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EVALUATION OF THE RAPID IMMUNOCHROMATOGRAPHIC TEST AGAINST GOLD STANDARD RT-PCR IN GENITAL C.TRACHOMATIS INFECTION IN SEXUALLY ACTIVE WOMEN.

Microbiology

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ABSTRACT

Chlamydia trachomatis is the leading cause of sexually transmitted infections (STIs) worldwide. Chlamydial infection if undiagnosed and untreated can result in Pelvic Inflammatory Disease (PID), ectopic pregnancy, and infertility. The present study aimed at detecting the presence of C.trachomatis in endocervical sample of symptomatic women attending gynecology OPD.50 cases of sexually active women were included in the study and samples were tested by both rapid immune chromatographic test and RT-PCR.The rapid test was evaluated against RT-PCR in detection of C.trachomatis in endocervical samples. In this study the prevalence of C. trachomatis as detected by PCR was 14%Screening symptomatic women in reproductive age group along with syndromic management can able to reduce the burden of the disease in this community.The sensitivity of POC rapid test used in this study was found to be low.Hence it is necessary to improve the standards of POC rapid test which would be much useful for screening in developing countries like india.

KEYWORDS

chlamydia infection , sexually transmitted disease, endocervix, PCR.

1.INTRODUCTION

Sexually transmitted infections (STI) are a major global cause of acute illness with severe medical and psychological consequences[1]. Complications of STDs with severe sequale may results in chronic pelvic pain, tubal pregnancy and the costliest complication of infertility[2] . Hence timely and precise diagnosis of these infections is mandatory.Chlamydia trachomatis is the leading cause of sexually transmitted infections (STIs) worldwide. Chlamydial infection if undiagnosed and untreated can result in Pelvic Inflammatory Disease (PID), ectopic pregnancy, and infertility. Chlamydia is a risk factor in HIV infections by increasing in the transmission rate from 3 to 6 times and it is also has a cofactor in the development of cervical carcinoma[345]. Symptomless patients are the reservoir for the C.trachomatis. During pregnancy, chlamydial infection may cause complications such as spontaneous abortion, premature rupture of fetal membranes, premature delivery, low birth weight and neonatal infections like conjunctivitis and pneumonia[6]. Economic burden of testing and poor performance of the available tests are the major obstacles to Chlamydia screening programs in India. Instead of relying on microbiological testing, clinics in resource-poor settings continue to employ syndromic management for sexually transmitted infections , that lacks specificity for C. trachomatis infection[7]. Blind use of antibiotics for C. trachomatis also enables resistant strains to evolve rapidly. Therefore, a vital need is felt to actively diagnose the disease. The polymerase chain reaction technique has been used in the diagnosis of Chlamydia

2.AIM

- Find out the prevalence of genital C.trachomatis infection in sexually active women.
- To evaluate the rapid immunochromatographic test against gold standard RT-PCR.

3.MATERIALS AND METHOD.

The present study was conducted at the Department of Microbiology, Tirunelveli Medical College, Tirunelveli detect the prevalence of genital chlamydial infection in sexually active women attending gynaecology OPD of Tirunelveli medical college and to evaluate the efficiency of rapid immunochromatographic test [ICT] in C. trachomatis antigen detection and also to compare its performance against Polymerase chain reaction [RT-PCR] the gold standard test. A total of 50 samples collected from symptomatic individuals from Tirunelveli medical college, gynecology OPD.

Inclusion criteria.

Sexually active women (18-35 years) attending general gynaecology, outpatient department, having pathological vaginal discharge, perineal itching, lower abdominal pain and other STD related symptoms.

Exclusion criteria

Sexually in active women i.e.<20 years or > 45 years, pregnancy, women who were menstruating, women with cancerous or precancerous lesion of cervix and history of antibiotics intake in last 10 days. Before testing patients were checked whether they have used vaginal creams or sprays in the past 24 hrs. The patients were not supposed to urinate for 1 hour before the specimen collection.

Study sample collection.

after explaining the procedure patient was put in lithotomy position. The vulva and cervix were examined for evidence of lesions and vaginal/cervical discharge. After cleaning the exocervix with a cotton swab/Dacron (Hi Media), two endocervical swabs were taken from each patient in order to avoid the swab sample variation. Large or small Dacron swab was inserted into endocervical canal until most of Dacron tip is not visible. Swab was rotated for 5-10 seconds inside endocervical canal and withdraw without touching any vaginal surfaces. Two swab materials were collected. First one for immune-chromatographic test and second swab material was for PCR. All the 50 samples were tested by rapid immunochromatography card test (Insight Chlamydia test by Tulip Diagnostics).Rapid test cassette has a letter of "T" for C.trachomatis Test line and "C" as Control Line on the surface of the device. All the 50 cervical specimens were tested with RT-PCR by the kit provided by Helini Biomolecules, Chennai, India and the procedure was done according to the manufacturer's guidelines.All the laboratory works were carried out as per standard laboratory procedures and Bio-safety norms in class II biosafety cabinet.Before starting the purification reaction, water bath was set to 56^{o}C and the elution buffer was warmed up to to 56^{o}C.

4.RESULTS

Table 1: Comparison Of RICT With Rt-pcr In Detection Of Chlamydia Trachomatis.

Test	Samples tested	Positive		Negative	
		Cases	Percentage	Cases	Percentage
RT-PCR	50	7	14	43	86
ICT		1	2	49	98

The above table shows all the 50 samples were tested with RT-PCR and ICT for C. trachomatis from genital samples. Out of this 14% were positive by RT-PCR and 2% by ICT.

Table 2 : Evaluation Of Rapid ICT Against RT-PCR

ICT	RT-PCR	
	positive	negative
positive	1	0
negative	6	43
Total	7	43

Table 3: Age Wise Distribution Of Chlamydia Trachomatis Positive Cases

AGE (years)	Positive cases		Negative cases		Total		$\chi 2$	df	Sig
	No	%	No	%	No	%			

20-25	5	71 %	8	19%	13	26%	9.162	3	p<0.027
26-30	2	29%	23	53%	25	50%			
31-35	0	0	8	19%	8	16%			
36-40	0	0	4	9%	4	8%			
Total	7	100 %	43	100%	50	100%			

The above table(fig 7) shows that majority of positive cases are in the age grup of 20-25 years. Out of 7 samples tested positive by RT-PCR 5 (71%) were in the age group of 20-25 years. Association of this age group with C. trachomatis positivity was statistically significant , P value <.027.This is followed by a higher incidence in the age group of 26-30(29%)years.

5. DISCUSSION

C. trachomatis is the most common cause of sexually transmitted disease in women of reproductive age group. The major causes of concern are due to sequelae like pelvic inflammatory disease and infertility. In developing countries like India syndromic management is in progress to treat the sexually transmitted disease. Various studies imply that syndromic approach in treating genital discharge syndrome has poor performance. So in countries like India with low return rates of patients, efforts are made to improve diagnostic tools on the basis of WHO (ASSURED) criteria for POC tests. In this background this study attempts to evaluate a rapid immunochromatographic test with the gold standard RT-PCR.Sexually active females are prone for Chlamydial infections and the major morbitity is infertility so the population taken for this study included patients between 20 to 40 years This was similar to the prevalence study done in Chennai by Prabhu N et al This is because younger age group is often related to difference in sexual behavior.Vaginal discharge was the major clinical symptoms in the study group followed by dysuria, lower abdominal pain and dyspareunia . Mucopurulent cervicitis was the predominant sign followed by cervical ectopy and erosion The prevalence of Chlamydia trachomatis was 14% by RT-PCR in this study This is comparable to the study by Pandya et al in Gujarat where the prevalence was found to be 12%.The study by jayanti et al, found Chlamydial prevalence to be 20% among lower genital infectionsThe study by sood et al found the prevalence of genital Chlamydia as 11.3%[3] However a study conducted in high risk population by Joye et al in chennai had a prevalence of 32.3%. But the study done by Joyee et al among normal population showed a prevalence of 1.1%.This varied prevalence rates may be due to different diagnostic methods and different population.The introduction of a commercial nucleic acid amplification assay that can be integrated into routine clinical microbiological laboratory is an important advance in diagnosing chlamydial infections. Since PCR has improved performance over culture and antigen based tests with highest sensitivity and specificity it makes the test as gold standard diagnosis for C.trachomatis.PCR detects even a single copy of DNA in a clinical sample. RT-PCR has an advantage of quantification of the chlamydial load in the specimen. It can be done by CT values which are inversely proportional to the chlamydial load . Though plamid based primers are more sensitive we used major outer membrane protein(MOMP) as a target for primer because of emerging plamid less strains.Horizontal and vertical PCR contamination with amplicons is an essential drawback of PCR especially when processing more numbers of samples. This contamination may be nullified by using closed systems such as the one that was used in this study. (Bio-Rad) .In this study, the sensitivity of ICT was 14.2%, which is very low where as the specificity was 100% when evaluated against RT-PCR . This is similar with a study reported by Van Dommelen et al where the sensitivity of three rapid EIAs was extremely low (12% to 27%) compared to PCR[72]. The study by Rani R et al evaluated a rapid test and observed that it had a sensitivity of 25% and 100% specificityThe study done by Sabidó M et al from Guatemala on a rapid test showed that the sensitivity and specificity were 62.96% and 99.60% against PCR[20]**.** However Lourdes Mahilum-Tapay et al evaluated a rapid test and observed that it had high sensitivity and specificity, 83.5% and 98.9% respectively. In contrast Navjyot K. Vidwan et al did a study from vellore and evaluated the FDA approved ICT[73]. He concluded that it had very poor performance in low prevalence community with 0% sensitivity and 90% specificity.In this study the prevalence of C.trachomatis was 14 % among symptomatic sexually active females. ICT has detected 2% of the positive cases. Urdea et al stated that a test that requires no laboratory infrastructure could save 4 million Disability- adjusted life years and avert >16.5 million incidence of Chlamydia infections[80]. Vickramen et al stated that point of care with moderate sensitivity could identify more infections than gold standard tests. POC tests support the use in scenarios where it would be difficult to ensure a high return rate, failure to trace the contact and where there is high potential for further STI transmission. However our results showed poor sensitivity of 14%. Thus it may fail to detect the true positive cases and facilitates Chlamydial transmission in the community.The ideal screening test for STIs should be 100% sensitive and specific. However few tests are perfect. In conjunction with this WHO promoted the development and evaluation of STI diagnostics that meet specific criteria known by the acronym “ASSURED” and it stands for “Affordable by those at risk of infection; Sensitive-few false negatives; Specific-few false positives; User-friendly-simple to perform; Rapid and Robust-to enable treatment at first visit (rapid) and does not require refrigerated storage (robust); Equipment-free-easily collected non invasive specimens; Delivered-to end users”.Though PCR is highly sensitive and specific, the high price of the commercial kits and lack of proper infrastructure are the major drawback for using them for large screening programmes in developing countries like India. A POC setting using a test that meets the criteria of “ASSURED” to afford an rapid diagnosis and to initiate prompt and proper treatment, seems to be best policy in facing chlamydial infections. Thus there is an urgent call for improved and cost effective diagnostic tests that will decrease the burden of C.trachomatis infections in the developing countries. The present study aimed at detecting the presence of C.trachomatis in endocervical sample of symptomatic women attending gynecology OPD.50 cases of sexually active women were included in the study and samples were tested by both rapid immune chromatographic test and RT-PCR.The rapid test was evaluated against RT-PCR in detection of C.trachomatis in endocervical samples.

6.CONCLUSION

C.trachomatis is one of the important cause of STDs in symptomatic female aged 20-40 years.In this study the prevalence of C. trachomatis as detected by PCR was 14%Screening symptomatic women in reproductive age group along with syndromic management can able to reduce the burden of the disease in this community.The sensitivity of POC rapid test used in this study was found to be low.Hence it is necessary to improve the standards of POC rapid test which would be much useful for screening in developing countries like india.Further molecular studies are essential to know the accurate information of sero types which will be helpful in formulating vaccines in future for at least reproductive age group.

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