Microbiological profile of leucorrhoea in patients attending a tertiary care hospital at Jalna

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Abstract

Introduction and Objective: Leucorrhoea is an abnormal white, yellow or greenish vaginal discharge associated with irritation, is non-hemorrhagic in nature and signifies some underlying pelvic pathology. It is common complaint of patients attending Obstretic and Gynaecology department. There are three main causes of vaginal discharge -1.Bacterial vaginosis (BV) 2.Vulvo-vaginal candidiasis (VVC) 3. Trichomoniasis. Symptomatic vaginal discharge needs to be evaluated to ascertain the cause so as to institute proper treatment. So the present study was undertaken to screen abnormal vaginal discharge in women to diagnose BV, VVC and Trichomoniasis.

Materials and Methods: We included 175 patients with main complaints of leucorrhoea .The study was carried out in the the Department of Microbiology, Indian Institute of Medical Science and Research Jalna, for a period of 1 year. High vaginal swabs were taken from these patients and assessed by wet mount, gram staining, Nugent's score and culture on respective media followed by identification of candida on Hicrome agar.

Results: Majority of patients belonged to the reproductive age group (15-45years). Out of the 175 patients, 67(38.28%) patients showed normal flora in which no etiology could be elicited. Remaining 108(61.71%) patients had a definite etiological agent amongst which bacterial vaginosis was diagnosed in 68 (38.85%)patients as per the Nugent's Score.22(12%) patients revealed candidiasis, and 18 patients (10%) showed *Trichomonas vaginalis* on wet mount examination. When further speciation of *Candida* were done, on Candida Hicrome agar, it was found that 13 (59.1)were *C.albicans*,5 (22.72%)were *C glabrata*,3 (13.63%)were *C parapsilosis* and only one strain was *C krusi*(4.5%)

Conclusion: Bacterial vaginosis remains the most predominant cause of leucorrhoea in sexually active women. For confirmation of clinical diagnosis, microbiological investigations have to be performed so as to improve the treatment outcome.

Keywords: Leucorrhoea, Bacterial Vaginosis, Vulvovaginal Candidiasis, Nugent's Score.

Introduction

Leucorrhoea is excessive vaginal discharge commonly encountered in females of reproductive age group (15-45 years). It may be observed in physiological conditions like pregnancy, premenstrually, or during ovulation. Pathological vaginal discharge occurs in 1-14% of all women in the reproductive age group and is responsible for 5-10 million OPD visits per year throughout the world.[1] Three main causes of pathological leucorrhoea are bacterial vaginosis, candidial vulvo vaginitis and trichomoniasis.[2] Bacterial vaginosis (33-47%)[3] is the most common cause of symptomatic vaginal discharge, followed by candidiasis (20-40%) and trichomoniasis (8-10%).[4,5] These three conditions account for 90% of all etiologies of abnormal vaginal discharge. Multiple infections can also coexist.[3]

Bacterial vaginosis is termed vaginosis rather than vaginitis because this is associated with alteration in normal flora rather than due to any specific inflammation. There is considerable decrease in number of lactobacilli in the vagina and altered pH and is characterised by increased growth of other bacteria. Common agents of bacterial vaginosis include *Gardnerella vaginalis*, moblincus, bacteroides, saprophytes and *Mycoplasma hominis*, etc.[6] Vulvovaginal candidiasis is characterized by pruritis and

cotton cheese like discharge caused by candida species.[7]

Vaginal trichomoniasis is associated with a copious yellow or green sometimes frothy discharge caused by *Trichomonas vaginalis*.[7]

Leucorrhoea predisposes to significant morbidity in the form of pelvic inflammatory diseases, infertility, endometriosis, cuff cellulitis, urethral syndrome, pregnancy loss, and preterm labour.[8] The management of vaginal discharge is largely syndromic and empirical, it is usually based on naked eye examination of vaginal discharge and that is unsatisfactory because the diagnostic accuracy is lost without microscopic examination.[9] It not only has a financial and social impact leading to noncompliance on the part of patients, but also contributes to overall emergence of resistance.[10] Considering all these issues ,the present study was undertaken to screen all the women presenting with leucorrhoea and determine the microbiological profile of symptomatic vaginal discharge in them.

Materials and Methods

This is an observational kind of descriptive study, carried out in the Department of Microbiology, Indian Institute of Medical Science and Research Jalna, a tertiary care hospital, for a period of 1year from 1st June

2015 to 31st May 2016. After obtaining ethical committee clearance, a total of 175 women having complaints of white discharge per vaginum, abdominal or back pain, pruritis and dysuria that were referred from the department of Obstetric and Gynaecology were included in the study. Informed consent was taken from them.Patients on antibiotic treatment, those who had genital prolapse, who were pregnant or who had delivered or aborted six weeks prior were excluded from the study.

Collection of sample

A sterilized Cusco's speculum was inserted into the vagina to visualize the vagina and cervix. The amount, colour, character and smell of the vaginal discharge in the vagina were noted. Vaginal pH was recorded by using pH strips with in a range of pH 2.0 - 10.5 from the mid-lateral vaginal wall. Three vaginal swabs were collected with sterile cotton swabs from the posterior fornix and transported to the Microbiology laboratory for further processing.

Microscopic Examination
A. **Saline Wet Mount:** One swab was used immediately to prepare a wet mount with one to two drops of normal saline on a glass slide and was examined by light microscopy for the presence of motile pear shaped trichomonas and pus cells.
B. **Gram smear examination:** The second swab was taken and smears were made on a clean and grease free glass slide, heat fixed by just passing the glass slide over the flame. Then the smear was stained by Gram's method and observed under oil immersion objective. The smear was evaluated for the presence of pus cells, vaginal epithelial cells, various bacterial morphotypes, clue cells & yeasts. Large gram-positive rods were taken as lactobacillus morphotypes, smaller gram-negative to gram-variable rods were considered as *G. vaginalis and Bacteroides spp.* Nugent's score(Table 1) was applied for the diagnosis of bacterial vaginosis.[11] The sum of the 3 scores was taken and a score of 7 or more was considered the "operational definition" of bacterial vaginosis.

Table 1: The Nugent scoring system for diagnosis of *Bacterial vaginosis*

Score	Lactobacillus morphotype per field	Gardnerella morphotype per field	Curved bacteria (mobiluncus) per field
0	>30	0	0
1	5-30	<1	1-5
2	1-4	1-4	>5
3	<1	5-30	5-30
4	0	>30	>30

C. **Isolation by Culture Methods:** Third vaginal swab was inoculated on Blood agar, Maconkeys

agar, Chocolate agar, Sabourauds dextrose agar(SDA). *Candida species* were identified after 48hrs of incubation, depending on colony morphology from SDA agar. Gram stain and germ tube testing were done for confirmation of *Candida albicans*. For species identification of candida, HiCrome candida differential agar was used. *Candida albicans* produced green colonies, *Candida tropicalis* produced blue coloured colonies, *Candida parapsilosis* cream coloured colonies and *Candida krusi* purple coloured colonies on Candida hicrome agar.

Results

A total of 175 patients were evaluated during a period of one year. Majority of the patients were in the reproductive age group, and were married.36.57% of patients were in the age group of 26-35 years followed by 34.28% patients in age group of 15-25 years.(Table2)

Table 2: Distribution of cases according to their age

Age	No. of patients n=175	Percentage
15-25 years	60	34.28
26-35	64	36.57
36-45	28	16
Above 45	23	13.14
Total	175	100

No abnormal finding was revealed in 67 patients (38.25%)(Table3). They showed normal flora i.e. epithelial cells, lactobacillus or few pus cells etc. which did not satisfy the Nugent's score, so these patients were considered to be suffering from physiological leucorrhoea. Clue cells were observed in 27.42% of cases on gram stain indicating bacterial vaginosis. Budding yeast cells were seen in 16% and *Trichomonas vaginalis* in 10.28%. *Trichomonas vaginalis* were seen as motile pear shaped protozoa on wet mount along with abundant polymorphoneutrophils.

Table 3: Comparison of wet mount and gram staining results

Wet mount /gram stain	No. of patients	Percentage
Pus cells	20	11.42
Trichomonas vaginalis	18	10.28
Clue cells	48	27.42
Budding yeast cells	28	16
Inconclusive	67	38.25

As per Nugent's criteria, bacterial vaginosis was confirmed in 68(38.85%) patients (Table 4). 28 cases revealed budding yeast cell in wet mount and gram stain, of which 22(12%) was isolated by culture on SDA. When further speciation of these were done, on Candida

Hicrome agar. It was found that 13(59.1%) were *C.albicans*,5(22.72%) were *C glabrata*,3 (13.63%)were *C parapsilosis* and one strain (4.5%)was *C krusi*. (Table 5)

Table 4: Classification of patients as per etiological agents

Etiological agents	No. of patients	Percentage
Bacterial vaginosis	68	38.85
Candida species	22	12.57
Trichomonas vaginalis	18	10.28

Table 5: Speciation of *Candida* on Hicrome agar n=22

Species of candia	No. of isolates	Percentage
Candida albicans	13	59.1
Candida glabrata	5	22.72
Candida parapsilosis	3	13.63
Candida krusei	1	4.5

Discussion

Leucorrhoea is a very distressing problem in women of childbearing age group. In the present study, 175 women from the Obstetric and Gynaecology department were followed for assessment of leucorrhoea. Majority of cases were found to be in the age group of 25-35years (36.57%) followed by 15-25 years (34.28%) This represented the reproductive age group in our rural population with clustering of cases around 20-25 years. Similar peak age incidence have been reported by Rekha et al, Lavanya et al and Masand et al.[8,2,12] This could be because of increased sexual activity, childbirths and the use of contraceptive devices at this age.

Bacterial vaginosis was found to be the most common cause of leucorrhoea(38.85%) as calculated by the Nugent's score. Various studies have revealed bacterial vaginosis as 32.3%[13] 26%[14] 28%.[7] Chaudhari et al have reported incidence of bacterial vaginosis as (36.68%).[15] Our study corresponded with these findings.

Candidiasis was observed in 22(12%) patients, though on direct smear, a few more cases were detected. We considered culture as more definitive than direct smear, hence only culture positive cases were included in this study. Sevitha et al have reported Candidiasis in 15% of patients with leucorrhoea,[16] Masand et al have reported Candidiasis in 14% of patients[12] while Lavanya et al[2] have reported Candidiasis in 22.4% of patients. The main symptom in these patients was itching but the character of discharge could not be elucidated properly in these patients. On speciation on candida Hicromeagar, *Candida albicans* was the most common isolate followed by *Candida glabrata, Candida*

parapsilosis and Candida krusi. Similar findings have been elucidated by Aring et al.[17]

Finally *Trichomonas vaginalis* as a causative agent was found in 18(10%) of patients which corresponded to the studies done by Nishat et al.[18] Overall prevalence of trichomonal vaginosis varies from place to place and from study to study and ranging from 6-14.9%.[2] Another consistent finding, we observed was increase in polymormhoneutrophils in patients suffering from trichomoniasis.

Conclusions

Bacterial vaginosis remains the most predominant cause of leucorrhoea in sexually active women. Microscopic wet mount and Gram staining can be used as reliable test for determining abnormal vaginal discharge. Amsel's criteria[19] of characteristic thin homogenous consistency, pH determination, Whiff test and clue cells to diagnose abnormal discharge correlated with most of our cases, but the definitive diagnosis could only be established by microbiological evidence. Hence, physical diagnosis by Amsel's criteria can still be followed in resource poor setting but if facilities are available, clinicians must bank on microbiological evidence for proper treatment.

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