Cytomorphology of vaginal pap smears- A spectrum of lesions in a tertiary hospital

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Abstract

Background: Pap test is an important and easy diagnostic tool to detect any abnormalities on vaginal cytology. It is routinely done in women of reproductive age group. In addition to detecting cellular and epithelial abnormalities; certain infections, rare entities including neoplastic and non-neoplastic conditions can also be identified.

Aims and Objectives: 1. To study the spectrum of abnormalities on vaginal pap cytology as per The Bethesda System (TBS) 2001.2. To study the utility of pap smears in early diagnosis of premalignant lesions.

Materials and Methods: This prospective study was conducted in Dr. B.R. Ambedkar Medical College, Bangalore where routine pap smears referred from the Obstetrics and Gynaecology Department were studied. Important clinical data like vaginal discharge, inter menstrual bleed, post–coital bleed and lower pain abdomen were collected. Pap smears were analysed, interpreted and categorised based on TBS2001. Importance of detecting premalignant lesions were highlighted in our study.

Results: 525 pap smears were interpreted in a 2 year prospective study out of which 400 cases were analysed after excluding 125 unsatisfactory case. The age range was 18-78 yrs (mean age of 48 yrs). 120 (30%) cases of nonspecific inflammation were identified. Specific infectious etiologies included 186 (46.5%) cases range including most common candida and Bacterial vaginosis to 2 rare infections, one case each of filariasis and granulomatous cervicitis. Significant number(82 cases) of premalignant conditions were diagnosed. 12 malignancies-10 SCC and 2Adenocarcinomas were reported.

Conclusions: Certain rare infectious entities were detected in our study which pose challenge due to infrequent occurance in our daily practice. Close follow- up and histological examination are necessary in premaligant lesions to avoid spread and untimely death of the patient. Thus emphasising effective utility of this simple and cost-effective tool.

Keywords: Vaginal pap cytology, TBS 2001, NILM, Rare infections, Premalignant, Invasive cancers.

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Introduction

Pap test is a simple and inexpensive method to screen in women for squamous dysplasia and carcinoma of uterine cervix. In addition to the primary use of pap smear for detecting cellular and epithelial abnormalities, certain rare entities of neoplastic and non- neoplastic conditions pose challenges in daily practice resulting in important diagnostic pitfalls.[1] Uterine cervical cancer accounts for 6% of all carcinomas in women.[2] Carcinoma cervix is the 2nd leading cause of cancer related deaths among women.[3,4,5] The most current reporting system recommended for reporting vaginal pap cytology is the TBS 2001. TBS continues to update its recommendations and guidelines.[6]

Materials and Methods

This is a prospective study carried out during a period of 2 years in a tertiary care hospital. All the female patients referred to Obstetrics and Gynaecology Department of DR. B. R. Ambedkar Medical College and Hospital, Bangalore, with clinical symptoms such as lower abdominal pain, bleeding per vagina, post-coital bleeding, intermenstrual bleeding, dyspareunia, vaginal discharge, irregular menstruation etc were included in the study after informed consent. Relevant history of illness was obtained from the patient and recorded on the proforma. Pap smears were prepared using Ayre's spatula/ brushes, fixed in 95% ethyl alcohol and stained with rapid pap technique, analysed and reported. The cytological interpretation of the smears were made according to The New Bethesda System 2001.

Results

A total of 525 cases of pap smears were included in our study during the time period of two years, out of which 400 cases were analysed after considering the exclusion criteria (125 cases of unsatisfactory/ inadequate samples, some with obscured morphology due to blood/ mucus or dense inflammation). The age range of the patients was 18-78 yrs (mean age of 48 yrs). Mean age of invasive carcinoma was 70.5 yrs. The most common presenting symptom was abnormal discharge per vaginum(Table 1). The general categorization of spectrum of lesions (Fig. 1) included, 120 (30%) cases of nonspecific inflammation. Of these cases, inflammatory smear with squamous metaplasia(56.7%) were more common than with

reactive atypia(30.8%). Only few cases showed atrophic vaginitis(10.9%).We also found 2 cases(1.6%) of post chemotherapy induced reactive changes.

Lesions with specific etiologies (Fig. 2) included 186(46.5%) cases of which bacterial vaginosis (29%) was the most common followed by candidal vaginitis (23.2%), lactobacillary vaginitis (5.9%), trichomonas vaginalis(7.6%), leptothrix infection (4.4%), HPV infection (1.6%), HSV infection (1%) and actinomycosis(1%). We also reported mixed infections of bacterial vaginosis, candida, trichomonas and leptothrix (25.3%). We encountered 2 rare infections in our institute, one case each of filariasis (0.5%) and granulomatous cervicitis (0.5%).

We found significant number of premalignant conditions(Fig. 3) in our study. Out of 82 (20.5%) cases, 36 cases of ASCUS were diagnosed, of which 32 were ASCUS-NOS type and 4 cases of ASCUS favouring neoplasia. 14 cases of ASC-H were interpreted. 8cases of LSIL, 11 cases of HSIL and 6 cases of SIL were identified. A total of 7 cases of AGC were also diagnosed.

We observed 12 (3%) cases of malignancies(Fig. 4) of which 10 cases were SCC (8 cases of keratinizing type) and 2 case of adenocarcinoma.

A cluster of endometrial cells was identified in a single vaginal pap smear in a patient below 40 yrs of age.

Discussion

The pap smear was originally designed to detect malignant cervical lesions, subsequently used to diagnose precancerous and other lower genital tract infections of the uterine cervix including Human papilloma virus (HPV).[1] It is estimated that by the age of 60 years, approximately 30% females undergo hysterectomy, approximately 85% of these cases are performed for benign diseases and 15% for carcinoma cervix.[2] The lower genital infections of the uterine cervix are closely related with age, marital status, promisquity, socio-economic status, malnutrition and genital hygiene. High incidence of infections, in general, are also attributed to lowered immune status of females due to varied physiological conditions of the body like menarche, pregnancy, lactation and pathological states like diabetes and AIDS. Hygiene of lower genital tract is related to the infections and inflammations of the genital organs of females.[7,8] In developing countries where hygiene is poor, incidence of infections is high when compared to developed countries.[9] The frequency of genital tract infections in our study showed 46.5% comprising of candida(Fig. 5), gardnerella vaginosis(Fig. 6), trichomonas vaginalis(Fig. 7), microfilariae(Fig. 8), actinomycosis(Fig. 9), leptothrix(Fig. 10), human papilloma virus(Fig. 11) and herpes simplex virus(Fig. 12). These infections accounted for 20.2% in normal women during routine screening programme as reported earlier also.[10,11]

Candidiasis is the most common fungal infection in women.[12,13] Diagnosis of candida on pap smear correlates poorly with clinical disease and is part of the normal flora in 10-20% of women. Candida should be treated only if the patient is symptomatic. Evidence of presence of sexually transmitted diseases may be specific in some cases, e.g.: finding a trichomonad and non-specific in some with only inflammatory cells. Bacterial vaginosis can be detected by the presence of clue cells.[14]

2 cases of actinomycosis were reported in our study. Predisposing factors for abdominopelvic actinomycosis include a previous history of surgery, GI tract perforation, GI or genitourinary foreign body (particularly intrauterine devices in women) and neoplasia[15]. Our patients had nonspecific constitutional symptoms including chronic fever, weight loss and vaginal discharges. The infection is indolent and spreads through soft and bony tissue planes. It can cause rare but serious forms of endometritis and PID.[15,16]

Over 120 million people are currently infected with about 40 million disfigured and incapacitated by filariasis. Such deformities lead to social stigma as well as financial hardship from loss of income and increased medical expences.[17] Walter A et al found microfilariae in the cervicovaginal smears in the absence of clinical filariasis.[18] Filariasis should be considered in the differential diagnosis of tissue parasites even in the non-endemic areas. High index of suspicion is necessary in the part of the cytologist in identification of parasitic organisms for early diagnosis and prompt institution of appropriate treatment.[19]

Patients with cervical carcinoma have consistently been showed to demonstrate the presence of higher levels of HSV-2 antibody than the controls. 2 hypothesis have been devised to explained the ‘hit and run’ hypothesis and synergism between HSV and HPV.[20]

HPV infection can be reliably diagnosed if both nuclear and cytoplasmic changes are present.[14] The current estimates indicate approximately 1,32,000 new cases diagnosed and 74,000 deaths annually in India, accounting to nearly one–third of the global cervical cancer deaths. 6.6% of women are estimated to harbour cervical HPV infection of which HPV serotypes 16 & 18 account for nearly 76.7% of cervical cancer in India.[21]

Long term use of hormonal contraceptives, high parity, early initiation of sexual activity, multiple sex partners, tobacco smoking, HPV infection, coinfection with HIV, chlamydia trachomatis, HSV-2, immunosuppresion, low socio-economic status, poor hygiene, diet low in antioxidants are the potential risk factors. Genetic and immunological host factors, viral

factors such as the variants of the type, viral load, viral integration are also important.[21,22]

ASCUS(Atypical squamous cells of unknown significance) (Fig. 13) in pap smear indicates mildly abnormal cells of the cervix. Most commom causes are benign conditions such as infection/inflammation. If it is caused by HPV it requires further monitoring and possible treatment to prevent cervical carcinoma. For adolescents and young women (age 20 and less) with Ascus test is repeated at 12 months. In adult females pap test is repeated at 6 and 12 months or have a reflexive HPV DNA co testing. A reflexive HPV DNA test utilizes the same sample used for PAP smear and eliminates need for another sampling.[23]

In the literature, the incidence of atypical glandular cell (AGC) ranges from 0.1 to 2.1%.[24] Abnormal glandular cells can also be seen in cases of endometrial, tubal or ovarian neoplasms. Studies of series of women with AGC suggests the occurrence of dysplasia or carcinoma in atleast 30% of the patients.[25]

The mean age for LSIL is 44.5yrs, HSIL 51.5 yrs and for invasive carcinoma 70.5 yrs in our study. The most common presenting complaint is abnormal vaginal discharge.

A pap smear triage threshold to LSIL or more severe dysplasia for women with prior ASCUS or LSIL on pap smear results was clearly ineffective for detecting high grade cervical precancerous lesions. In contrast, when repeat pap smear repeat triage threshold was expanded to include persistent ASCUS as abnormal, 83% of women with CIN 2/3 were detected. Women with ASCUS and particularly LSIL pap smears should be referred for a colposcopy examination until better triage methods become available.[26]

In our study, we experienced ambiguity in accurately diagnosing carcinoma –in-situ due to the overlapping features of the cells with HSIL and invasive carcinoma. Though pap smears is a routine screening test, the overall sensitivity in detection of HSIL is 70-80%.[3]

Microinvasive carcinoma is the earliest stage in the genesis of an invasive carcinoma that can be recognised cytologically and histologically. The negative field stimulus described above also apparently influences the columnar epithelial cells of the endocervical canal. The co- occurrence of abnormal columnar cell changes together with abnormalities of the squamous and metaplastic epithelium is becoming increasingly frequent.[27]

The most common complaint associated with premalignant and malignant lesions were abnormal discharge per vagina followed by post menopausal bleed. The colposcopic correlation in these lesions were predominantly erosions followed by cervical bleed on touch, inflamed and congested cervix and very few with hypertrophied cervix.[28]

In SCC(Fig. 14 a and b), tumor cells appear singly, elongated, caudate or bizzare forms admixture with cellular debri, blood cells (especially in exophytic growth). They may be often associated with excessive keratinisation. From a practical standpoint, a single well-formed epithelial pearl includes a neoplasm in the category of a keratinizing carcinoma. Highly keratinized cells or cells showing high pleomorphism are important to recognise SCC. Some cells show hyperchromasia while others appear pale. Often the background is as important as the cytology of the cells.[29]

2 HPV vaccines are commercially available in India and approved by the DRUG CONTROLLER GENERAL of INDIA(DCGI), US FOOD and DRUG ADMINISTRATION, EUROPEAN MEDICINE AGENCY and prequalified by the WHO.[21]

Invasive endocervical adenocarcinoma(Fig. 15) shows features of heavy blood staining, abundant abnormal glandular epithelium, super crowding of sheets, small 3-D groups, papillary clusters, tumor diathesis, single malignant cells with nuclear pleomorphism, macronucleoli and mitotic figures.[30,31,32]

To reduce the consequences of false negative results, it may be advisable to repeat the smear one year after the initial smear. After two negative smears, a longer interval of three years or even five years seems justified.[33,34]

Carcinoma cervix has a long latent phase during which it can be detected as identifiable and treatable premalignant lesions which precede the invasive disease and the benefit of conducting screening for carcinoma cervix exceeds the cost involved.[35]

If diagnosed in the early stages and treated early, cervical cancer is a curable disease. Several screening methods are studied and many researches have emphasized the importance of pap smear as one of the easy and important screening methods for cervical cancer.

Endometrial cells(Fig. 16) may be more common on pap test in HRT users compared to non-users.[36] The atypical glandular cells are difficult to differentiate from atypical endometrial cells especially when cells degenerate. Atypical endometrial cells generally occur in small 3-D clusters of about 5- 20 cells exhibiting mild to moderate nucleomegaly. However nuclei are generally smaller than atypical endocervical cells and adenocarcinoma cells.[37,38]

2 cases(1.6%) of post chemotherapy induced reactive changes(Fig. 17) were also interpreted in our study one was a case of adenocarcinoma (Fig. 17 a) and the other a case of keratinizing squamous cell carcinoma(Fig. 17b).

Not all high risk HPV types are equally responsible for cervical carcinoma.[39] The 8 most common HPV types detected in carcinoma cervix are 16, 18, 45, 31, 33, 52, 58, & 35 which are responsible for 90% of carcinoma cervix worldwide.[40,41] Oncogenic HPV types replicate in the higher levels of stratified

squamous epithelium where the cells no longer divide, whereas the low risk types replicate in lower levels where there is still cell division.[42,43] 2 cases(1.6%) of post chemotherapy induced reactive changes(Fig. 17).

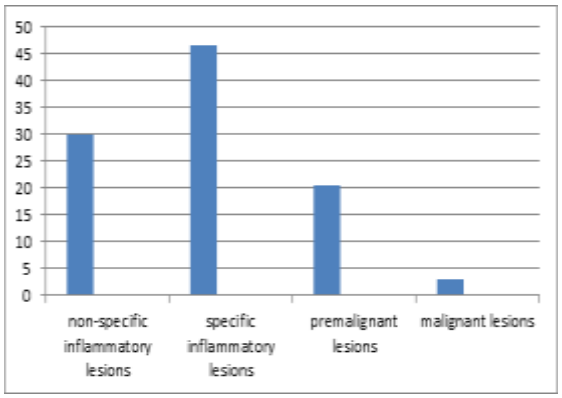


Fig. 1: Categorisation of spectrum of lesions

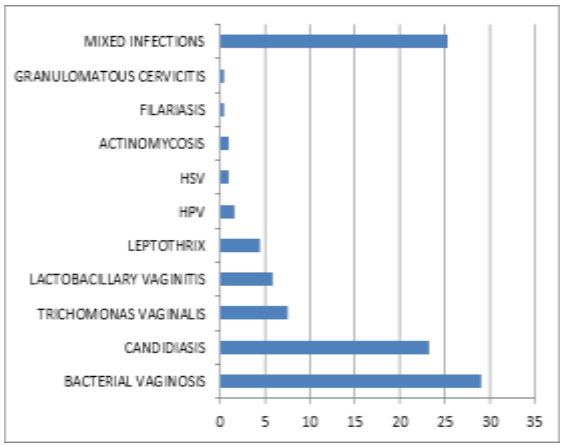


Fig. 2: Inflammatory lesions with specific etiology

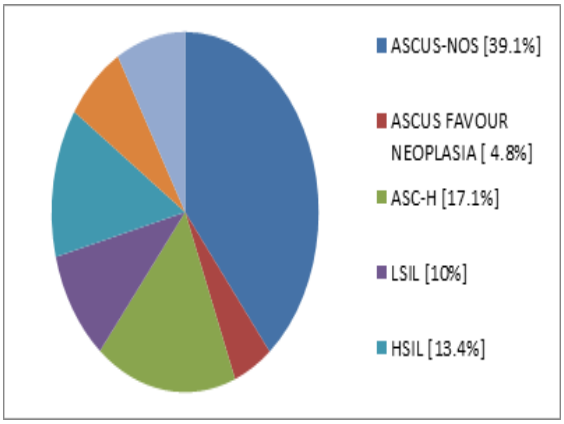


Fig. 3: Premalignant lesions of the cervicovaginal smears in our study

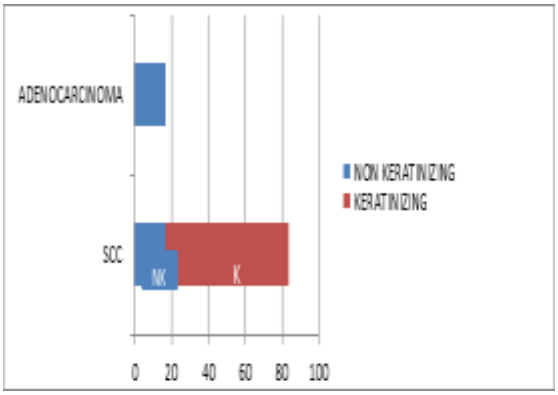


Fig. 4: Malignant lesions reported

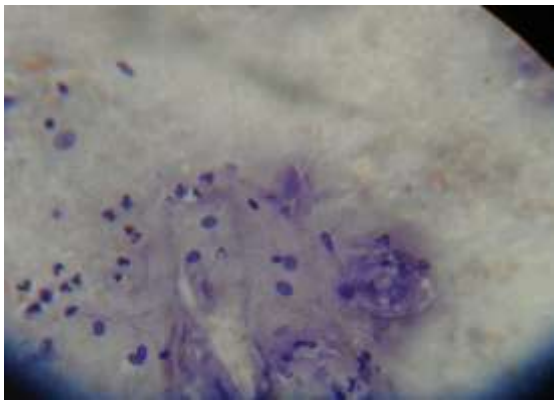


Fig. 5: Candidal Hyphae with yeast forms

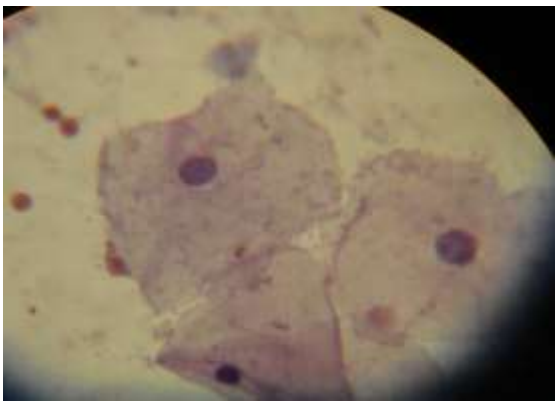
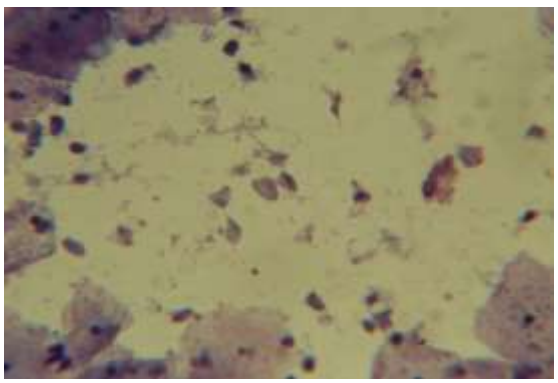


Fig. 6: Clue cells in Bacterial Vaginosis



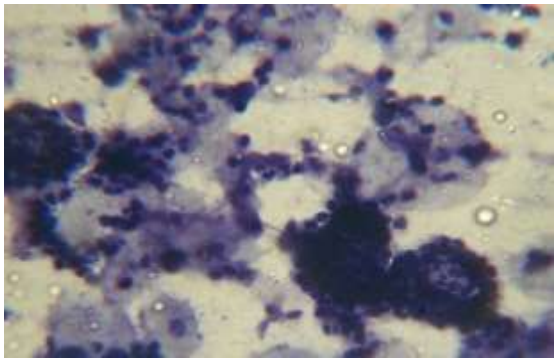


Fig. 7: Trichomonas Vaginalis

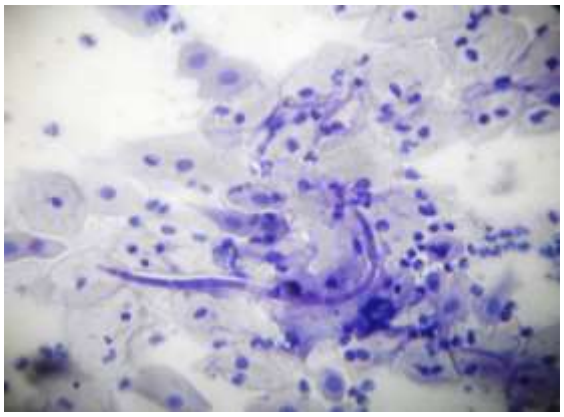


Fig. 8: Microfilariae of W. Bancrofti

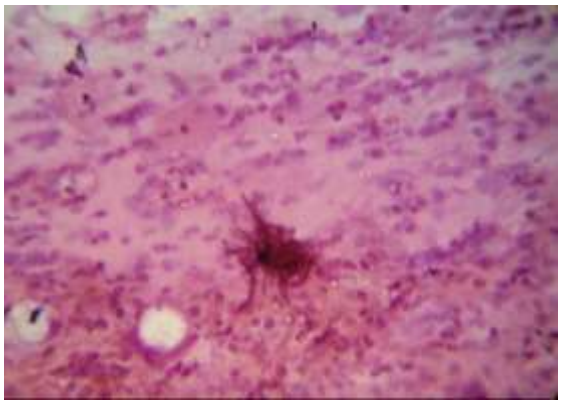


Fig. 9: Actinomycetes

Fig. 10: Leptothrix

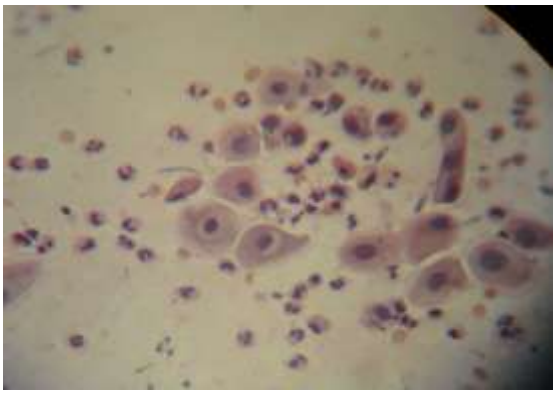


Fig. 11: Koilocytes in HPV infection

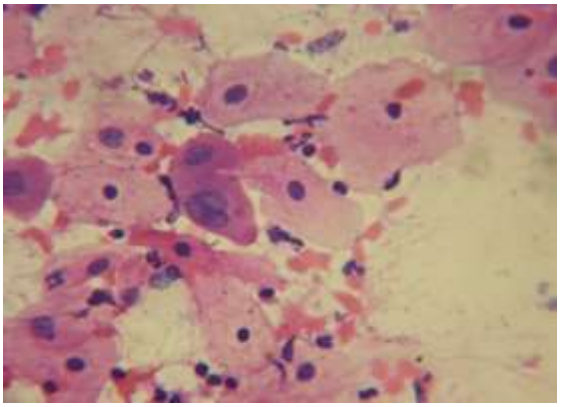


Fig. 12: Multinucleation in HSV Infection with LSIL features

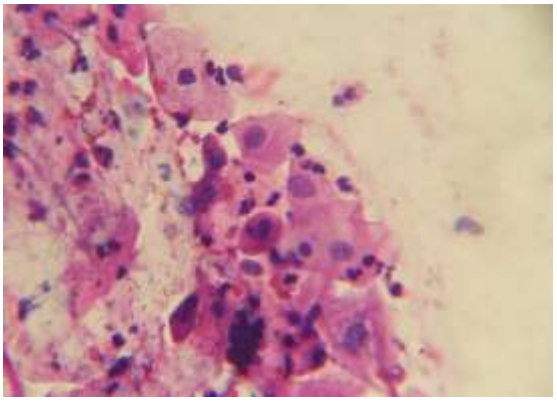


Fig. 13: ASCUS

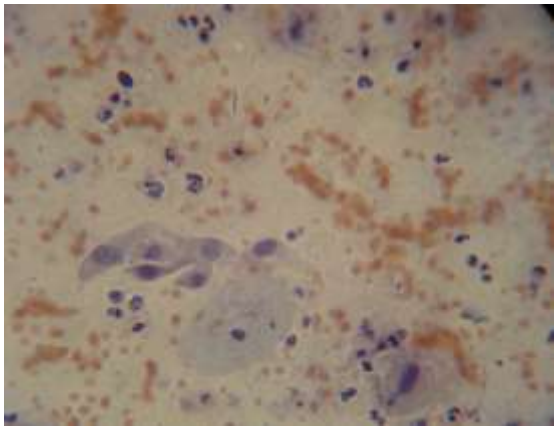


Fig. 14 a: Foci of keratinizing SCC cell custers

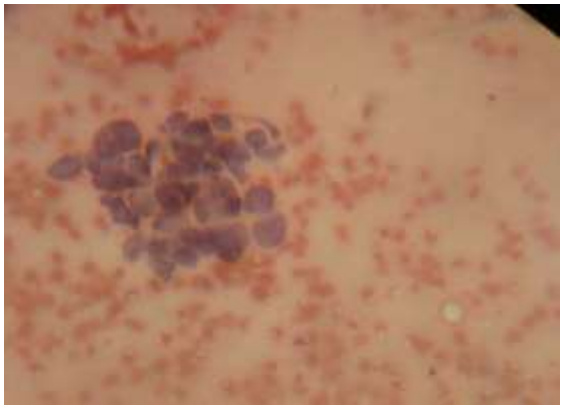


Fig. 14 b: Non-keratinizing SCC

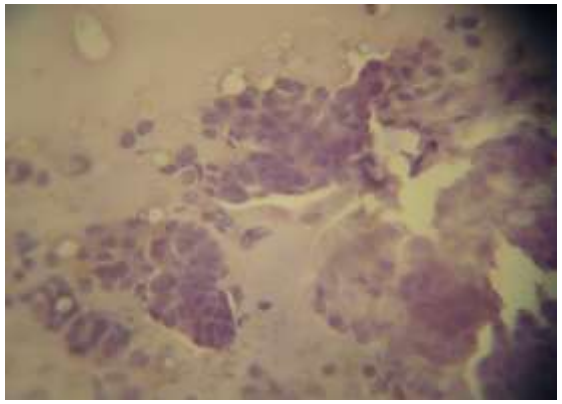


Fig. 15: Adenocarcinoma of the cervix

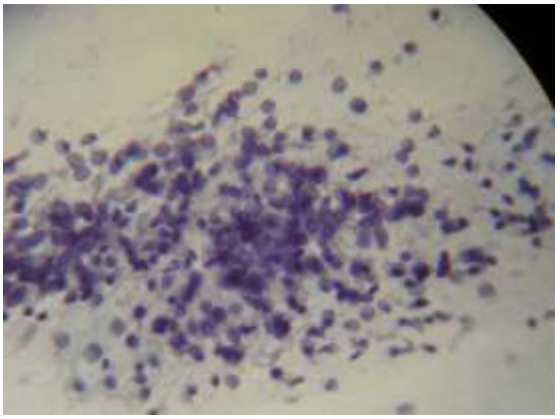


Fig. 16: Endometrial cell clusters in 39 Years old woman

a)

b)

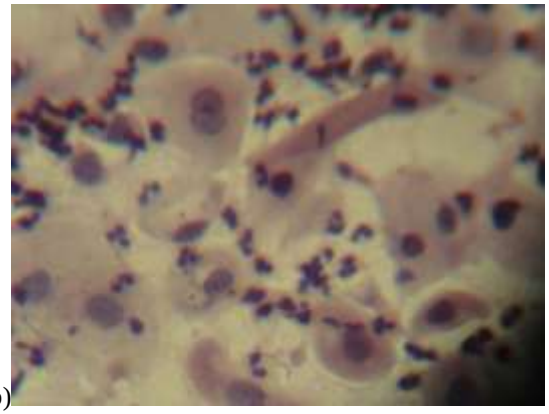


Fig. 14: Post-chemo radiotherapy in a) Adeno ca b) SCC

Table 1: Frequency of clinical presentation in our study

	Clinical Presentation	Percentage
1.	White discharge per vagina	58.1%
2.	Chronic pelvic pain	30.1%
3.	Dyspareunia	2.2%
4.	Intermenstrual bleed	1.0%
5.	Postmenopausal bleed	3.4%
6.	Mass per vagina	2.0%
7.	Postcoital bleed	3.2%

Conclusion

Detecting and accurately diagnosing rare entities including non-neoplastic conditions, rare infections, premalignant and neoplastic conditions is of utmost importance to prevent diagnostic pitfalls in routine reporting of vaginal pap smears. Histological follow up of uterine cervix samples is necessary in all the patients that have cytological results of ASCUS favouring neoplasia and AGC/ glandular atypia due to the possibility of their association with premalignant and malignant lesions.

Unlike most of the malignancies, carcinoma cervix is readily preventable when effective programmes are conducted to detect and treat its precursor lesions. With the introduction of pap test, the world wide incidence and mortality of carcinoma cervix has been dramatically reduced. However in India, routine cervical screening programme is not done significantly in the peripherals and hence rural women are to be encouraged to step forward to utilize these programmes.

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