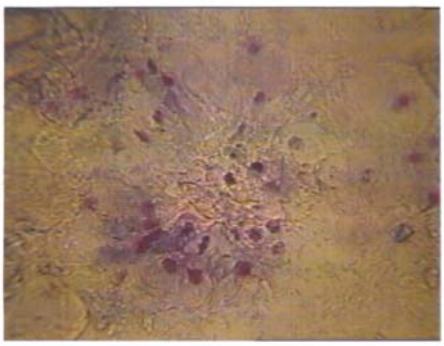


Plate-3 Giemsa staning Chlamidia nagative

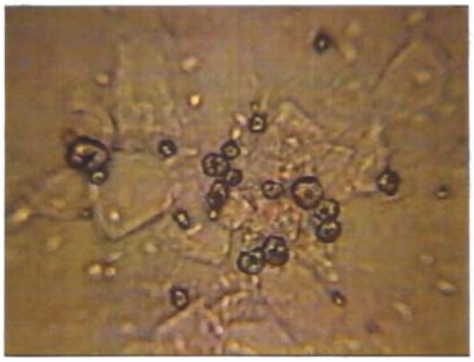


Plate – 4 Trachomatis lps antigen test Chlamidia positive

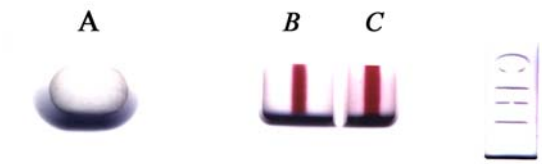
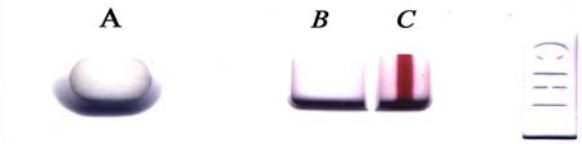


Plate-5 Trachomatis lps antigen test Chlamidia positive



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