

Role of cervical/vaginal cytology in the diagnosis of cervicitis /vaginitis in a rural setupAnuja Mishra¹, Tanwi Singh^{1*}, Nidhish Kumar¹, K.M. Hiwale²¹Senior Resident, Department of Pathology, Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India²Professor, Department of Pathology, Jawaharlal Nehru Medical College, Wardha, Maharashtra, India

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Abstract

Introduction: Vaginal discharge in the reproductive age group is the most common complaint encountered everyday. Among the cases of symptomatic vaginal discharge Bacterial vaginosis is commonest cause followed by candidiasis and trichomoniasis. Multiple infections can also coexist but these three conditions account for majority of all etiologies of abnormal vaginal discharge. **Material and methods:** There were 100 women recruited for the study between 20-45 years with the objectives to recognize the cytological and microbiological culture manifestations in vaginitis/cervicitis by correlating the cytological findings with diagnostic culture method to confirm the diagnosis. All the patients with signs and symptoms of vaginitis, who attended the obstetrics and gynecology outpatient department, were subjected to detailed clinical examination and vaginal smearing with a prior consent, a comprehensive history, general examination and gynecological examinations. After that, the papanicolaou test was done on these patients. Three air dried smears for gram stain, giemsa stain, PAS stain and one wet fixed smear for pap stain was prepared along with a wet mount preparation and sent to the department of pathology immediately. **Results:** The most common organism causing vaginitis was Gardnerella vaginalis which causes bacterial vaginosis (20%), next common organism detected was E.coli (16%), the least commonly detected organisms in our setup was candida albicans, S. epidermidis, actinomycetes, and trichomonas vaginalis. The culture method determined the organisms and helped in coming to the accurate diagnosis of the cervical smear and hence it was taken as gold standard. **Conclusion:** It was concluded that women with inflammation on pap test had a significant decrease in the percentage of the negative culture and increase in the percentage of a positive culture. Thus, the role of cervical or vaginal cytology in the diagnosis of cervicitis/ vaginitis should be considered in the normal treatment protocol

Keywords: Vaginal discharge, Bacterial vaginosis, Candidiasis, Trichomoniasis, Culture method.

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Introduction

The cervical and vaginal infections are not uncommon in our population especially in young and sexually active women.¹ These infections are common problems in women of a reproductive age group that incurs the substantial cost and are associated with clinical complaints of vaginal discharge, odor, and pruritus^[2]. Abnormal vaginal discharge is a characteristic feature of vulvovaginal infections (VVI). It occurs in 1-14% of all women among the reproductive age group around the world and its prevalence in India is estimated to be 30%.³ The most common documented causes of symptomatic vaginal discharge include bacterial vaginosis followed by vulvovaginal candidiasis (VVC) and trichomoniasis^[4]. Earlier studies have reported the co-existence of these VVI. Vaginal infections are known to increase the susceptibility to sexually transmitted infections including HIV and are associated with complications like low birth weight and premature birth. The prevalence and incidence of and risk factors for vaginal infections are not well studied in many settings. Further, the aforementioned consequences of these infections are

highly prevalent in low income settings providing ample justification for additional research required to confirm the diagnosis and associated risk factors for these vaginal infections. Vaginal complaints are one of the most common reasons for women to seek medical advice. The diagnosis of vaginitis typically begins with a history of the vaginal symptoms and physical examination. It has been noted that early diagnosis by a trained specialist would help women to get timely and efficient treatment limiting the cost of diagnostic tests, the adverse effects of inadequate treatment, and unnecessary anxiety. This study was undertaken to estimate the prevalence of vaginitis / cervicitis in a rural setup and compare the results of vaginal culture method considered as a gold standard test for diagnosing vaginitis/ cervicitis with the cytological pap test. With backdrop of the gap in understanding the role of cervical cytology in diagnosing cervico-vaginal infections; a research question is created as, "Is cervical cytology adequate at detecting the infection for its ideal early treatment?" The hypothesis is constructed for the relevance as; cervical cytology is not diagnostic enough to detect the offending organisms therefore auxiliary tests are needed. The aim of current modern medicine era is to diagnose the disease at its early course. The present study provides the various investigative modalities so as to avoid the complications, improve the prognosis and to change the treatment protocol as required by co-relating the cytodiagnosis technique with the help of different types of stain. It will be helpful to find out the diagnostic accuracy of each one of the test done during the study.

Materials and Methods

Source of data: Material for this study was collected from the patients attending the out-patient clinics of the department of

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Obstetrics and gynecology of A.V.B.R.H, and its cytological and culture diagnosis was carried out in the department of Pathology & department of Microbiology respectively, Jawaharlal Nehru Medical College, DMIMS, Wardha, Maharashtra.

The details including name, age, sex, MRD number, diagnosis were included after due permission from ethical committee of the college.

Study period: 1st July 2014- 31st July 2016, Sample size: 100 cases.

Inclusion criteria: All the patients, clinically having the symptoms of cervicitis & vaginitis, were included in the study.

Exclusion criteria: Patients in menstrual period and patients who had taken antibiotics or received any treatment for vaginitis within the previous month were excluded from this study.

Method of collection of data: All the patients with signs and symptoms of vaginitis who attended the obstetrics gynecology outpatient department, were subjected to detailed clinical examination and vaginal smearing. With the prior consent, a comprehensive history, general examination and gynecological examination were initially carried out. A pap smear, also known as papanicolaou smear, is a microscopic examination of cells scraped from the cervix and is used to detect cancerous or pre-cancerous or infectious conditions of the cervix or other medical conditions. The cervix is composed of columnar epithelium, which lines the endocervical canal and squamous epithelium which covers the ectocervix. The point at which they meet is called the squamocolumnar junction Metaplasia advances from the original squamocolumnar junction inwards toward the external os and over the columnar villi which establishes an area called the transformation zone. It is this area from where the sample is taken for the Pap smear. A pap test should be performed during the second half of the menstrual cycle (Day 14). Sample collection usually begins with the appropriate instruction to the patient. Patients must abstain from sexual intercourse and avoid using any vaginal medication or contraceptives 48 hours before sample collection. The patient is placed in lithotomy position and the cervix is visualized by means of a speculum. The smaller end of the Ayre's spatula is introduced through the external os and the squamocolumnar junction is scraped by rotating the spatula to 360°. The scraping is then evenly spread onto a glass slide, which is immediately fixed using 95% ethyl alcohol and ether to avoid air-drying artifacts. Three air dried smears for gram, periodic-acid schiff, giemsa stain & one wet fixed smear for papanicolaou stain are prepared along with a wet mount preparation with normal saline and sent to the department of pathology immediately. All the smears are carefully examined for the various cytological manifestations and the causative agent. Wet microscopy provides the simplest, practical, inexpensive and yet objective means for the evaluation of the disturbances of vaginal flora in women.

Saline wet mount: A small proportion of vaginal discharge is placed on a microscope slide and one drop of saline is added. The specimen is then covered with a glass cover and examined under a microscope. Initially, read at 10x for trichomonas and yeast. Scan the whole coverslip for Trichomonas. Clue cells are epithelial cells of the vagina whose borders are difficult to see because so many bacteria are found on the surface of the cells. The clue cell was one of the clinical criteria introduced by Gardner and Duke. These cells

were the clue to the diagnosis. The vaginal culture swab from the posterior vaginal vault or vaginal orifice is collected and transported to the laboratory as soon as possible

Vaginal Culture Studies: The vaginal swabs collected simultaneously were cultured on blood agar and Sabouraud's glucose agar (pH 5.4). The blood agar plates were evaluated following 24-48 hours incubation at 37°C in micro aerophilic conditions, and sabouraud's glucose agar were evaluated after 2-3 weeks incubation at 37° C. Positive cultures for *G. vaginalis* were defined as bacterial vaginosis. Positive culture on sabouraud's glucose agar showed the positive culture for candida.

Observations: A prospective study of consecutive 100 vaginal swabs from women with vaginal symptoms was studied over the 2 years period for Vaginitis/ cervicitis; the age range was 20-45 years with a mean of 35.56 years (Table 1). Table 2 shows the different types of isolated organisms in 100 cases in which lactobacilli acidophilus (53) (the normal commensal found in vagina) was maximum in number followed by the bacterial pathogen *Gardnerella vaginalis* (20) which causes bacterial vaginosis, *E. coli* (16), *C. Albicans* (4), *S. epidermidis* (3) and the least common pathogen found was *Actinomycetes* (2) and *Trichomonas vaginalis* (2). Table 3 shows a correlation of pap smear finding with gold standard culture method. When compared with culture method, there were 39 patients found to be true positive on pap stain and 12 patients were false positive. While 41 patients were found to be true negative & only 8 patients were found to be false negative. Table 4 shows the validity of the pap smear for different organisms which are stated below-

Gardnerella vaginalis shows sensitivity of 90%, specificity of 84.38%, positive predictive value of 78.26%, & negative predictive value of 93.10% with the diagnostic accuracy of 86.53%.

E. coli shows sensitivity of 75%, specificity of 83.78%, positive predictive value of 66.67% & negative predictive value of 88.57% with the diagnostic accuracy of 76.74%.

S. epidermidis shows sensitivity of 66.67%, specificity of 100%, positive predictive value of 100% & negative predictive value of 97.78%.

Actinomycetes show sensitivity of 50%, specificity of 100%, positive predictive value of 100% & negative predictive value of 97.83%.

Candida albicans shows sensitivity of 100%, specificity of 97.73%, positive predictive value of 80% & negative predictive value of 100% .

Trichomonas vaginalis shows 100% each for sensitivity, specificity, positive predictive value, & negative predictive value. Table 5 shows the correlation among pap stain smear, gram stain smear, wet preparation. In each of the findings sensitivity, specificity, positive predictive value, negative predictive value, pap stain smear has a high value when compared to the values of gram stain smear and wet preparation

Pap stain was found to be highly useful as it helped in detecting total 39 true positive cases of vaginitis by showing the clue cells, the presence of organisms and inflammatory smear present in a cervical smear. Giemsa stain, gram stain, wet mount smear, PAS stain appeared complimentary in finding certain cytomorphological features.

Table 1: Age- wise distribution of patients undergoing pap test

Age Group(yrs)	No of patients	Percentage(%)
20-29 yrs	27	27
30-39 yrs	42	42
40-49 yrs	31	31
Total	100	100
Mean Age	35.56±7.52(20-45 years)	

Table 2:Percentage of organisms isolated

Organism Isolated	N	Percentage(%)
Actinomycetes	2	2
Candida Albicans	4	4
E.Coli	16	16
Gardnerella Vaginalis	20	20
Lactobacilli Acidophilus	53	53
Trichomonas Vaginalis	2	2
S. Epidermidis	3	3
Total	100	100

Table 3: Correlation of PAP smear result with culture method

PAP Stain Smear↓	Culture		χ ² -value	p-value
	Positive	Negative		
Positive	39	12	43.47	0.0001,S
Negative	8	41		
Total	47	53		

Table 4 : Validity of the Papanicolaou Smear in the Diagnosis of organisms

Diagnosis	Sensitivity	Specificity	PPV	NPV	Accuracy
Gardnerella Vaginalis	90.00%	84.38%	78.26%	93.10%	86.53%
E-Coli	75.00%	83.78%	66.67%	88.57%	76.74%
S. Epidermidis	66.67%	100%	100%	97.78%	97.87%
Actinomycetes	50%	100%	100%	97.83%	97.87%
Candida Albicans	100%	97.73%	80%	100%	97.94%
Trichomonas Vaginalis	100%	100%	100%	100%	100%

Table 5: Correlation of pap stain, gram stain, wet mount preparation

	PAP Smear		Gram Stain Smear		Wet Preparation	
	%	95% CI	%	95% CI	%	95% CI
Sensitivity	82.98%	69.19-92.35%	78.72%	64.34-89.30%	63.83%	48.52-77.33%
Specificity	83.02%	70.20-81.25%	83.02%	70.20-91.93%	75.47%	61.72-86.24%
PPV	81.25%	67.37-91.05%	80.43%	66.09-90.64%	69.77%	53.87-82.82%
NPV	84.62%	71.92-93.12%	81.48%	68.57-90.75%	70.18%	56.60-81.57%
Accuracy	80%		81%		70%	

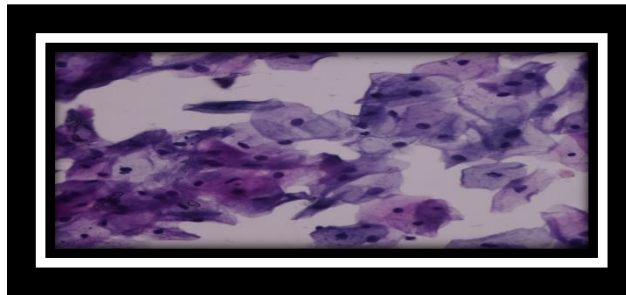


Fig 1: Cervical Cytology- Normal pap smear (pap stain, 40X);Smear shows superficial and intermediate squamous cells.

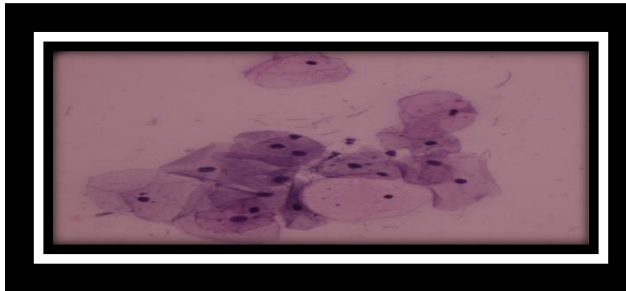


Fig 2: Cervical cytology- shows lactobacilli along with superficial and intermediate cells. (pap stain ,40X)

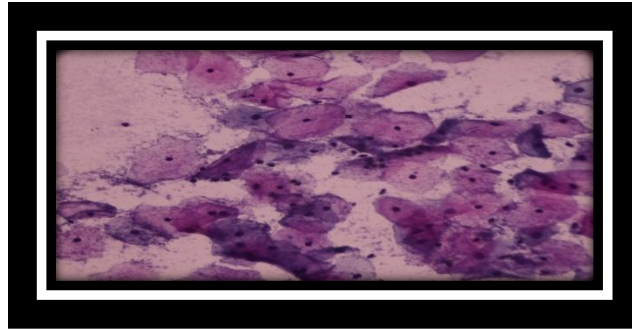


Fig 3: Cervical cytology- Bacterial vaginosis shows presence of clue cells (pap stain ,40X)

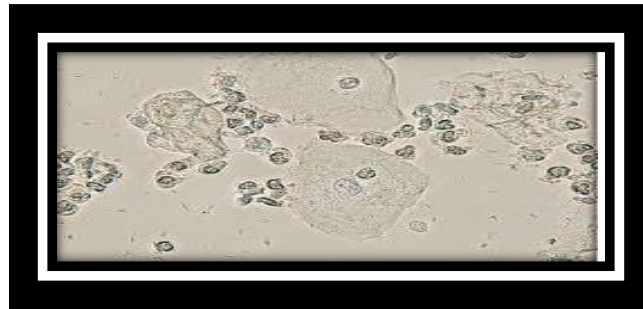


Fig 4: Wet preparation- Bacterial vaginosis- floating clue cells (Wet preparation, 40X)

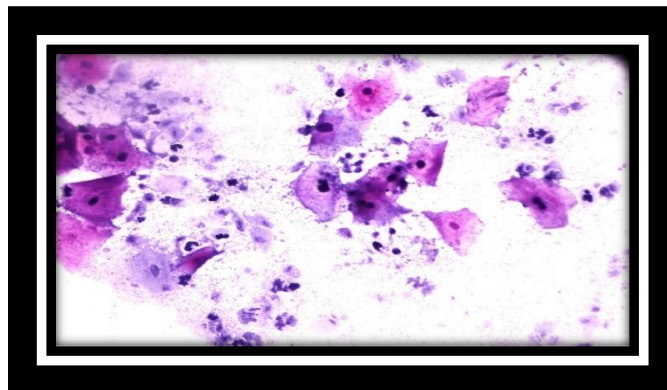


Fig 5: Cervical cytology - Trichomonas vaginalis shows pear shaped organism (pap stain, 40X)

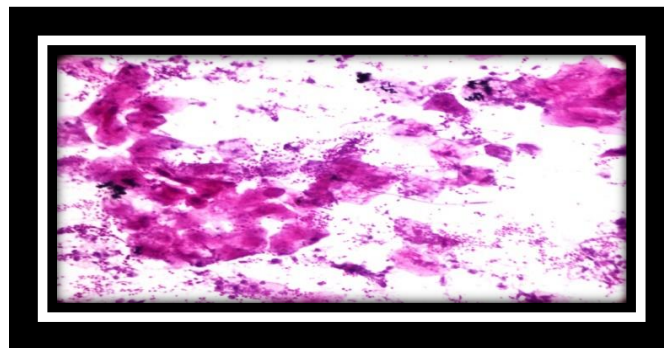


Fig 6: Cervical cytology- Candidiasis shows yeasts and filaments of candida (pap stain, 40X)

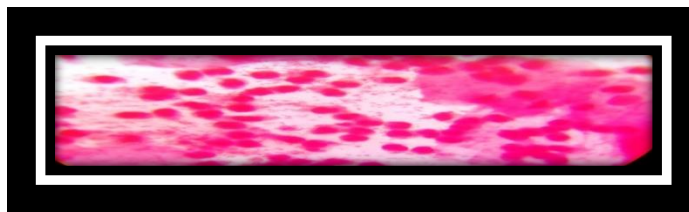


Fig 7: Cervical Cytology- Gram stain shows gram negative cocci (Gram stain, 100 X)

Discussion

There were a total number of hundred subjects deemed fit to be included in the study with the application of inclusion and exclusion criteria. The age distribution of the patient varied from 20 to 45 years with a mean age of 35.56 years. When these infections were matched against the age, those in the 30-39 years age group were among the most infected group. Similar findings of mean age were seen in the study of Kelly et al[5], Konje et al[6], and Vijayalakshmi et al[7] which was 38, 36.8, 34.3 years respectively. Vaginal and cervical cultures were carried out in this study for the detection of a wide range of microorganisms in women with and without inflammation on pap test in order to estimate the predictive value of this finding for the presence of pathogens in suspected women. This study determined that 69.64% of women presenting with inflammatory changes on cervical smear tests had positive cultures for different pathogens. Kelly et al[5] Wilson et al[8] in their study also demonstrated the similar findings. However, 18.18% of women with no inflammatory changes on smear testing had positive cultures. Previous studies demonstrated that women with no inflammatory changes on cervical smears can harbor genital tract pathogens. Baka et al[9] also observed that positive cultures are seen both in women with inflammatory changes & in those without inflammatory changes. The determination of cervicovaginal microbial flora is essential for the detection of potential pathogens for correct diagnosis and treatment. Batra et al[10] emphasized the role of cytology as rapid, non-invasive confirmative diagnosis in patients of vaginitis. On contrary, the sensitivity of pap stained smear for detecting cervicovaginal infections in this study is 82.98%. The sensitivity of pap stained smear suggests that there is a need for an additional gold standard culture method for the confirmation of diagnosis which was concordance with the findings of Audisio et al[11] which said that sensitivity of pap stained smears in the diagnosis of vaginitis is inadequate for screening. The present study showed gram-stained smears of vaginal fluid may be the adequate diagnostic criteria when it is positive but it is less sensitive technique than culture for the detection of organisms in the vaginal smear. The direct examination of a clinical specimen by wet mount is a rapid and cost-effective diagnostic aid in the laboratory. The visual examination by the laboratory assists the clinician in quickly identifying the causative agent of infection so treatment may begin. The wet mount preparation is an inexpensive and quick test. Banneheke et al[12] state that sensitivity reported by a meta-analysis ranges from 58-82% and our result falls within the stated range that is 63.83%. The sensitivity of wet mount microscopy is highly dependent on the skills and experience of the microscopist.

Conclusion

The bacterial flora complicates microenvironment in vagina and cervix which consists of different bacterial species variable in quantities and proportion. Auxiliary methods of giemsa stain, PAS stain, gram stain & wet preparation help in understanding the etiology and fore it to early treatment protocol. Vaginitis is a condition, where an impressive micro-biological alteration of vaginal flora takes place, characterized by decreased lactobacilli species and overgrowth of anaerobes together with potentially

Conflict of Interest: Nil Source of support: Nil

pathogenic bacteria. The incidence of primary infertility can be reduced by identifying the vaginitis/ cervicitis in early stage. The results of this study states that pap smear is at par to the gram stain in identifying vaginitis. In a rural setup, where cost of treatment is a major constraint for early diagnosis and treatment of the patients. The mandatory inclusion of the pap smear in the initial evaluation of vaginitis can be a landmark in the treatment modality for vaginal infections, supported by the microbiological culture method.

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