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Original Research Article

Detection of protozoal *Trichomonas vaginalis* and abnormal vaginal flora in high vaginal smear

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ABSTRACT

Background: Vagina is the fibro muscular membrane sheath communicating with the uterine cavity and to the exterior at the vulva. Infection of female genital tract results from vaginal flora, extraneous agents and sexually transmitted diseases. The objective of the study was: a) high vaginal smear screening to find out incidence of abnormal vaginal flora of micro-organisms, b) prevalence of protozoal *Trichomonas vaginalis*.

Methods: Rural women from in and around Ammapettai in the age group of 20-50 years attending Gynaecology OPD of SSSMC&RI for vaginal discharge and itching vulva. Exclusion criteria were age less than 20 and above 50 and pruritis vulva due to other causes the study size comprised of 50 women. After getting Institutional ethical committee's approval and written informed consent from the patients, detailed history regarding type of discharge, odour, itching, and associated bleeding was taken. Per speculum and per vaginal examination done. High vaginal smear for aerobic bacterial profile and *Trichomonas* infection were taken and transported to microbiology laboratory for staining and culture. Wet mount microscopic examination of *Trichomonas vaginalis* and *Candidiasis* with saline and KOH mount done.

Results: Out of 100 smears 9 were normal vaginal flora organisms, aerobic pathogens 43%, *Trichomonas vaginalis* (TV) 30%, and *Candida albicans* 5% mixed infections on 7 smears. *E. coli* was seen in 23 samples, *Klebsiella* in 14, *Pseudomonas aeruginosa* 4, *Staphylococcus* in 6 samples. Gram negative organisms were 100% sensitive to imipenem, amikacin and gentamycin and 100% resistant to cefazolin, 80% to ceftazidime, 74% to cefotaxime. Gram positive *staphylococcus* 50% were resistant to gentamycin, ampicillin.

Conclusions: This study reflects on our responsibility to create awareness among women regarding abnormal vaginal discharge through proper health education routine screening targeted treatment to envisage healthy quality life.

Keywords: Aerobic microbes, *E. coli*, *Staphylococcus aureus*, TV

INTRODUCTION

Vagina is the fibro muscular membrane sheath communicating with the uterine cavity and to the exterior at the vulva. Infection of female genital tract results from vaginal flora, extraneous agents and sexually transmitted diseases. Vaginal flora has complex microbacteria in varying quantities and relative proportions maintaining intricate balance of gram negative aerobic and gram

positive anaerobic microbes. Most important microbe that maintains vaginal flora is *Lactobacilli*. In the *Lactobacillus* dominant flora, various clinical effects are caused by variation of pH, activation of polymicrobial agents and sexually transmitted protozoa, *Trichomonas vaginalis*. Smears deficient in *Lactobacillus*, positive for cocci with clue cells are diagnosed as bacterial vaginosis¹. Deficient Lacto smears with positive for cocci, coarse bacilli, para epithelial cells and vaginal

leucocytes are diagnosed as gram positive aerobic vaginalis. Aerobic gram negative microbes in vaginal flora are *E.coli*, *Klebsiella pneumonia*, *Oxytocica*, *Pseudomonas aeruginosa*, *Staphylococcus pyogenes*, *Staphylococcus aureus* and *epidermidis*.¹

The study was conducted in sexually active rural women in reproductive age to find out the incidence of aerobic microbes and protozoal *Trichomonas vaginalis* and to formulate modalities for proper health education, screening and pre-targeted treatment.

METHODS

50 sexually active women living in and around Ammapettai village, Tamil Nadu, India in the age group of 20-50 years attending gynaecology OPD of Shri Sathya Sai Medical College and Research Institute, over a period of 6 months for vaginal discharge and itching in the vulva were recruited as study subjects. Age <20 years and >50 years, itching in the vulva due to other causes were excluded from the study. After getting institutional ethical committee's approval and informed written consent from the study subjects, detailed history regarding type of discharge, odour, itching and associated bleeding was taken. Per speculum and per vaginal examination was done. High vaginal smear for aerobic bacterial profile and trichomonas infection were taken and transported to microbiology laboratory for staining and culture.

High vaginal swab for aerobic pathogens was cultured into Macconkey blood, chocolate and Sabouraud's dextrose agar. Incubated at 37°C for 48 hours. Culture plates were examined for colony growth and processed upto species level identification and antibiotic sensitivity. Direct wet mount with saline and KOH was examined by microscopic examination. The culture was performed with Kupferberg media. The swab was inoculated into the media and incubated at 37°C for 7 days. The same was inoculated into Sabouraud's dextrose agar and fungal colonies grown were identified upto species level. Both wet mount, microscopic and Giemsa's staining was done for *Trichomonas vaginalis*.

RESULTS

Analysis of vaginal discharge in sexually active women from rural Ammapettai, Tamil Nadu, India, attending Gynaec OPD of SSS Medical college and Hospital lead to wide range of microbial profile including bacterial, parasitic, fungal agents.

Aerobic gram negative pathogens were the main study pathogens. 100 high vaginal smear from 50 women in the age group of 25-50 years were cultured for aerobic and anaerobic pathogens. Wet mount microscopy in KOH and saline for *Candida* and *Trichomonas vaginalis*, special stain giemsa and culture with Kupferberg media for *Trichomonas* infection was done.

DISCUSSION

Prevalence of aerobic pathogens is shown in Table 1. In present study of 9 smears showed normal vaginal flora 7 mixed infections, 30 *Trichomonas vaginalis*, 5 *Candidiasis* 43 were positive for aerobic and 6 for anaerobic pathogens. Gilbert GG Donders from Belgium has reported in his study 10% were aerobic pathogens.⁵ Sandhya R from Andhra Pradesh found aerobic microbes were 14% and Shameem Mumtaz from Abbottabad, Pakistan has reported 21-30% of aerobic microbes and Karthika from silk city 54%.²⁻⁴

Table 1: Prevalence of aerobic pathogens.

	Prevalence
Normal vaginal flora	-9
Mixed infections	-7
<i>Trichomonas vaginalis</i>	30
<i>Candida</i>	5
Positive for aerobes	43
Positive for anaerobes	6

Table 2: Age groups.

Age group	
20-25 years	20
26-30 years	21
31-35 years	33
36-40 years	15
41-45 years	07
46-50 years	04

Table 3: Age group correlation with.

Years	No. of TV	No. of <i>Candidiasis</i>
20-30	21	nil
31-40	4	5
41-50	4	nil
51 and above	nil	2
Total	30	7

TV more common in 20-30 years. TV infections was more common in young age group and candidiasis in the middle age group. These women present with severe pruritis vulva with DM.

Incidence of *Trichomonas vaginalis* was assessed by wet mount microscope, culture and giemsa stain. Many studies have been done showing varying incidences. In present study 30% women showed TV +ve smear, Gilbert 10-30%, Sandhya R 30-35%, Shameem 45-50% in Pakistani women, Karthika-30% in Indian women, Barry L Harnes 14-35% in South Carolina women and Yasmeen 13.6% TV in Pakistani women.¹⁻⁶ Prevalence of STD, protozoal, TV infection was 30% average in women from various countries.

Candidiasis was studied in wet mount KOH. In present study 7% women had vaginal candidiasis these were

obese, diabetic, presented with curdy white discharge and bleeding PV Shameem from Pakistan had 6-10% smear showing candidiasis.³ Karthika incidence was 2% and Bary L Harnes 17-39% *Candidiasis* from South Carolina women.^{4,5} Yasmen from Pakistan has quoted TV incidence 13.6%.⁶ Distribution of aerobic and anaerobic pathogen shown in Table 4. Analysis of vaginal discharge in the present study, bacteria was positive in 49 women out of which 43% was aerogenic gram negative and 6% gram positive microbes. Normal vaginal flora 9%, mixed infection in 7% were seen. Most common aerobic pathogen was *E.coli* and next was *Klebsiella*. In the present study *E.coli* was seen in 23 smears. *Klebsiella oxytoca* in 8, *Klebsiella pneumonia* in 6 < *Pseudomonas aeruginosa* in 4. Gram positive *Staphylococcus aureus*

seen in 6 smears. Incidence of *E.coli* in Gilbert's study 35– 40%.¹ Sandhya 35-45%, karthika 21%, Shameem 54%.^{2,4} All these observation by various authors or similar as ours. Next common gram negative pathogen was *Klebsiella* in present study. 14% smears were positive for *klebsiella* in Gilbert study 7-8% *klebsiella* and 6-8% were *Staphylococcus aureus*.¹ Sandhya *Staphylococcus aureus* 4-6% *Klebsiella* 13%.² Shameem *Klebsiella* was seen 9-10% *Staphylococcus aureus* in 5% smears Karthika *Klebsiella* in 36 smears and *Staphylococcus aureus* in 16%.^{3,4} In present study *Staphylococcus aureus* gram positive organism was seen in 3 smears *Staphylococcus epidermidis* 1 *pyogenes* 1 enterococcus 20%. 23% smears were positive for *E.coli* pathogen.

Table 4: Distribution of micro-organisms.

Gram positive aerobes	No	Gram negative aerobes	No	Miscellaneous	No
<i>Staphylococcus</i>		<i>E.coli</i>	23	Normal vaginal flora	9
1) <i>aureus</i>	3	<i>Klebsiella oxytoca</i>	8	Mixed infections	7
2) <i>epidermidis</i>	1	<i>Klebsiella pneumoniae</i>	6	TV positive	30
3) <i>pyogenes</i>	1	<i>Pseudomonas aeruginosa</i>	4	<i>Candidiasis</i>	5
<i>Enterococci</i>	1	<i>Acetobacter bowmanii</i>	2		
Total	6		43		

Prevalence of gram negative aerobes 43%, Prevalence of gram positive anaerobes 6%

Sensitivity pattern in aerobic and anaerobic microbes is shown in Table 5.

We had 43 smears positive for gram negative aerobes. Sensitivity pattern of aerobic pathogens showed 100% sensitivity to imipenem, amikacin and gentamycin. They were 100% resistant for cefazolin 80% ceftazidime and

75% for cefloxime. Gram positive staphylococci were 100% sensitive to oxacillin 50% were resistant to gentamycin and ampicillin. Similar observations have been made by Sandhya R, Shameem, Karthika where *E.coli* was the predominant aerobic gram negative bacilli shown in vaginal smears and they were highly sensitive to amikacin and gentamycin.²⁻⁴

Table 5: Sensitivity pattern.

Name of bacteria isolated	Sensitivity pattern					
	IMI%	AK%	G%	CZ%	CA%	CE%
<i>Escherichia coli</i>	100	100	100	0	20	26
<i>Klebsiella oxytoca</i>	100	100	100	0	20	26
<i>Klebsiella pneumoniae</i>	100	100	100	0	20	26
<i>Pseudomonas aeruginosa</i>	100	100	94	0	15	22
<i>Acinetobacter baumannii</i>	100	100	90	0	10	20
<i>Staphylococcus epidermidis</i>	-	90	100	10	100	100

IMI- imipenem AK- amikacin G- gentamycin CE- cefazolin CA-ceftazidime CE- cefotaxime. *Staphylococcus epidermidis* 100% sensitive for oxacillin and vancomycin, resistant 50% to gentamycin, ampicillin. Gram negative bacilli- resistant for cefazolin 100%, ceftazidime 80% and cefotaxime 74%.

CONCLUSION

The analysis shows the correlation between bacterial and parasitic infections common among sexually active women. Women with poor personal hygiene, malnutrition, anemia come with recurrent episodes of

vaginitis. Unprotected coitus and unhygienic practices help in transmitting STD, HIV AND HSV infections. Recurrent vaginal infection is common in women because of close proximity of vaginal wall, urethra and anus. In the present study the incidence of abnormal vaginal flora was 43%. STD protozoals *Trichomonas*

vaginalis 30% anaerobic vaginal anaerobes were 6% *Candidiasis* 5% 23% of aerobic gram negative microbes was *E.coli* followed by *Klebsiella* 14%. In gram-negative anaerobes were 100% sensitive to imipenem, amikacin and gentamycin. *Staphylococcus* were 50% resistant to amikacin and gentamycin but 80% sensitive to ampicillin and amoxicillin. This study reflects on our responsibility to create awareness among women regarding abnormal vaginal discharge through proper health education routine screening targeted treatment to envisage healthy quality life.

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