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REPRODUCTIVE TRACT INFECTIONS IN WOMEN ATTENDING A TERTIARY CARE HOSPITAL IN NAVI MUMBAI,WITH FOCUS ON C.TRACHOMATIS.

Microbiology

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ABSTRACT

The asymptomatic nature of Chlamydial genital infections coupled with the occurrence of severe sequela in untreated patients makes the laboratory evaluation of great importance in the diagnosis of the disease. Genital chlamydial infections are the leading cause of preventable sexually transmitted infections (STIs) worldwide, with 43 million new cases in Southeast Asia. The present study was designed to determine the prevalence of genital chlamydial infection in women attending a OBGY outpatient department in a tertiary care hospital in Navi Mumbai and to determine the association of the disease with other STIs. A total of 100 female patients were enrolled for the study. Genital discharge specimens (endocervical and vaginal swab)s , together with blood/serum samples were collected from all the patients. The patients were investigated for the presence of antigen and antibody of *Chlamydia trachomatis* with the help of the Polymerase chain reaction (PCR) and the Enzyme Linked Immunosorbant Assay (ELISA), respectively. Investigations for aetio-pathogens of other STIs were carried out using the standard methods. Chlamydial infection was found in 3.5% to 16.7% (as per PCR) 1.8% to 33.3%(as per IgG ELISA) test. The overall incidence of other aetio-pathogens was low. *Chlamydia trachomatis* was found to be most commonly associated with *Candida albicans and Bacterial Vaginosis*..However, there was no co-infection of *Chlamydia trachomatis* with *Neisseria gonorrhoeae,Syphilis or Vaginal Trichomoniasis* .

KEYWORDS:

INTRODUCTION

In developing countries, sexually transmitted diseases (STDs) are a major cause of morbidity and mortality particularly in women and neonates.[1] Among women of childbearing age,the health problems caused by the STDs include pelvic inflammatory disease, ectopic pregnancy, tubal infertility, carcinoma of the cervix, preterm premature rupture of membranes and postpartum infections.[2,3] In neonates, purulent ophthalmia, congenital syphilis, pneumonia, low birth weight and perinatal deaths reflect the most significant consequences of the STDs carried by their mothers.[4] It is now well documented that the presence of STDs facilitates the acquisition of human immunodeficiency virus (HIV) infection.[5,6] For all these reasons the management of the STDs in developing countries is a priority today as part of reproductive health programmes and, moreover, in the campaign against the expansion of HIV epidemic. Considering the limited technical and financial resources available in developing countries,the World Health Organisation (WHO) has suggested a syndromic approach based on the complaints of the people who suffer from genital symptoms for STD management.[7,8] Furthermore STDs in general, *Chlamydia trachomatis*(CT) and *Neisseria gonorrhoeae* (NG) infection particularly, are frequently asymptomatic in women.[9]

The cervico-vaginal mucosa represents a portal of entry for different pathogenic microorganisms. In healthy women of childbearing age, the protective mucosa in the vagina is populated with microflora typically dominated by lactobacilli and their dominance over pathogenic anaerobes is positively associated with vaginal health.

C. trachomatis is the most common sexually transmitted bacterial infection worldwide with an estimated 92 million new cases every year and it is a frequent cause of cervicitis and salpingitis in women (Geisler et al., 2013).[10]

Considering that around 75% of the Chlamydia positive women are asymptomatic[11,12]routine screening and treating for this condition are obligate for populations at high risk. It is associated with an increased risk for the transmission or acquisition of HIV as well as other STIs (Sexually Transmitted Infections) and is also attributed to be a risk factor for the development of cervical carcinoma.[13]

MATERIALS AND METHODS

Type of study: Prospective and Experimental

Period of study: 3 years(Aug 2015 to July 2017)

Place of study: Hospital Microbiology Laboratory,MGM Medical College and Hospital,Navi Mumbai and Department of Infectious Disease & Biology, National Institute of Research in Reproductive Health. Mumbai.

Ethical Aspects: The study was submitted to research and ethics committee of the institute and obtained approval in 27th March 2015.

Sample size: 100 samples was taken and analyzed.

Selection of Cases:

Inclusion criteria – Women attending the OBG Dept. of MGM Hospital, Navi Mumbai with an age limit of 16-45 years. The group comprised of women with histories of Spontaneous Abortions, Infertility,Lower Genital Tract Infections and Pregnant Women attending ANC .

Exclusion criteria-Women having per vaginal bleeding and or on recent antibiotic use.

A total of 100 subjects were included in this study over the time period from April 2015 to May 2017.The clinical history and presentation in each group was as follows:

1. Bad Obstetric History(BOH): Women having history of one ,two or more than two spontaneous abortions , those operated for ectopic pregnancies and pre term delivery cases were included in this group.
2. Infertility: Women who were living with their husbands but not conceiving and desirous of having a child were enrolled in this group.
3. Lower Genital Tract Infections(LGTI): Women with signs and symptoms of cervicitis and vaginitis on per speculum examination or having complaints like burning micturation, leucorrhoea, abdominal pain and lower backache were included in this group.
4. Asymptomatic: Women attending AnteNatal Care(ANC) clinic

without any of the above mentioned conditions and with a confirmed pregnancy of more than 12 weeks gestation were enrolled in this group.

Specimen collection & processing:

1. Collection of Vaginal swabs-

Vaginal collection typically includes careful insertion of the swab approximately 2 inches into the vaginal opening and gently turning/rubbing the swab against the posterior fornix and both lateral walls of the vagina followed by swab removal and insertion into the collection tube (being careful not to touch the swab to any surface prior to placing into the collection tube).

2. Collection Of Endocervical swabs-

The cervical os is first wiped/cleaned of secretions. Collection of an endocervical swab specimen for use in (Nucleic Acid Amplification Tests) NAAT as well as for culture testing procedures involves the use of a speculum and involves the use of three swabs, one for initial cleaning of the cervix to remove excess mucus from the cervical os and surrounding mucosa (this swab is then discarded), and the remaining two for specimen collection. Once the cleaning swab has been used and discarded, the remaining swabs are used to collect the specimen by insertion of the swab into the endocervical canal followed by gentle rotation of the swab. The swab is then withdrawn while avoiding contact with the vaginal mucosa.One of the swab was used for isolation of N.gonorrhoea and or Candida albicans by culture.The second swab was stored at -25^{o}C before NAATs.

3. Collection of Blood/Serum-

Blood was collected by venepuncture and placed in Red top(Plain) blood collection tubes. Serum samples were then stored at -25^{0}c until they were processed .Thawed samples were mixed well before antibody testing.

MICROSCOPY

A. WET MOUNT EXAMINATION

1.For detection of *T.vaginalis* :

Wet mounts of High Vaginal Swabs were used for the detection of *T.vaginalis*. This was performed within 10 to 20 min of collection of the sample, or the organisms tend to lose viability. The organisms are about the size of a white blood cell and may be actively motile or may be seen beating their flagella at rest.

2. For detection of Candida :

10% KOH wet mounts of High Vaginal Swabs were used for the detection of C.albicans. Candida was seen as yeast cells with or without budding / pseudohyphae.

B. GRAM STAIN EXAMINATION

1. For detection of Bacterial Vaginosis(BV):

For detection of BV infection, specimens were collected from the posterior fornix of the vagina and a smear was prepared on a slide. The smear was Gram stained.A standardized 0-10 scoring system (Nugent's criteria) was used to evaluate BV on the basis of the presence of large Gram-positive rods (*Lactobacilli*), small Gram-negative rods (*Gardnerella*) and *Mobiluncus*. A Nugent's score of ≥7 was considered as positive for BV. Smears were also examined for Neutrophils.

2. For detection of Vaginal Candidiasis and *Neiserria gonorrhoea*:

For detection of Vaginal Candidiasis and *N.gonorrhoea* endocervical swabs as well as vaginal swabs were obtained and Gram stain was performed.The presence of gram positive budding yeast cells with or without pseudohyphae was considered to be positive for vaginal Candidiasis while the presence of Gram Negetive intracellular Diplococci in the presence of (Polymorpho nuclear Lymphocytes) PMNLs was considered to be positive for *N.gonorrhoea*

CULTURE :

FOR ISOLATION OF CANDIDA:

1. **Morphology:** Candida are Gram positive budding yeast cells about 10-12 µm in diameter.
2. **Culture Media:** Plating Media: Sabouraud's Dextrose Agar (SDA) slants/Chocolate Agar.

On SDA *Candida* produces creamy,smooth, pasty and convex colonies which may become wrinkled on further incubation.

On Chocolate Agar produces pasty, yellow-white colonies from which "feet" extend out from the margins into the surrounding agar.

2. FOR ISOLATION OF *N.gonorrhoea*:

Morphology: *N.gonorrhoea* are Gram Negative oval or spherical cocci ,0.6-0.8 µm in diameter ,arranged in pairs with adjacent sides concave typically kidney shaped.

Culture Media: Plating Media: Modified Thayer Martin Agar Plate w/VCNT and Chocolate Agar .

On Chocolate Agar colonies are small,round,translucent,convex with a granular surface and lobate margins.

On Thayer Martin colonies are small, grayish-white to colorless mucoid colonies.

Detection of Treponema pallidum:

RAPID PLASMA REAGIN TEST

Syphilis is a sexually transmitted (venereal) disease caused by the spirochete *Treponema pallidum.* After infection the host forms Treponemal antibodies to *Treponema pallidum,* in addition, the host also forms Non Treponemal antilipoidal antibodies in response to the lipoidal material released from the damaged host cell. These antibodies are traditionally referred to as 'Reagins.' The Rapid Plasma Reagin (RPR) / Carbon Antigen test is a macroscopic non-Treponemal flocculation test for the detection and quantitation of antilipoidal antibodies.

Detection of C.trachomatis

1. Antibody detection by ELISA(Nova Tech Immunodiagnostica, Germany):

For detection of serum anti-*chlamydial* IgG antibody in serum samples

***2. C.trachomatis(Major Outer Membrane Protein)*MOMP gene detection by PCR.**

3. Confirmation of *C.trachomatis* PCR by Southern Blotting

RESULTS AND CONCLUSION

***A. C.trachomatis* infection in defined population**.

The **prevalence** of acute *C.trachomatis* infection in the study population varied from 3.5% to 16.7% (as per PCR test) while with respect to past infection the prevalence varied from 1.8% to 33.3%(as per IgG ELISA test)**-(Table- 1)**

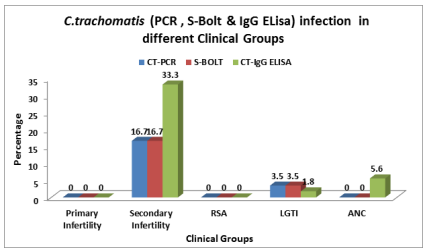
IgG antibody was detected in 4 cases while 8 cases showed Intermediate Positive results. Intermediate samples were not taken into consideration.

Table –1 : Prevalence of *C.trachomatis* (PCR & IgG ELisa) infection in different groups.

Parameters	Clinical Groups	N	Acute *C.trachomatis* infection (CT-PCR)		*C.trachomatis* infection (S BLOT)		Past *C.trachomatis* infection (CT-IgG ELISA)	
			Positive Finding	Percentage	Positive Finding	Percentage	Positive Finding	Percentage
Infertility	Primary	13	0	0.0	0	0.0	0	0.0
	Secondary	6	1	16.7	1	16.7	2	33.3
BOH	RSA	6	0	0.0	0	0.0	0	0.0
LGTI	LGTI	57	2	3.5	2	3.5	1	1.8
ANC	ANC	18	0	0.0	0	0.0	1	5.6
Total		100	3	3.0	3	3.0	4	4.0

CT-PCR:*Chlamydia trachomatis* PCR , CT-IgG ELISA- *Chlamydia trachomatis* Immunoglobulin G ELISA.S Blot-Southern Blotting

Figure –1 : Prevalence of *C.trachomatis* (PCR & IgG ELisa) infection in different groups.



B. Presence of other Reproductive Tract Infections (RTIs).

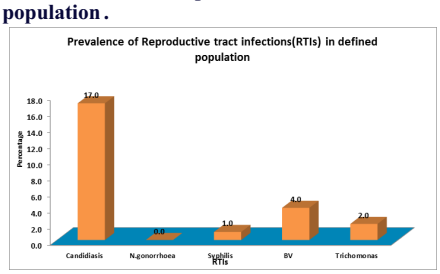
Microscopy,culture and serological tests were put up for the detection of other Reproductive tract infections (RTIs). Results showed that the Control/ANC subjects had a high rate of Vaginal Candidiasis(33%) while Bacterial Vaginosis(BV)(16.7%) and Trichomonas(3.5%) was more common in the BOH and LGTI group respectively. Only 1 case of Syphilis was detected while Gonorrhea was absent **(Table – 2,Figure-2).**

Table –2 : Prevalence of Reproductive tract infections(RTIs) in defined population .

Parameters	Clinical groups	N	Candidiasis	N.gonorrhoea	Syphilis	BV	Trichomonas
			N(%)	N(%)	N(%)	N(%)	N(%)
Infertility	**Primary**	13	4(30.8%)	0	0	1(7.7%)	0
	Secondary	6	0	0	0	0	0
BOH	**RSA**	6	1(16.7%)	0	0	1(16.7%)	0
LGTI	**LGTI**	57	6(10.5%)	0	0	2(3.5%)	2(3.5%)
ANC	**ANC**	18	6(33.3%)	0	0	0	0
Total		100	17(17.0 %)	0	1(1.0 %)	4(4.0%)	2(2.0%)

BV-Bacterial Vaginosis

Figure –2 : Prevalence of Reproductive tract infections(RTIs) in defined population .



C.trachomatis and associated infections

Association of *C.trachomatis* with other common RTIs was studied. It was seen that 14.3% of *C.trachomatis* infected subjects had concomitant BV and Candida. There was no association of *C.trachomatis* with Gonorrhea, Trichomonas or Syphilis**(Table-3,Figure-3).**

Table 3- C.trachomatis and associated infections.

Infections	BV N(%)	Candida N(%)	Trichomonas N(%)	Syphilis N(%)	N.gonorrhoea N(%)
C.trachomatis N=7(3 PCR+ 4 ELISA)	1(14.3%)	1(14.3%)	0	0	0

Figure 3- C.trachomatis and associated infections

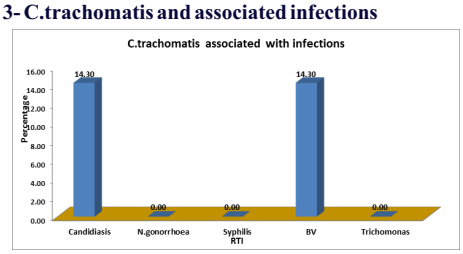


Table 4 and 5 shows the Association variables of acute as well as chronic C.trachomatis infection with other RTIs. Table 26 shows an association between past C.trachomatis infection with BV (OR:10.33,p=0.029).

Table – 4 : Association variables of Acute C.trachomatis (CT-PCR) with Other RTI's

RTIs	CT -PCR			Chi square test	P value	Significant At 5% level	Odds Ratio (95% CI)	
	Positive	Negative	Total				Row (1/2)	Col.1 (+ve value)
Candidiasis								
Positive	1	16	17	0.585	0.444	No	2.531 (0.216 to 29.616)	2.441 (0.234 to 25.420)
Negative	2	81	83					
Total	3	97	100					
N.gonorrhoea								
Positive	0	0	0	0	1.00	No	0.0	0.0
Negative	3	97	100					
Total	3	97	100					
Syphilis								
Positive	0	1	1	0.031	0.860	No	0.0	0.0
Negative	3	96	99					
Total	3	97	100					
BV								
Positive	0	4	4	0.129	0.720	No	0.0	0.0
Negative	3	93	96					
Total	3	97	100					
Trichomonas								
Positive	0	2	2	0.063	0.802	No	0.0	0.0
Negative	3	95	98					
Total	3	97	100					

p>0.05 CT-PCR: *Chlamydia trachomatis* PCR

Table –5 : Association variables of Chronic C.trachomatis (CT-IgG) with Other RTI's .

RTIs	CT -PCR			Chi square test	P value	Significant At 5% level	Odds Ratio (95% CI)	
	Positive	Negative	Total				Row (1/2)	Col.1 (+ve value)
Candidiasis								
Positive	0	17	17	0.853	0.356	No	0.0	0.0
Negative	4	79	83					
Total	4	96	100					
N.gonorrhoea								
Positive	0	0	0	0.00	1.00	No	0.0	0.0
Negative	4	96	100					
Total	4	96	100					
Syphilis								
Positive	0	1	1	0.042	0.837	No	0.0	0.0
Negative	4	95	99					
Total	4	96	100					
BV								
Positive	1	3	4	**4.785 ***	**0.029**	**Yes**	10.33(0.816 to 130.82)	2.18 (0.37 to 13.02)
Negative	3	93	96					
Total	4	96	100					
Trichomonas								
Positive	0	2	2	0.085	0.771	No	0.0	0.0
Negative	4	94	98					
Total	4	96	100					

p<0.05

CT-IgG ELISA- *Chlamydia trachomatis* Immunoglobulin G ELISA.

CONCLUSION

1. Acute *C.trachomatis* infection in the study population varied from 3.5% to 16.7% while with respect to past infection the prevalence varied from 1.8% to 33.3% in the defined population.
2. A higher proportion of Secondary Infertility women had *C.trachomatis* infection as compared to Primary Infertility cases while infection rate was low in the BOH, LGTI and ANC/Control groups.

3. Vaginal Candidiasis was more common in the ANC Group while Bacterial Vaginosis(BV) and Trichomonas was more common in the BOH and LGTI group respectively. Only 1 case of Syphilis was detected while Gonorrhea was absent.
4. 14.3% of *C.trachomatis* infected subjects had concomitant BV and Candida. There was no association of *C.trachomatis* with Gonorrhea, Trichomonas or Syphilis.
5. Past *C.trachomatis* infection could be a risk factor for the acquisition of BV.

DISCUSSION

Timely detection and effective management of cervical infection due to *Chlamydia trachomatis,* in women, provide critical intervention opportunities. The prevalence of genital infection with Chlamydia varies depending upon the population studied and the sensitivity of the laboratory methods used.

The present study carried out in a hospital reveals 3.5- 16% for current and 1.8- 33% for past *Chlamydia trachomatis* infection. in contrast to a previous study in Mumbai,in which 8.8% prevalence was observed.[14] Similar prevalence, i.e., 30.8% has recently been reported in Chennai, India.[15] A study from a UK hospital,where male partners of females with Chlamydial infection, both symptomatic as well as asymptomaticcases, were taken, have reported the prevalence as high as 44%.[16]

Among the other STIs, the most common infection was candidiasis (17%) followed by bacterial vaginosis (4%), Trichomoniasis (2%), Syphilis (1%), and gonorrhoea (0%). In a study in patients with genital discharge the incidence of candidiasis (26%), trichomoniasis (13%), gonococcus (1%), and bacterial vaginosis (48%) was reported from India.[17]

Coinfection of Chlamydia with other STIs, especially bacterial vaginosis, highlights the importance of early laboratory diagnosis and specific treatment of the condition as they increase the risk many folds when the infections exist together.[18]The observations of the current study reinforce the importance of routine screening for *Chlamydia trachomatis* as a necessary intervention to decrease the burden of chlamydial disease and to reduce the risk of HIV and its spread.

IMAGES

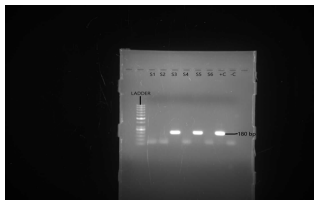


Figure 1- AGE of C.trachomatis PCR products

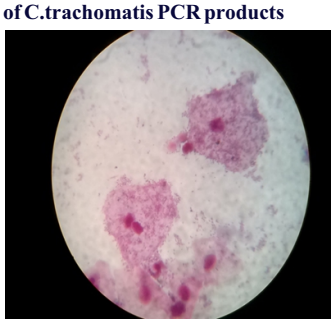


Figure 2-Gram Stain Showing Vaginal Clue Cells.

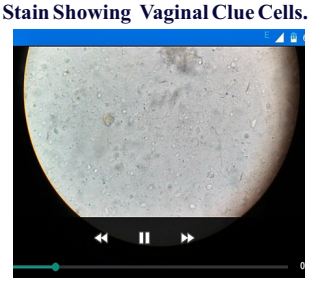


Figure 3-Wet mount showing Vaginal Trichomoniasis

Figure 4-C.albicans on SDA

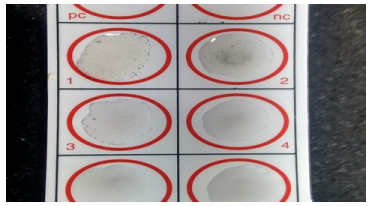


Figure 5-Positive (RAPID PLASMA REAGIN) RPR Test.

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