International Journal of Reproduction, Contraception, Obstetrics and Gynecology
Murugesan M et al. Int J Reprod Contracept Obstet Gynecol. 2016 Nov;5(11):3987-3992
www.ijrcog.org

pISSN 2320-1770 | eISSN 2320-1789

DOI: http://dx.doi.org/10.18203/2320-1770.ijrcog20163876

Original Research Article

Screening for lower genital tract infections in women of reproductive age group attending a tertiary care hospital

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Received: 12 September 2016
Accepted: 07 October 2016

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ABSTRACT

Background: Lower genital tract infections are the major cause of gynecological morbidity and a great public health concern in India. Inadequate laboratory diagnostic facilities in all the levels of health care, limited resources in material and manpower, stigma and discrimination associated with RTI services are some of the reasons of lack of exact incidence/prevalence rate of RTI in India. Hence this study was conducted to provide a reliable laboratory based data on the occurrence of lower genital tract infections.
Methods: A prospective study was conducted on 110 women attending Gynecology OPD at a tertiary care teaching hospital over a period of one year (June 2014 to May 2015). After getting informed consent and brief history, vaginal swab and endocervical sample was collected and used for microscopic examination and culture. All the endocervical samples were subjected to Real Time PCR for detection of Chlamydia trachomatis.
Results: Among 110 samples, laboratory diagnosis of lower genital tract infections was positive in 43 subjects (39.09%). By Real time PCR assay among the 110 samples, 9 (8.8%) of the samples were positive for Chlamydia trachomatis infection. Candida sp., (17, 35.42%) was the most common organism identified followed by Escherichia coli (10, 20.83%).
Conclusions: Laboratory screening is must in all the symptomatic women in order to avoid the unnecessary treatment, which warrants the patients' reliability. Chlamydia trachomatis screening is mandatory for all the child bearing age group women to avoid consequences like PID and infertility.

Keywords: Laboratory diagnosis, Genital infections, Reproductive age group, RTI, STI

INTRODUCTION

Lower genital tract infections are the major cause of gynecological morbidity and a great public health concern in India. The infections affecting the lower genital tract includes sexually transmitted diseases such as Chlamydia, Gonorrhea, Trichomoniasis, Syphilis, endogenous infections such as vulvovaginal candidiasis or bacterial vaginosis and iatrogenic infections which are associated with medical procedures.[1] WHO statistics indicate that the total number of new cases of Reproductive Tract Infections (RTI) in adults between the ages of 15 and 49 was estimated to be 498.9million including lower genital tract infections. Among them are 105.7 million cases of *Chlamydia trachomatis*, 106.1 million cases of *Neisseria gonorrhoeae* and 276.4 million cases of *Trichomonas vaginalis.*[2] The common causes of lower genital tract infections which presents with Discharge per vaginum are *Chlamydia trachomatis*, Candidiasis, Trichomoniasis, Bacterial vaginosis, Gonorrhea, Syphilis, Human papilloma virus (HPV) infection, Herpes simplex virus (HSV) infection, Lymphogranuloma venerum, Chancroid and other bacterial infections.[3]

The gold standard method for the diagnosis of Candidiasis is culture with clinical correlation of symptoms like itching and burning micturition. For Trichomoniasis, the specific method is culture using Kupferberg`s STS medium or Modified Diamond medium.[4] Diagnosis of Bacterial Vaginosis can be done either by Amsel criteria or Nugents' criteria.[5] Nugent et al., made a scoring system ranged from 0 to 10 based on his criteria. The sensitivity and specificity is 90% and 94% respectively.[6] Culture is the "Gold Standard" for the definitive diagnosis of gonorrhoea. The sensitivity of culture is 85−95% in case of acute infection and the specificity is 100%, whereas in chronic infections it is 50% sensitivity.[7] In case of syphilis, the nonspecific test like VDRL, RPR are used for screening and specific tests are used for confirmatory. TPI test is the gold standard test and FTA-ABS is the standard reference test.[8] The sensitivity of TPHA is 85 to 100% and the specificity is 98 to 100%.[9]

In females, the asymptomatic Chlamydial infection, if left untreated leads to Pelvic Inflammatory Disease (PID), infertility and its complications. Henceforth, screening women for *Chlamydia trachomatis* helps in prevention of PID, ectopic pregnancy and infertility. The gold standard test for diagnosing *Chlamydia trachomatis* infection is the detection of nucleic acid in samples i.e., Nucleic Acid Amplification Test (NAAT). Inadequate laboratory diagnostic facilities in all the levels of health care, limited resources in material and manpower, stigma and discrimination associated with RTI services are some of the reasons of lack of exact incidence/prevalence rate of RTI in India, which forms an important data tool for determining intervention and treatment strategies.[10] Moreover, epidemiological studies in RTI are not abundant in South India.[11] Hence this study was conducted to provide a reliable laboratory based data on the microbiological profile of lower genital tract infections. The main aim of the study is to isolate and to study the etiological agents of lower genital tract infections in women of reproductive age group and also to determine the proportion of *Chlamydia trachomatis* among the study group by Real-time PCR assay.

METHODS

A prospective study was conducted on 110 women attending Gynaecology OPD at a tertiary care teaching hospital over a period of one year from June 2014 to May 2015.

The inclusion criteria were age group 18-45years, women with history of vaginal discharge, lower abdominal pain, itching, burning micturition and infertility.

The exclusion criteria were unmarried women, pregnancy, women on menstruation, women who have undergone hysterectomy, women on antimicrobial therapy, on chronic illness. After getting informed consent, brief history was obtained by structured questionnaire related to socio-demographic profile, presenting complaints and past history. Procedure for obtaining vaginal and endocervical swabs was explained to the patient beforehand.

Table 1: Methods used for the diagnosis of STI/RTI.

Name of STI/RTI	Preliminary test	Confirmatory test
Candida albicans	Gram staining KOH mount Germ tube test	Sabourards dextrose agar (Culture) Dalmau plate culture (For speciation)
Trichomonas vaginalis	Saline Wet mount	-
Bacterial vaginosis	Gram staining – Nugent scoring	Gram staining – Nugent scoring
Neisseria gonorrheae	Gram staining – For intracellular Gram negative diplococci Transport media	Culture in modified Thayer Martin medium followed by placement in CO2 jar in the field
Chlamydia trachomatis	-	Real Time PCR assay
Genital ulcer	Gram staining – Pus cells, Organisms	Culture according to the Gram staining morphology
Aerobic culture	MacConkey agar plate and blood agar plate and incubated at 37^0C for 24 hours with 10% CO_2.	Organism was identified by Colony morphology, Gram staining, Catalase test, Oxidase test and other biochemical reactions.

In lithotomy position, under aseptic precautions, vaginal discharge was collected for wet mount, then three swabs from posterior vaginal fornix with the help of sterile cotton swabs and one sample from cervical os using Ayer`s spatula was collected. Vaginal discharge was immediately utilized for performing wet mount and Gram staining.

The vaginal swabs were transported to the diagnostic microbiology laboratory as early as possible for culture. A sterile swab was then introduced into the cervix to remove the cervical mucus secretions. The endocervical brush was inserted 1 to 2 cm into the endocervical canal, rotated against the wall for 10 to 30 seconds, withdrawn without touching the vaginal surfaces and then placed in the appropriate transport medium. One endocervical brush was inoculated immediately in Modified Thayer

Martin medium and incubated in candle extinction jar. Another endocervical brush was inoculated in a sterile aliquot tube containing 2ml of 99% ethanol for detecting Chlamydial nucleic acid by Real-time PCR. The aliquot tubes were carried in an ice-packed carrier and stored in deep freezer (-20^{0}C) immediately.

Real time PCR was done by HELINI PureFast®Bacterial DNA mini spin purification kit, HELINI *Chlamydia trachomatis* Real- time PCR kit, Instrument used was Agilent MX3000P Real time PCR machine. Endogenous control = FAM channel (Human RNase P gene), *Chlamydia trachomatis* = FAM channel. Real time interpretation was done by the amplification plots and by the ct value.

Data was formulated in terms of frequency distribution for different variables. As the data are categorical variable, Fischer test was employed as test of significance for testing associations. Multivariate logistic regression model for statistically significant predictors of lower genital tract infections were also tested. The data was analysed using Epi-Info software (7.1.0.6 version; Center for disease control, USA) and Microsoft Excel 2010.

RESULTS

A total of 110 samples were collected over a period of one year which included vaginal swab and endocervical swab. The samples were processed and the results are shown as follows. Age wise distributions of the subjects were analysed. The range of age was 20 to 43 years. The median age was 26. The majority (43.63%) of the study population were in the age group of 21 to 25 years, followed by the age group of 26 to 30 years (33.64%). Among them, parity wise distribution showed that the majority of the study group (49.1%) had two children. 9.09% was nulliparous women and 2.73% had history of abortion/still born. Discharge per vaginum (82, 74.55%) was the major presenting complaint, followed by abdomen pain (56.36%), itching (23.64%) and then burning micturition (17.21%). Among 110 samples, laboratory diagnosis of lower genital tract infections was positive in 43 subjects (39.09%). Among 43 subjects who had laboratory findings for lower genital tract infections, 36 (83.72%) presented with single infection and 7 (16.28%) were presented with mixed infections. Laboratory diagnosis of bacterial vaginosis (6.97% positivity) was based on Nugent`s score.

Candida sp. (17, 35.42%) was the most common organism identified followed by Escherichia coli (10, 20.83%). Among the isolated Candida sp., Candida albicans accounted for 8 (47.05%), Candida glabrata 3 (17.35%), Candida tropicalis 3 (17.35%) and Candida kefyr 3 (17.35%). By Real time PCR assay among the 110 samples, 9(8.8%) of the samples were positive for C.trachomatis infection. The distribution of the various microbiological agents causing lower genital tract infections in the study population is depicted in the Table 1.

Table 1: Distribution of study group with positive laboratory findings for lower genital tract infections.

Type of infections	Cases positive for laboratory diagnosis	No. (n=43)	%
Single infections	Candida sp.	12	27.91
	Chlamydia trachomatis	5	11.63
	Trichomonas vaginalis (TV)	3	6.97
	Bacterial vaginosis (BV)	3	6.97
	Staphylococcus aureus	1	2.33
	Streptococcus sp.	1	2.33
	Escherichia coli	7	16.28
	Klebsiella pneumoniae	4	9.30
Mixed infections	Candida + Chlamydia	1	2.33
	Candida + Staphylococcus aureus	1	2.33
	Candida + Escherichia coli	2	4.65
	Candida + Chlamydia + Escherichia coli	1	2.33
	Chlamydia + Citrobacter koseri	1	2.33
	Chlamydia + Klebsiella pneumoniae	1	2.33
	Total	43	100

Candida sp., was the commonest agent (75%) causing lower genital tract infections in study group ≤ 30 years and aerobic bacterial agents were the commonest agent among >30 years age group study population whereas, Poly microbial infections were seen only in age group ≤30 years (Table 2). Over all, lower genital tract infections were common among women ≤30 years with significant p value (0.0037). Candida sp. was the most common agent affecting all the parity groups with majority of study group belonging to L1 (41.67%). Aerobic bacteria was commonly identified among all groups of parous women (L1, L2,>L2). In nulliparous women (n=10), Candidiasis (n=1), TV and BV each (n=1) and polymicrobial infections (n=2) are seen. Over all, lower genital tract infections were common among the women with multi parity with significant p value (0.0797) (Table 3).

DISCUSSION

Our country is in a state of epidemiological and demographical transition where morbidity and mortality rates may differ from previous demographic studies. In the last few years, community based studies on reproductive tract infections have been carried out in various parts of our country. Yet, South Indian studies are not widely available.

Out of 110 vaginal swabs which were subjected to wet mount microscopy, Gram staining, aerobic and anaerobic culture, fungal culture and the endocervical swabs which

were subjected to culture for *N. gonorrhea* and Real time PCR assay for the detection of C.*trachomatis*, 43 (39.09%) of the cases were positive for any one of the agents commonly causing lower genital tract infections. This correlates well with a study conducted in an urban health centre, where the prevalence of RTI was 34.3% based on the laboratory findings whereas 40.4% based on only the symptoms.[12] Prabha MLS et al. have estimated the prevalence of 33.1% in their study population.[13] The higher prevalence in urban areas is due to knowledge and awareness about genital tract infections and easy accessibility to tertiary care centres.

Table 2: Distribution of laboratory diagnosed cases in relation to age group.

Age	Candida sp.		Chlamydia trachomatis		Trichomonas vaginalis		Bacterial vaginosis		Aerobic bacteria		Poly microbial	
	No	%	No	%	No	%	No	%	No.	%	No	%
≤ 30 years	9	75	5	100	2	66.67	3	100	7	53.85	7	100
> 30 years	3	25	0	0	1	33.33	0	0	6	46.15	0	0
Total	12	100	5	100	3	100	3	100	13	100	7	100

Table 3: Distribution of laboratory diagnosed cases in relation with parity.

Parity	Candida Species		Chlamydia Trachomatis		Trichomonas vaginalis		Bacterial Vaginosis		Aerobic bacteria		Poly microbial	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Nulliparous	1	8.33	0	0	1	33.33	1	33.33	0	0	2	28.56
Abortion	0	0	0	0	0	0	1	33.33	1	7.69	1	14.29
One child (L1)	5	41.67	2	40	0	0	1	33.33	4	30.77	3	42.86
Two child (L2)	4	33.33	3	60	2	66.67	0	0	5	38.46	1	14.29
>2 child (>L2)	2	16.67	0	0	0	0	0	0	3	23.08	0	0
Total	12	100	5	100	3	100	3	100	13	100	7	100

The findings of laboratory diagnosed cases were evaluated and it was estimated that in our study about 83.72% had single infection and 16.28% had mixed infections. The differences in the figures in our study with comparison to other studies is due to lesser sample size, hospital based study while all the comparative study groups are community based studies.[13,14]

Bacterial vaginosis, as such is a clinical term diagnosed by Amsel criteria. The sensitivity and specificity of Amsel criteria is 37% and 99% respectively (Sha BE et al.[14] We have estimated 6.97% of BV cases by the gold standard laboratory diagnostic method i.e., Nugent scoring. There is a low level of estimation in our study when compared to other studies (Rohit Chawla et al., 32.86% and Modak T et al., 24%).[16,17] Since only laboratory method was employed, correlation between Amsel criteria method and Nugent scoring method could not be obtained in our study. The consequences of BV include abortion, still birth; preterm deliveries and co infections are also more common. Hence in addition to screening of high risk groups, all child bearing age group should be screened for BV to avoid such complications.

The agents causing the lower genital tract infections were analyzed individually and it was estimated that 35.42% were *Candida sp*., among them, 47.05% *Candida albicans*, 17.65% *Candida glabrata*, 17.65% *Candida tropicalis*, 17.65% *C. kefyr*. There is slightly increased prevalence when compared to other studies. Jindal et al., estimated 23.4% culture positive for *Candida sp*., with 74.4% of *Candida albicans*, 11% *Candida glabrata*, 6% *Candida tropicalis*, 3.6% *Candida krusei* and 2.43% *Candida parapsilosis* and *Candida guillermondi* each.[18] Anis ahmad et al., estimated 20.47% prevalence of *Candida sp.*, with *Candida glabrata* 36.7%, *Candida parapsilosis* 10.2%, *Candida tropicalis* 2.8%, *Candida kefyr* 1.8% and *Candida krusei* 1.4%.[19] 75% of *Candida* isolates affected ≤30years which gets correlated with a study conducted by Sujith D.Rathod et al.[20] Vulvovaginal candidiasis (VVC), as such is not a sexually transmitted disease but the majority of women presenting with leucorrhea is diagnosed with VVC. *Non albicans* species

of *Candida* is in increased prevalence for the recent years. The risk of recurrent infections is more common if the infection is not properly treated. *Chlamydia trachomatis* (18.75%) was the proportion obtained from our study. The prevalence rate of *C.trachomatis* by PCR is 7.04% (Dwibedi et al.,) and 23% (Saluja D et al).[26] The increased proportion of *Chlamydia trachomatis* in our study is attributed to the molecular diagnostic technique employed i.e., Real-time PCR detection method which has higher sensitivity when compared to other methods. Jaton K et al., have identified that the sensitivity and specificity of Real time PCR is 95.7% and 100% respectively.[23] As *Chlamydia trachomatis* presents as asymptomatic infection, untreated genital tract infection can lead to ramifications for the childbearing age group women. It is a must to screen all the child bearing age group women for *C.trachomatis* infection with cost effective methods. The available diagnostic techniques like ELISA are not a reliable indicator and at least in resource rich areas, molecular methods are advisable to estimate the exact prevalence of *C.trachomatis* infection. Similar to others findings, *Chlamydia trachomatis* (100%) affected the age group ≤30 years in our study.[24,25] The reasons for the significance of this age group and *C.trachomatis* infections is due to early coitarche, inconsistent use of barrier methods and multiple sexual partners. There is a need to educate the young sexually active females to avoid risky sexual practices and to undergo periodic screening for *C.trachomatis*. Health programmes should be implemented to screen the clinically silent *C.trachomatis* infections at an early age (<25 years) to safeguard the reproductive health of women.

6.25% of lower genital infections were contributed by *Trichomonas vaginalis* in our study. This is in accordance with 8.5% estimated by Madhivannan et al.[26] As TV is a treatable infection, the consequences like preterm deliveries, transmissibility to sex partner, increased exposure for other sexually transmitted infections can be prevented when appropriate etiological diagnosis is obtained. In developing countries, the screening modalities are mainly aimed at high risk groups leaving behind the study population belonging to community, where the exact prevalence data is submerging. In our study, only wet mount method was used as the modality of diagnosis of TV. The sensitivity of wet mount method is 55% (Sood s et al.) Shetkar S has estimated that the prevalence of TV by wet mount and broth culture method was 0.5% and 3% respectively.[27,28] There is an underestimation of the TV cases in our study and if broth culture method has been employed, the actual data could be obtained. However, our estimation reflects the methodology employed in a developing country like India, where wet mount is the universal method of screening (NACO guidelines).

Among the aerobic bacterial infections, 6.25% (4.66% as single infection, 1.59% as mixed infection) caused by Gram-positive cocci and 33.33% (25.58% as single infection, 7.75% as mixed infection) by Gram negative bacilli. The difference between colonization and infections was established by the presence of active symptoms, presence of pus cells in wet mount and Gram staining, presence of inflammation in Pap smear findings and remission of symptoms following treatment.

In our study, the age group ≤30 years were more prone for poly microbial infections (16.28%) with significant p value of 0.0037. The findings of parity analysis in our study showed that mixed infections were equally distributed in almost all the types of parity groups.

CONCLUSION

The findings of our study have shown that laboratory screening is must in all the symptomatic women in order to avoid the unnecessary treatment, which warrants the patients' reliability. The symptomatic treatment should be supported by the etiological diagnosis to know the exact burden and distribution of various agents causing RTI. As *Chlamydia trachomatis* is the most common cause of treatable RTI worldwide, screening is mandatory for all the child bearing age group women to avoid consequences like PID and infertility. Hence it is recommended to take a prudent action on implementing a nationwide screening programme for *Chlamydia trachomatis.*

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Murugesan M, Arumugam V, Gomatheeswari N, Sowmya AV. Screening for lower genital tract infections in women of reproductive age group attending a tertiary care hospital. Int J Reprod Contracept Obstet Gynecol 2016;5:3987-92.