

Original Research Article

Diversity of Vaginal microbial communities and role of PAP smear in its detection

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ABSTRACT

Keywords

Vaginal microbial communities, Vaginitis, Candidiasis, HPV-4 Cases, HSV-1 case, Leptothrix-2 cases

Vaginitis is among the most common condition for which woman seek medical care, accounting for approximately 10 million hospital visits each year. To recognise the cytological manifestations in vaginitis, identify the possible causative organism and correlating the clinical diagnosis with the cytological diagnosis. 150 cases of vaginitis were studied in department of pathology over a period of 2 year. After detailed clinical examination, posterior fornix was swabbed with a cotton tip applicator. pH was determined. Whiff amine test was done. Wet mount preparation was immediately made and smears were prepared, one each for gram and pap stain. Majority were in age group 21–30 years. Commonest clinical manifestation was homogenous greywhite vaginal discharge. Cytological manifestations suggested Bacterial vaginosis (70 cases) as the most common infective cause of vaginitis followed by Candidiasis (25 cases), Atrophic vaginitis(20cases), Trichomonas vaginalis-12 cases, HPV-4 Cases, HSV-1 case, Leptothrix-2 cases, Aspergillosis-1 case. 89.18% cases showed positive clinicocytological correlation. Clinico-cytological correlation was more important than considering only the clinical findings so as to reach to the proper conclusion and institute appropriate antimicrobial therapy to treat these conditions and prevent long term sequelae. So this study was done to emphasize the role of cytology as rapid non-invasive confirmative diagnosis in patients of vaginitis as clinical diagnosis alone can lead to false interpretation.

Introduction

Vaginitis is a clinical syndrome characterized by vaginal discharge, vulvar irritation, or malodorous discharge (2003). These patients complain of an excessive

vaginal discharge, which may be white (leucorrhoea), discolored or blood stained. This may not be always due to infection, since hormonal changes and allergies may

be associated with similar manifestations (2003)

The normal flora of the vagina varies from person to person, but lactobacilli are usually the preponderant organism. Bacterial flora also contains other aerobic and anaerobic microorganisms. Some of these are considered to be pathogenic. Many of these organisms can be identified in cytologic preparation (2006). It is recognized that, bacterial vaginosis may be associated with pelvic inflammatory disease, preterm premature rupture of the membranes (PROM), post operative gynaec infections and the secret of successful management of vaginal discharges or infections is in the diagnostic approach. If a proper diagnosis is made, treatment follows easily. The crux of the diagnosis of vaginal infections and discharges rests with the microscopic examination of the vaginal discharge as these infections can lead to various inflammatory changes in the epithelial cells which may be cytoplasmic abnormalities of diagnostic importance (1993). This has indicated the need to take up the study of diversity of vaginal microbial communities and role of pap smear in its detection

Materials and Methods

Study of clinico-cytological correlation of vaginitis was conducted in the Pathology department, KVG Medical College, Sullia, Karnataka, over a period of two years from 1st August 2010 to 1st August 2012 after due permission from ethical committee of the college.

Method of collection: With the prior consent, a comprehensive history, general examination and gynecological examination were initially carried out. Posterior vaginal fornix was swabbed with a cotton tipped applicator. The pH was measured using

indication papers ranging from 2 to 10. Whiff amine test was done as one or two drops of vaginal discharge taken on a clean glass slide and one or two drops of freshly prepared 10% potassium hydroxide solution was added to it. Both were mixed and smelt immediately. Wet mount preparation was immediately made by adding a drop of discharge mixed in a drop of normal saline on a clean slide and topped with a cover slip. This was then observed under microscope.

A second sample was obtained with another swab and two smears were made, one each for Grams stain and Papanicolaou stain. All the smears were carefully examined for the various cytologic manifestations and the causative agent. Data obtained was analysed for the statistical significance using Chi square test.

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

Exclusion criteria: Patients in menstrual period and who had taken antibiotics or received any treatment for vaginitis within the previous month were excluded from this study as this will lead to false positive results.

Results and Discussion

Salient features observed in this study were as follows

Clinical features: The age range varied from 19 to 70 years. Majority were in age group of 21–30 years (58%), followed by 31–40 years (26.6%) and 41–50 years (8.66%). 61–70 years age group was least commonly affected in the present study.

The commonest clinical symptom associated with vaginitis was history of homogenous grey white discharge observed

in seventy cases (n=70). Itching was the second common symptom observed in sixty nine cases (n=69). Other clinical manifestation were thick white discharge, in fifty one cases (n=51). Curdy discharge was present in fifteen cases (n=15), greenish yellow discharge in ten cases (n=10). Also seen were burning micturition, pain abdomen, menstrual disturbances in twenty (n=20), twelve (n=12) and six (n=6) cases, respectively.

Clinical diagnosis based on symptoms were Non Specific Vaginitis-60 cases (40%), Bacterial Vaginosis-30 cases (20%), Candidiasis-25 cases (16.66%), *Trichomonas* 13 cases (8.66%), Senile Vaginitis 20 cases(13.33%) and HPV-2 (1.35%) cases.

Biochemical features: Mean pH ≥ 5 was observed (n=102) in Bacterial vaginosis (70 cases), *Trichomonas* (12 cases) and 20 cases of Atrophic vaginitis, pH of 4.5(n=33) in Candidiasis 25 cases, HSV 2 cases, HPV 4 cases, 1 case of Aspergillosis and Leptothrix each. Normal pH seen was in 15 cases of normal flora. The difference in pH was highly significant. $\chi^2=81.549$ $p<.001$ vhs. Whiff's Amine test was positive in 53 cases(75.7%) of Bacterial vaginosis out of total 70 cases and among 12 cases of *Trichomonas*, 4(33.3%) were positive, the difference in result of amine test was highly significant. $\chi^2=81.549$ $p<.001$ vhs.

Cytological diagnosis

Cytological diagnosis based on wet mount, Gram stain and Pap stain were Normal Flora 15 cases(10%), Bacterial infections 71 cases (Bacterial Vaginosis 70 cases (46.66%), Leptothrix-1 case (0.66%); Fungal infections-26 cases (Candidiasis-25cases (16.66%), Aspergillosis-1 case (0.66%); Parasitic infections- *Trichomonas vaginalis*-12 cases (8%); Viral infections-5 cases

(Koilocytic atypia-4 (2.66%) cases, HSV -1 case (0.66%) and Atrophic vaginitis-20 (13.33%) cases.

Saline (wet) mount showed 67 cases (45.6%) to be positive (Figure 1).

Nugent scoring based on Gram stain revealed 45 patients to be positive for Bacterial vaginosis, 25 patients were at high risk of transition to Bacterial vaginosis. 15 patients showed overgrowth of *Lactobacilli* (Table 1).

Pap stain showed (n=150), Bacterial vaginosis in 70 cases, *Candida* in 25 cases, *Trichomonas* in 12 cases, Atrophic vaginitis in 20 cases, overgrowth of lactobacilli in 15 cases, HPV was diagnosed in 4 cases. Aspergillosis, Leptothrix, HSV was found in 1, 2, 1 cases, respectively.

15 cases were cytologically diagnosed as Normal flora with over growth of *Lactobacilli*. Features of cytolysis were seen in seven cases (n=7). Karyolysis was seen in 2 cases. Inflammation was seen in all 15 cases.

Pap smear showed mainly shift in bacterial flora suggestive of Bacterial vaginosis with absence of lactobacilli in 45 (n=45) cases. Shift in flora with occasional lactobacilli were seen in 25(n=25) cases. Clue cells (Figure 2) were seen in fifty eight cases (n=58).

25 cases were diagnosed as Candidiasis. Pap stain revealed budding forms in 12 cases and pseudohyphae form (Figure 3) in 13 cases. Sheesh kebab appearance of *Candida* was seen in 15 cases.

12 cases were diagnosed as having *Trichomonas*. All the patients were in the third decade. Pap smear showed cytoplasmic eosinophilia in all 12 cases. BB shot effect was seen in 11 cases (Figure 4).

Trichomonas organism was seen in all 12 cases.

Twenty cases were diagnosed as Atrophic vaginitis. Smear showed degenerated parabasal cells in all Twenty (n=20) cases (Figure 5). Pseudoparakeratosis was seen in twelve cases (n=12). Inflammation was seen in 10 cases. Four (n=4) cases of HPV was identified, all were in the third decade and presented with homogenous vaginal discharge. In all the cases pH was ≥ 4.5 . In the smear mild cytolysis and karyolysis with cytoplasmic vacuolation was observed in all 4 cases. Binucleation and multinucleation was observed in 2 cases.

One case of *Leptothrix* was diagnosed, the patient was in the third decade and presented with foul smelling discharge. Pap smear showed *Leptothrix* organism in long strands and loops in an inflammatory background. 2 cases of HSV were diagnosed on Pap stain showing multinucleated giant cells along with nuclear moulding. One case of Aspergillosis was diagnosed showing septate hyphae along with inflammatory exudates (Figure 6)

In the present study of 150 cases, all the cases of Candidiasis (n=25) and Atrophic vaginitis (n=20) correlated well with the clinical findings (100% correlation was found). Out of 70 cases of Bacterial vaginosis, only 30 cases correlated with the clinical diagnosis (42.9%), remaining 40 cases (57.1%) were considered as NSV. All the 12 cases of *Trichomonas* correlated well with the clinical diagnosis (100%). Koilocytic atypia (n=4) cases showed 50% clinicocytological correlation as 2 of them were considered as NSV. Normal flora (15 cases), Aspergillosis (1 case) and HSV (1 case) showed no correlation with the clinical diagnosis as all were diagnosed as NSV. False positive diagnosis of *Trichomonas* was made clinically which turned out to be

Leptothrix on cytology. Difference in the correlation was statistically significant. $p < .01$. In this study vaginal discharge was a common presenting symptom seen in 21-30 years age group. Similar peak incidence was noted in other studies. All the 150 patients in this study had complains of vaginal discharge in which majority had homogenous grey white discharge, followed by thick grey white discharge. Sobel et al also observed vaginal discharge as the commonest symptom (1994).

Cytological diagnosis offered were Normal flora, Bacterial vaginosis, Candidiasis, *Trichomonas*, Senile vaginitis, *Leptothrix*, Koilocytic atypia, Herpes simplex virus and Aspergillosis. Other principle causative factors were not observed this study. The variations in the results are consistent with the fact that all the authors have not used identical methods in diagnosing the cases (1992).

pH of the vaginal discharge in bacterial vaginosis was more than 4.5. Similar observation was made in other studies. Present study showed clue cells in 58 cases (n=70) of bacterial vaginosis. Bacterial vaginosis may be diagnosed clinically by Amsel's criteria (1983). This is in agreement with others that clue cells per se, are very sensitive indicators of the presence of *Gardnerella Vaginalis* (2008).

Whiffs amine test was positive in 53 of these patients. Whiffs test was first described by Pheifer et al, involved mixing the suspect vaginal discharge with an alkali, potassium hydroxide as both sensitive and specific (1988).

Gram staining of vaginal discharge is a reliable means of establishing the aetiology of a vaginosis (greater than 40 Gram-negative or Gram-variable coccobacilli per microscopic field at 1000 x magnification

under oil immersion), especially when there is an anaerobic vaginosis in a study by Nugent *et al.* 1991. According to Spiegel *et al.* (1980), Gram stain is also a reliable means of establishing a shift in vaginal flora from lactobacilli to other organisms.

All the four cases (n=150) of koilocytic atypia in the present study were of age group of 21–30 years. Sadeghi *et al.* (1989) in his study of two year period and found that the prevalence of koilocytotic atypia was highest in women of ages 25–29 years. Misra *et al.* (2004) screened 30,415 women for sexually transmitted diseases and found that the incidence of HPV in 148 cases (0.6%). Herrero *et al.* (2000) examined 202 women aged between 17 and 66 years. 27 cases (13.4%) were showing koilocytosis of varying degree. We found in our study candidiasis in 25 patients. Curdy vaginal discharge and pruritis was the most common presenting symptoms. Other manifestations were burning micturition, pain abdomen and menstrual disturbances. Mean pH of discharge was 4.19. Siapco *et al.* (1986) observed complaints of discharge in 42%, irritation in 15% of patients. Microscopy showed pseudohyphae and budding yeast in all our cases.

Trichomonas vaginalis infestation was cytologically diagnosed in 12 cases. Greenish yellow discharge was the most common presenting complaints followed by pruritis, foul smelling discharge, pain abdomen, burning micturition, menstrual disturbances, dyspareunia. pH of discharge was elevated. Similar observations were made in other studies. We observed that the PMNLs surround the *T. vaginalis* organisms giving BB shot effect in 13 of our cases.

Atrophic vaginitis was diagnosed in 20 cases in our study. 15 among them presented with thin yellowish malodourous discharge, 5 presented with thin grey white discharge. All cases were not due to senile atrophy, 8 out of 20 cases presented in mean age of 35. Bachmann and Nevadunsky (2000) also stated that vaginal atrophy need not be an inevitable consequence of menopause and yellowish malodourous discharge is the characteristic discharge along with other features of atrophy. Multinucleated giant squamous cells are the characteristic cells in the HSV infection. These cells were first described in cervical smears by Stern and Longo (1963), who realized their potential value as markers of viral infection. Coleman *et al.* (1979) also stated that cytological diagnosis offers a rapid and convenient method of detection of HSV. Present study found only 1 case of HSV. This study showed Aspergillosis in one case as tangled cluster of branched hyphae accompanied by inflammation and necrosis. Sangoi *et al.* (2009) also concluded that pap stain remains as an effective cytological stain for aspergillosis.

15 cases were reported as normal flora with overgrowth of Lactobacilli and all cases showed cytolytic smear on pap stain. On saline wet preparation, trichomonads, clue cells and hyphae are absent, and few leukocytes are present. The pH is 3.5 to 4.0. Kaufman and Faro (1994) described the Leptothrix organisms as gram-positive anaerobic rods that are longer than Lactobacilli yet shorter than the filaments of *Candida*. A healthy woman had vaginal lactobacilli between 5 and 15 microns in length, whereas the *Lactobacilli* in the symptomatic patients ranged between 40 and 75 microns in length.

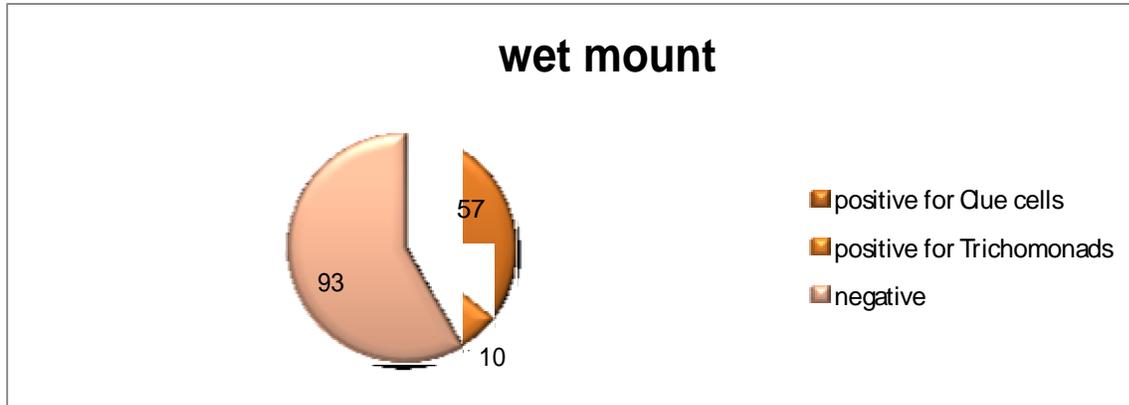


Figure.1 Showing distribution of cases on wet mount

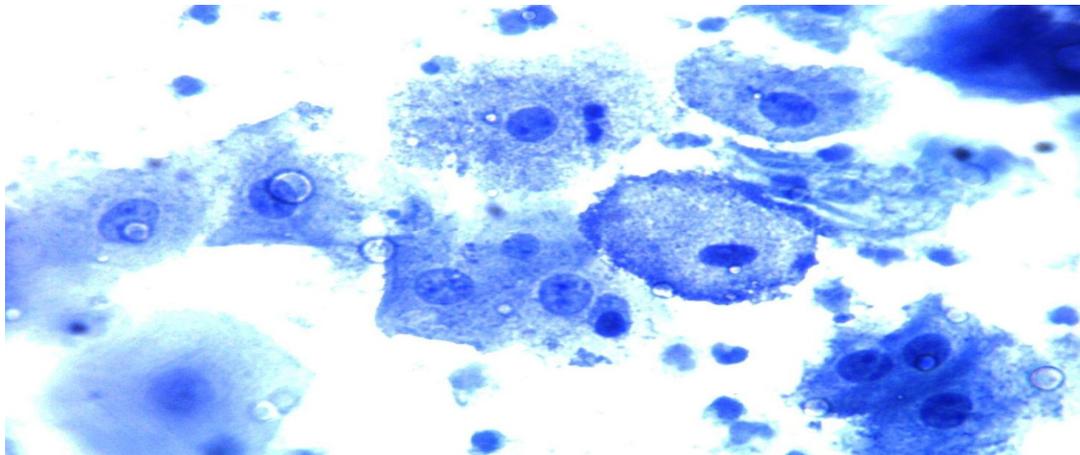


Figure.2 Photomicrograph showing clue cells in bacterial vaginosis (Pap stain x400)

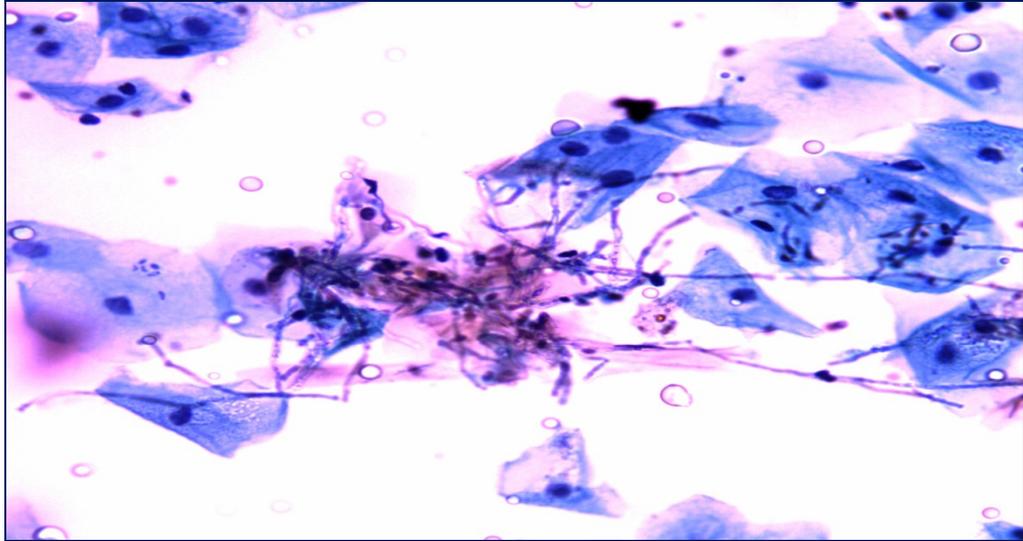


Figure.3 Photomicrograph showing pseudohyphae forms in candidiasis (Pap stain x400)

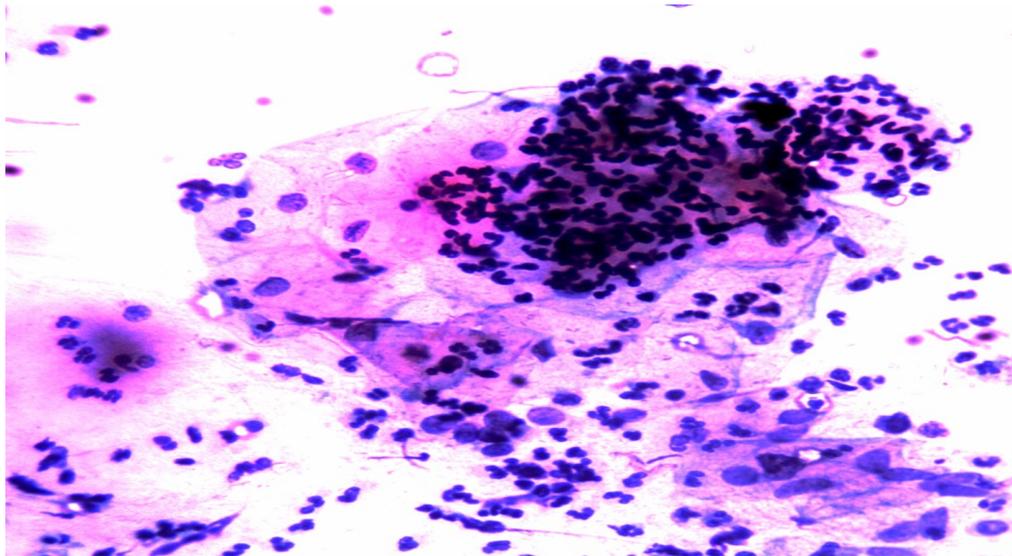


Figure.4 Photomicrograph showing BB shots and cytoplasmic eosinophilia in *Trichomonas vaginalis* (Pap stain x400)

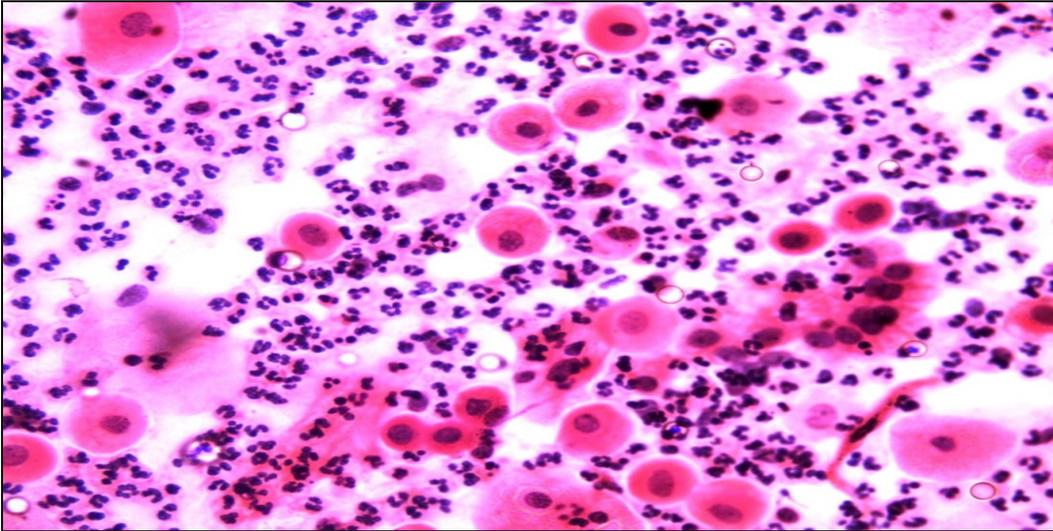


Figure.5 Photomicrograph of atrophic vaginitis showing degenerated parabasal cells with inflammatory background (Pap stain x400)

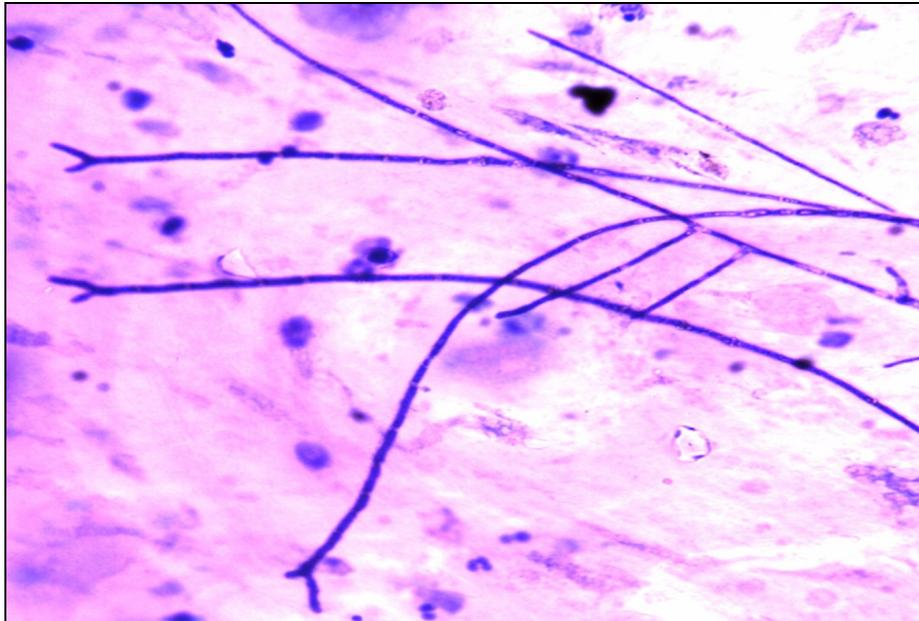


Figure.6 Photomicrograph-showing septate hyphae in aspergillosis (Pap stain x400)

Table.1 Showing Gram stain result based on Nugent score

SCORE	INTERPRETATION	NO OF PATIENTS
0-3	<i>Lactobacilli</i> and variable gram positive rods, few Bacteriods	15
4-6	<i>Gardnerella</i> , Bacteroids, other gram negative rods, occasional <i>lactobacilli</i>	25
7-10	Many <i>Gardnerella</i> , Bacteroides, <i>Mobiluncus</i>	45

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