

Evaluation of role of cytology and its pitfall in cytodiagnosis of vaginal discharge

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Abstract

Aim and Objectives: The present study was carried out with an objective 1) To find out the causes of discharge per vaginum, 2) To know the incidence of various causes of vaginal discharge, 3) To evaluate the role of cytology and its pitfall in cytodiagnosis of vaginal discharge, and 4) It can act as a screening procedure for cervical malignancies.

Methods: Total 150 cases of leucorrhoea were studied using pH determination, wet mount, KOH mount, amine test, Gram staining and papanicolaou staining methods, during a period of one year.

Results: Using all above methods we were able to detect non-specific vaginitis [70 (46.66%)], *trichomonas vaginitis* [24 (16.00%)], *senile vaginitis* [10 (6.66%)], *candida vaginitis* [13 (8.66%)] and *gonococcal vaginitis* [1 (0.66%)] while 24 (16.0%) cases were without any specific pathology. We found 3 (2.00%) cases suggestive of cervical erosion and 5 (3.33%) cases suggestive of squamous malignancies as a cause of discharge per vaginum. Out of 70 cases of non-specific vaginitis, 50 were suggestive of bacterial vaginosis. Pap stained smears were found to be 96.28% sensitive and 96.77% specific in diagnosing bacterial vaginosis while in diagnosing vaginal mycosis it was 48.22% sensitive and 100% specific as compared to cultures. KOH mount was 93.75% sensitive with 100% specificity in comparison to cultures.

Conclusion: In a low resource setting, primary clinical diagnosis based on simple microscopy, pH determination, wet and KOH mount, amine test, gram staining should be made prior to pap stained screening to reach appropriate diagnosis in patients of vaginal discharge.

Keywords: Cytodiagnosis, Vaginal discharge, Gonococcal vaginitis, Bacterial vaginosis, Malignancies.

1. Introduction

The complaint of vaginal discharge is a common presenting problem in any physician's office. It can be quite bothersome to the patient. Symptomatic vaginal discharge is caused by inflammation due to infection of the vaginal mucosa. It occurs in 1-14% of all women in the reproductive age group and the prevalence of vaginal discharge in India is estimated to be 30% [1]. Vaginal

discharge may be a normal physiologic occurrence or a pathological manifestation. It is often challenging to distinguish abnormal from normal discharge, both from the patient's and the health care provider's perspective. Moreover, normal physiologic variations occur due to biological or hormonal changes [2,3]. When considering the causes of vaginal discharge, it is useful first to distinguish between non-infectious and infectious causes, (Table 1).

Table 1: Causes of Vaginal Discharge

Non-infectious Causes		Infectious Causes	
Physiological	Non-Physiological	Vaginal	Cervical
Puberty	Foreign body	<i>Candida species</i>	<i>Neisseria gonorrhoea</i>
Menstrual cycle	Chemical	<i>Trichomonas vaginalis</i>	<i>Chlamydia trachomatis</i>
Sexual activity	Drug induced	Bacterial vaginosis (formerly nonspecific vaginitis)	<i>Herpes simplex virus</i>
Pregnancy	Gynecologic abnormalities		
Menopause			

However the vaginal discharge is a common symptom of genital infection in women, identifying its source can be challenging, because a large number of pathogens cause vaginal and cervical infection, and several infections may co-exist [4]. Patient history and physical examination findings may suggest a diagnosis. Most of the time a presumptive diagnosis is made based on the nature of the discharge (clinical diagnosis), which is often inaccurate and incomplete. This eliminates the laboratory component (Microbiological diagnosis) leading to treatment mismanagement [5]. To address the limitations of clinical diagnosis, the World Health Organisation (WHO) developed and advocated the syndromic management approach, but this management has some disadvantages.

The addition of a simple microscopic evaluation by Gram stain of the vaginal smear has evolved as a sensitive noncultural diagnostic technique for Bacterial vaginosis and Candidiasis [1]. Direct microscopy (wet smear) of the vaginal discharge to visualize the motile *Trichomonas vaginalis* has been determined to be as accurate as culture for the diagnosis of Trichomoniasis. Also, useful tests for etiological diagnosis include pH analysis of vaginal fluid, the "whiff test", wet mount examination, culture and Gram's stain [6]. Hence, the present study was carried out with an objective to find out the causes of discharge per vaginum and evaluate the role of cytology and its pitfall in cytodiagnosis of vaginal discharge.

2. Materials and Methods

Total 150 cases were selected from patients attending gynaecology outpatient department and family planning clinic. Formal consent for collection of specimen was sought and patients were requested to complete a short confidential questionnaire. Inclusion criteria of patients were- women should not use any vaginal medication 48 hour prior to sample collection, per vaginal examination should not be carried out prior to smear obtained. No coitus 48 hours prior to specimen collection. A detailed history, female genital system examination and all relevant investigations were done for all the patients. After using all aseptic precautions vaginal speculum is introduced inside and presence of vaginal discharge noted. Vaginal discharges distinguish in several criteria according to Eschenbach D. A. 1983 [7].

Indicator papers (Strips of papers impregnated with indicator substances) ranging from 4.5 to 7.5 in units of 0.5 were used for pH measured of vaginal fluid. They were touched to small amount of discharge and/or gently rubbed against vaginal wall. Color change was observed which was matched with the standard paper provided with the same pH indicator paper. *Amine Test*: It was done according to procedure given in Adler study [8]. Direct microscopy (wet mount) of the vaginal discharge was done

according to procedure given in Collee *et al* [9]. *KOH Mount*: To a drop of suspension of vaginal discharge in saline on a slide added a drop of fresh 10% KOH solution, after mixing two, cover slip was placed on top of it and slide warmed slightly. Slide was observed under high power object to note the presence of budding yeast form or pseudohyphae. KOH dissolves all cellular debris and makes the fungal identification easier. *Posterior Vaginal Cytology*: By using sterile cotton swab vaginal secretions were collected from posterior pool and smears were prepared on clean glass slide. Smears were immediately dropped in 95% ethyl alcohol for fixation and further carried to cytology laboratory for papanicolaou staining. *Cervical Cytology*: By using Ayres wooden spatula smear samples were collected from ectocervix and endocervix and smeared on clean glass slides. Smears were dropped immediately into 95% ethyl alcohol for fixation and further carried to cytology laboratory for papanicolaou staining. This staining was done as per method described in Hughes and Dodds [10]. *Gram Staining*: the dry smear which was carried to microbiology laboratory was fixed by passing it through Bunsen flame. Gram stained smear were also screened for gram positive yeast cells and pseudohyphae. Culture reports of the specimens were obtained from the patients.

3. Observations and Results

Among 150 cases, maximum number of patients was observed in age group of 21-30 years (70) followed by age group of 31-40 years (35), 41-50 years (30), above 50 years (16) and 15-20 years (07). Table 2 shows the possible etiologies of vaginal discharge in patients, of these etiologies non-specific vaginitis accounted for maximum number of cases (70) followed by *trichomonas vaginitis* and no specific pathology (24 patients in each).

Table 2: Possible etiologies of vaginal discharge in patients

Etiologies	No. of patients (%)
Non-specific vaginitis	70 (46.66%)
<i>Trichomonas vaginitis</i>	24 (16.00%)
<i>Candidial vaginitis</i>	13 (8.66%)
<i>Neisseria gonorrhoea</i>	1 (0.66%)
<i>Cervical erosion</i>	2 (1.33%)
<i>Senile atrophic vaginitis</i>	10 (6.66%)
Malignancy	6 (4.00%)
No=specific pathology (within normal limits)	24 (16.00%)

In table 3, cytologically 5 cases of squamous malignancies were obtained. 14 cases were suggestive of low grade squamous intraepithelial lesion (i.e. cervical intraepithelial neoplasia 1/mild dysplasia). 5 cases were suggestive of high grade squamous intraepithelial lesion (3 cases were showing moderate dysplasia and 2 cases were showing sever dysplasia).

Table 3: Cytological Findings in Patients

Etiologies	No. of patients (%)
Non-specific pathology	24 (16.00%)
Inflammatory smears	86 (57.33%)
<i>Senile vaginitis</i>	11 (7.33%)
Cervical erosion	03 (2.00%)
Squamous metaplasia (with ICC)	02 (1.33%)
Mild dysplasia with ICC	14 (9.33%)
Moderate dysplasia with ICC	03 (2.00%)
Severe dysplasia with ICC	02 (1.33%)
Squamous Malignancies	05(3.33%)

ICC= Inflammatory cell changes

Maximum incidence of bacterial vaginosis, *trichomoniasis* and *candidial vaginitis* was mainly seen in the age group of 21-30 years, (Table 4). Vaginal discharge seen in the patients with bacterial vaginosis have shown pH range between 5-5.9 while in *trichomoniasis* maximum pH range was also observed in between 5-5.9 as shown in table 4.

Table 4: Incidence of *G. vaginalis*, *T. vaginalis* and *Candida* as well as table show relationship between pH of vaginal discharge and infective organism

Age in Year	<i>G. Vaginalis</i>	<i>T. Vaginalis</i>	<i>Candida</i>
15-20	4 (8.33%)	00 (0.00%)	00 (0.00%)
21-30	22 (45.83%)	11 (45.83%)	08 (61.53%)
31-40	14 (29.16%)	08 (33.33%)	03 (23.07%)
41-50	4 (8.33%)	03 (12.50%)	02 (15.38%)
Above 50	4 (8.33%)	02 (8.33%)	00 (0.00%)
Total	48	24	13
pH range	<i>G. Vaginalis</i>	<i>T. Vaginalis</i>	<i>Candida</i>
4.4-9	05 (10.00%)	07 (24.13%)	06 (35.29%)
5-5.9	41 (82.00%)	19 (65.51%)	09 (52.94%)
6-6.9	04 (8.00%)	03 (10.34%)	02 (11.76%)
Total	50	29	17

The prevalence of individual criteria in diagnosis of bacterial vaginosis and the predictive value of the diagnostic criteria of Amsel and Spiegel were shown in table 5.

Table 5: Prevalence and predictive value of individual diagnostic criteria used for the diagnosis of bacterial vaginosis

Diagnostic criteria	Women with Bacterial Vaginosis	Women without Bacterial Vaginosis	Sensitivity %	Specificity %	PPV %	NPV %
pH >4.5	45	40	91.38	64.71	51.47	93.28
Clue cells on wet mount	49	06	100	95.48	92.28	100
Amine test + ve	50	03	100	96.38	95.44	100
Vaginal discharge (Homogenous, Gray)	51	23	97.14	82.17	71.21	98.10
Gram staining + Clue cells	44	00	90.35	100	100	95.23
Pap smear + Clue cells	48	02	96.28	96.77	95.14	98.14

PPV= Positive predictive value, NPV= Negative predictive value

Respective culture reports from 17 patients showed growth of *candida* on sabouraud's Agar. Out of 17, 15 patients were KOH mount positive. Cytology was positive in 8 patients and gram staining showed positivity of 94.11%, (Table 6). KOH mount and gram staining were

found to be more sensitive and highly specific but cytology was not found to be that sensitive, though specific, (Table 6). In case of *trichomonas vaginalis*, wet smear was positive in 27 cases while cytology was found to be positive in 12 cases.

Table 6: Method used in demonstration of candidiasis and sensitivity and specificity of criteria used in diagnosis of candidiasis

Method	No. of Patients	Percentage	Sensitivity (%)	Specificity (%)
Cytology	8/17	47.05	47.00	100.00
KOH mount	16/17	94.11	93.75	100.00
Gram stainig	16/17	94.11	93.75	100.00
Culture	17/17	100.00	100.00	100.00

Table 7 show the incidence of infective organisms according to contraceptive method used. In this table others

include patients with bilateral tubal ligation, using barrier contraceptive or vasectomy of husband.

Table 7: Incidence of infective organisms according to contraceptive method used

Contraception	<i>G. Vaginalis</i>	<i>T. Vaginalis</i>	<i>Candida</i>
IUCD	17	07	02
Oral pills	05	04	09
Others	22	12	03
None	06	05	01

4. Discussion

Total 150 women presented with chief complaint of white discharge per vaginum were included in the study. Vaginal infections commonly occur in women of

reproductive age i.e. between 25-35 years as noted in several other studies. In Rekha *et al* [11] age group was 26-30years while in Varghese *et al* [4] it was 26-35years. In present study we found most common in 21-30 years age

group. Etiological diagnosis was reached in all over the patients by using pH determination, wet mount, KOH mount, amine test, gram staining and papanicolaou staining method. The largest group comprised of 70 cases suggestive of non-specific vaginitis and second largest group comprised of 24 cases suggestive of T. vaginitis and 24 cases showing no specific pathology. Out of 70 cases of non-specific vaginitis, 50 were suggestive of bacterial vaginosis and 20 were suggestive of non-specific infection. Patients without any specific pathology seen on cytological smears were showing mostly normal flora, predominantly gram positive lactobacilli, without any local pathology. So these discharges may be due to physiologic leucorrhoea or may be due to frequent erotic stimulation, oral contraceptives or frequent vaginal douching [12]. No specific pathology (within normal limits) was classified according to Bethesda classification 1991. Vaginal discharge is a continuum and as such the concept of normality doesn't exist. Some patients have a copious discharge, others none or little. Only the patient can determine what her normal experience is [8].

Prevalence of bacterial vaginosis, trichomoniasis and candidiasis in the present study was 46.66%, 16%, 8.66% respectively; this finding was correlated with the finding of previous studies [11,13-15]. Bacterial vaginosis was the commonest diagnosis in this study. Among the individual criteria used for bacterial vaginosis, homogenous vaginal discharge found to be 97.14% sensitive and 82.17% specific. The overall pH of vaginal fluid shows that maximum pH ranges between 5-5.9. A raised pH i.e. >4.5 has shown sensitivity of 91.38% but less specificity of 64.71% in diagnosis of bacterial vaginosis. Clue cells in wet mount were found to be 100% sensitive and 95.48% specific. Amine test sensitivity was 100% and specificity was 96.38%. Detection of amine odour is observer dependent with wide person to person variability. The amine test is easily performed, rapid, inexpensive diagnostic test with good sensitivity and specificity which as suggested by previous studies, is ideally suited to clinical settings where microscopy is not available [16]. Gram staining was found to be 90.35% sensitive and 100% specific. So, all these methods (pH determination, wet mount, KOH mount, amine test, gram staining and papanicolaou staining) have adequate sensitivity and specificity and can alert pathologist to look for clue cells in pap stained smears. Papanicolaou smears are performed routinely; it would be of great benefit if this method could be used for bacterial vaginosis as the basis of routine screening. In present study, pap stained smears were found to be 96.28% sensitive and 96.77% specific in diagnosing bacterial vaginosis while in diagnosing vaginal mycosis it was 48.22% sensitive and 100% specific as compared to cultures. Despite the obvious superiority of cultures their routine use in practice is cumbersome and costly. Present

study showed that properly screened KOH mount should help to establish accuracy in the diagnosis of moniliasis. KOH mount was 93.75% sensitive with 100% specificity in comparison to cultures. Above findings of individual criteria were correlates well with the findings of Christensen *et al* [17].

The present study showed cytologic smear positivity in case of trichomoniasis as 46.6% in comparison with wet smear. Thus the study clearly demonstrated that the need to be extremely critical in evaluating possible trichomonads in the stained smears, as these microorganisms are reported in routine cervico vaginal smears much too often on the basis of in-conclusive morphologic and inflammatory cell changes, cultures though reliable need special media and cumbersome. The study also showed 14 cases suggestive of low grade squamous intraepithelial lesions (mild dysplasia/CIN I) associated with inflammatory changes. 12 of them after appropriate treatment were relieved and 4 of them were asked to come for follow-up, 3 returned and showed regression in mild dysplastic changes. 1 patient did not return back. The study showed 3 cases suggestive of moderate dysplasia, 2 cases suggestive of severe dysplasia and 5 cases suggestive of squamous malignancies. Patients with high squamous intraepithelial lesions (moderate dysplasia /CIN II and sever dysplasia/CIN III) were asked to come for follow up but none of them reported back. Hence in patients presenting with discharge per vaginum one should be cautious and careful so that one should not miss precancerous lesions as well as cervical malignancies. Rate of accuracy in the diagnosis can be increased if vagino-ecto-rndocervical smears are sent to cytology laboratory in patents who present with discharge per vaginum.

5. Conclusion

It is concluded that bacterial vaginosis common finding in our set up and it can be diagnosed early by Pap smears. In a low resource setting, primary clinical diagnosis based on simple microscopy, pH determination, wet and KOH mount, amine test, gram staining should be made prior to pap stained screening to reach appropriate diagnosis in patients of vaginal discharge.

The study suggested that the patients with the complaint of vaginal discharge and symptoms of vaginitis comprise a substantial proportion of those presenting with sexually transmitted infections. Sexual transmission of *Candida* species and bacterial vaginosis remains controversial, while it is a primary cause of the acquisition of trichimoniasis. Symptoms and physical findings are insufficient to distinguish specific etiologic agents, and as the treatment for each is different, diagnostic examination of smears is critical to the management of these patients. Further studies are needed to know the utility of the various

diagnostic approaches and the best approach that could be implemented in the rapid and accurate diagnosis of symptomatic vaginal discharge.

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