International Journal of Clinical and Diagnostic Pathology

ISSN (P): 2617-7226
ISSN (E): 2617-7234
www.patholjournal.com
2021; 4(3): 04-10
Received: 04-07-2021
Accepted: 07-08-2021

Dr. Shraddha Vardivale
Year resident, Department of Pathology, Gmers Medical College and Civil Hospital, Sola, Ahmedabad, Gujarat, India

Dr. Jignasa Bhalodia
Professor and HOD, Department of Pathology, Gmers Medical College and Civil Hospital, Sola, Ahmedabad, Gujarat, India

Corresponding Author:
Dr. Shraddha Vardivale
Year resident, Department of Pathology, Gmers Medical College and Civil Hospital, Sola, Ahmedabad, Gujarat, India

A study of cytopathological pattern of cervical papanicolaou smear examination in women between 20 - 75 years of age group

Dr. Shraddha Vardivale and Dr. Jignasa Bhalodia

DOI: https://doi.org/10.33545/pathol.2021.v4.i4a.414

Abstract
Background: Cancer of uterine cervix is a leading cause of mortality and morbidity among women worldwide. The primary cause of this cancer is infection with human papillomavirus. Pap smear is a simple, safe, non-invasive and cost effective method for the detection of pre-cancerous, cancerous and benign lesions of cervix.
Aim: To study the cytological patterns of cervical Pap smears in 766 cases and to classify the cytological abnormalities based on the Bethesda system 2014.
Materials and methods: A retrospective study of total 766 cases of cervical smears was done at Pathology department, GMERS Medical College and Civil Hospital, Sola, Ahmedabad over a period of 1.5 years starting from 1st January 2020 to 30th June 2021. Women between 20 -75 years of age group were included in the study. Cervical smears were taken by obstetrics and gynaecology department and were received at pathology department, stained by using rapid Papanicolaou staining technique and studied by using light microscopy.
Results: we studied total 766 cases out of which 26(3.40%) smears were unsatisfactory for evaluation. Out of these 740 cases, most common were inflammatory lesions-382 cases (49.86%).Cervical epithelial cell abnormalities including ASCUS-5 cases (0.67%), ASC-H -2 cases (0.24%), LSIL- 6 cases (0.78%), HSIL-1 case (0.13%) and AGC-NOS – 1 case (0.13%) were detected in our study. Majority of the cases were belonging to 31-40 years of age group -364 cases(47.50%) followed by 41-50 years-260 cases (33.94%).
Conclusion: Pap smear is an easy, simple, safe and noninvasive method of cervical examination. By using Pap smear as a screening test, it is possible to detect malignant lesions at an earlier stage.

Keywords: Cancer of uterine cervix, Pap smear, screening test, epithelial cell abnormalities, human papilloma virus

Introduction
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 conditions of the cervix or other medical conditions. It was named after Dr. George N. Papanicoloau, who first described it in 1928 [1]. Cancer of uterine cervix is a leading cause of mortality and morbidity among women worldwide. In developing countries it is the most common gynecological cancer and one of the leading causes of cancer death among women [2].
Cervical cancer is the third most common cancer and the fourth leading cause of cancer death in women worldwide. The primary cause of this cancer is infection with human papilloma virus [3]. According to the report published by the World Health Organization (WHO), 80% of deaths from cervical cancer were from developing countries because of poor screening facility in the society as well as poor awareness among women [4].
Every year cervical cancer is diagnosed in about 5,00,000 women globally and is responsible for more than 2,80,000 deaths annually [5]. It is estimated that in India, 1,26,000 new cases occur each year [6]. Even though national guidelines are there, the screening coverage in India is grossly inadequate and is mainly due to inequality between infrastructure, resources and outsized population. As a result, in most of the cases the diagnosis of cervical cancer is based on opportunistic screening or after the onset of symptoms [7].
Pap smear is a simple, safe, non-invasive and cost effective method for the detection of pre-cancerous, cancerous and benign lesions of cervix.

With the use of Pap smear as a screening tool for the detection of abnormal epithelial lesions in cervix, more cases can be diagnosed early and thus the morbidity and mortality of patients can be decreased [8]. Active participation of the target population is required for the success of the screening program [5].

The present study was done to study the cytological pattern in 766 cases of cervical Pap smears and to classify the cytological abnormalities based on the Bethesda system 2014.

Materials and Methods

A retrospective study of 766 cases was conducted at the department of pathology, GMERS Medical College and Civil Hospital, Sola, Ahmedabad.

Study period: Study duration was of 1.5 years starting from 1st January 2020 to 30th June 2021.

Inclusion criteria

- All female patients who presented at outdoor patient department of Obstetrics and Gynaecology of this hospital and gave informed oral consent for the procedure.
- Female patients with chief complaints of vaginal discharge, abdominal pain, dyspareunia, vaginal itching, postcoital bleeding, postmenopausal bleeding, backache were included in the study.

Exclusion criteria:

Patients who refused to give consent and patients with vaginal bleeding other than post coital or post menopasal bleeding were excluded.

The cervical smears were collected by Obstetrics and Gynaecology department of our hospital. Informed oral consent was obtained from all the female patients before carrying out the procedure. Relevant history (clinical complaints, age at first childbirth, parity, any past history of abortion or disease, husband suffering from any sexually transmitted disease) was obtained from all the female patients.

Two smears were prepared on a clean glass slide with the help of Modified Ayre's spatula/endocervical brush by transforming the material collected and immediately fixed by 95% ethyl alcohol for at least 15 minutes. These smears (one labelled as cervical and other labelled as endocervical) were received at the pathology department along with completely filled cytology requisition form. Rapid Papanicolaou staining technique was used for staining all the smears.

Smears were examined under light microscopy and lesions were classified according to the Bethesda system for reporting cervical cytology, 2014.

Observation and Results

A total 766 cases of cervical Pap smears were retrospectively analysed.

Women between age group of 20-75 years were included in the study. Out of these, maximum number of cases were found in the age group of 31-40 years (47.50%) followed by 41-50 years (33.94%), whereas least number of cases were found in the age group of 71-75 years (0.40%). [Table no.1]

Out of total 766 cases of cervical Pap smears, 26 smears (3.40%) were unsatisfactory for evaluation based on criteria applied according to the Bethesda system 2014. In the present study, out of remaining 740 cases, majority of the cervical smears examination showed presence of inflammation accounting for 382 cases (49.86%). In 22 cases (2.88%) inflammation was associated with reactive cellular changes. Trichomonas vaginalis was identified in 68 cases (8.87%) (Figure no.4). Shift in flora along with stippled epithelial cells and intense neutrophilic inflammation – Bacterial vaginosis was noted in 122 cases (15.96%). Fungal pseudo hyphae along with budding yeast-morphologically consistent with Candida was identified in one case (0.10%). 18 cases (2.35%) showed presence of atrophic smears consisting mainly of parabasal cells along with inflammation. Epithelial cell abnormalities were identified in 15 cases (1.95%) in our study.

These abnormalities include

- 5 cases of ASCUS (atypical squamous cells of undetermined significance)
- 2 cases of ASC-H (atypical squamous cells -cannot exclude HSIL)
- 6 cases of LSIL (low grade squamous intraepithelial lesion)
- 1 case of HSIL (high grade squamous intraepithelial lesion) and
- 1 case of AGC-NOS (atypical glandular cells-not otherwise specified).[table no.2]

Maximum number of these epithelial cell abnormalities were found in 31-40 years (6 cases) and 41-50 years (6 cases).No any single case of these abnormalities was found in 20-30 years of age group. [Table no.3]

In our study, vaginal discharge was commonest clinical symptom(18.60%) followed by abdominal pain(2.14%) and dyspareunia(9.8%).Complain of vaginal discharge was more commonly observed in cases of inflammatory conditions , whereas postmenopausal bleeding was more commonly seen associated with atrophy and epithelial cell abnormalities and premalignant lesions.[table no.4]

Table 1: Age group wise distribution of cases

Age group (in years)	No of cases	Percentage (%)
20-30	122	15.92
31-40	364	47.50
41-50	260	33.94
51-60	12	1.56
61-70	5	0.68
71-75	3	0.40
Total	766	100

Table 2: Cytological findings of cervical pap smear examination

Diagnosis	No. of cases	Percentage (%)
Unsatisfactory	26	3.40
Nilm	725	94.64
Inflammatory lesions	382	49.86
Inflammation associated with reactive cellular changes	22	2.88

Inflammation associated with trichomonas vaginalis	68	8.87
Inflammation associated with bacterial vaginosis	122	15.96
Inflammation associated with fungal organism-morphologically consistent with candida species	1	0.10
Atrophy	18	2.35
Epithelial cell abnormalities:	15	1.95
Squamous cell abnormalities:	14	1.82
Ascus	5	0.67
Asc-h	2	0.24
Lsil	6	0.78
Hsil	1	0.13
Glandular cell abnormalities		
Agc-nos	1	0.13

Table 3: Age wise abnormal findings (Cervical epithelial abnormalities)

Age group (in years)	Squamous cell abnormalities				Glandular cell abnormalities	No of cases (total: 15)	Percentage (%)
	ASCUS	ASC- H	LSIL	HSIL	AGC-NOS		
20-30						0	0
31-40	1	2	2		1	6	0.78
41-50	3		2	1		6	0.78
51-60	1		1			2	0.26
61-70			1			1	0.10
71-75						0	0

Table 4: Correlation of Pap smear findings with clinical findings

Clinical symptom (percentage)	Diagnosis (no. of cases)									
	Inflammatory lesions				Atrophy (18)	Ascus (5)	ASCH (2)	LSIL (6)	HSIL (1)	Agc-nos (1)
	Reactive cellular changes (22)	Trichomonas vaginalis (68)	Bacterial vaginosis(122)	Candida vaginitis (1)						
Vaginal Discharge (18.65%)	10	36	80	1	6	3	1	4	1	1
Abdominal Pain (12.14%)	8	32	38	0	9	1	2	2	1	0
Dyspareunia (9.8%)	5	24	23	0	10	3	0	3	0	1
Post coital Bleeding (6.4%)	6	15	20	0	0	2	2	3	1	0
Post-Menopausal Bleeding (1.40%)	1	0	2	0	4	2	0	2	0	0
Backache (6%)	5	10	4	0	4	0	1	2	0	0

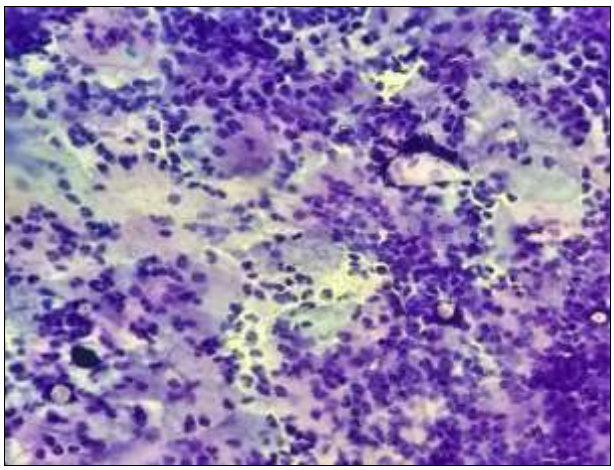

Fig 1: Pap smear showing intense neutrophilic inflammation (40x)

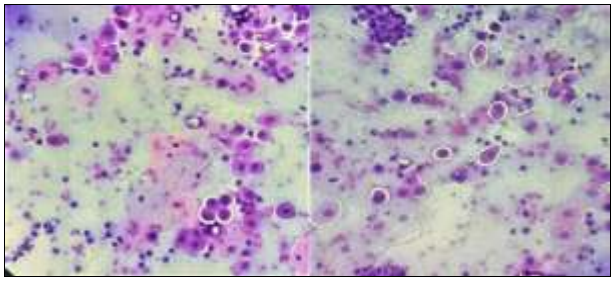

Fig 2: Pap smear showing predominantly parabasal cells along with neutrophilic inflammation-atrophic vaginitis (40x)

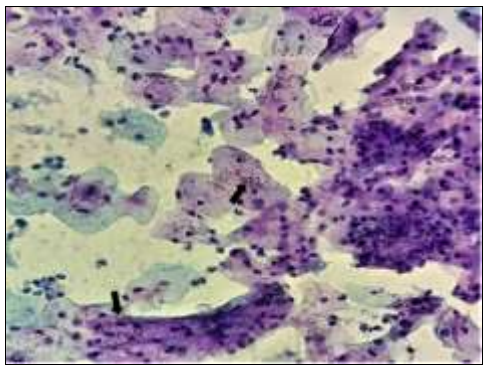

Fig 3: Pap smear showing budding yeast and hyphae- candida (40X)

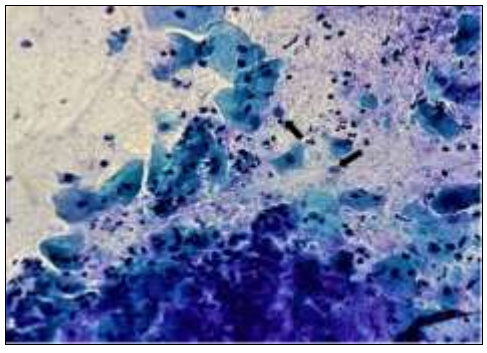

Fig 4: Pap smear showing pear shaped organism – trichomonas vaginalis

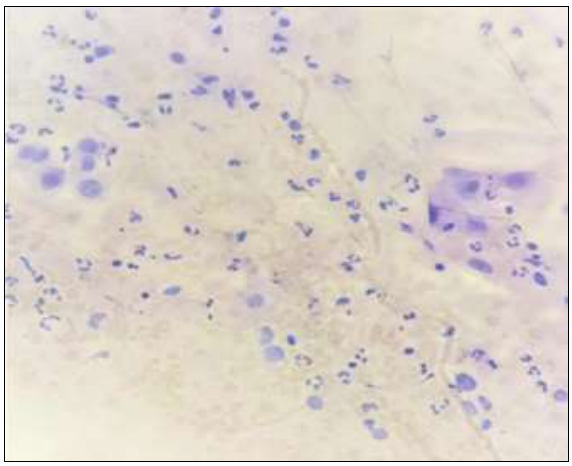

Fig 5: Ascus (40x)

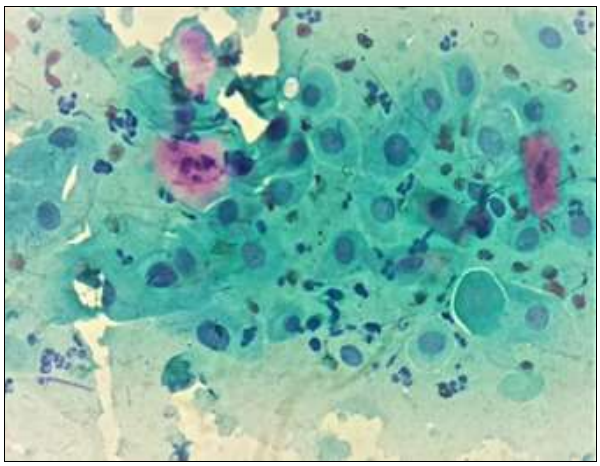

Fig 6: LSIL (40x)

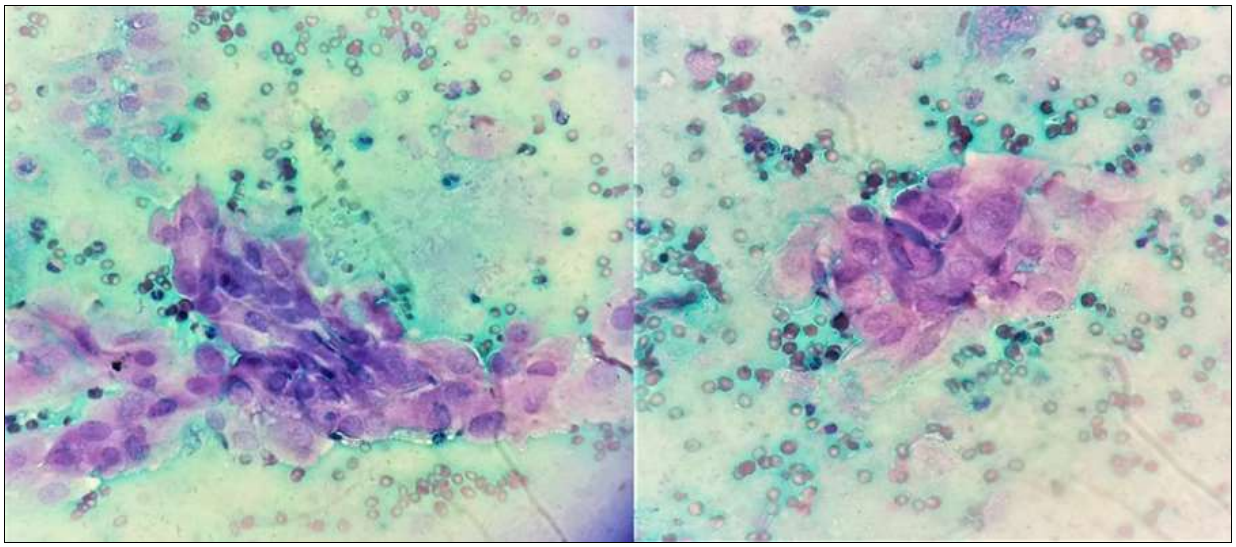

Fig 7(A) & (B): HSIL

Discussion

Human papilloma virus (HPV), the causative organism of cervical cancer is mainly sexually transmitted. Like other sexually transmitted infection, the peak incidence of HPV infection develops shortly after commencement of sexual intercourse. CIN (Cervical Intraepithelial Neoplasia), the precursor lesions of cervical cancer also peaks about a decade after the peak incidence of HPV infection and a decade earlier than invasive cervical cancer. Thus, screening for cervical cancer is recommended to start in the early 20s [10].

Currently, it is well known and proven that HPV 16 and 18 are the most virulent high-risk genotypes, causing about 70% of all invasive cervical cancer in the world. Natural history of CIN lesions is different depending on its grade. CIN1 is a low-grade squamous intraepithelial lesion (LSIL). CIN1 reflects a state of infection rather than a stage in disease development. CIN2 and CIN3 are considered high-grade dysplasia or high grade squamous intraepithelial lesion (HSIL); however, they are different whereby CIN2 less commonly progresses to cancer. CIN3 is considered a true precancer with the potential to progress to invasive cancer at a rate of 0.2% to 4% within 12 months [11].

The Pap smear test used as a screening method to detect cervical cancer is an effective way to prevent the development of cervical cancer, but awareness within the community about the Pap smear test is very low. According to the American Cancer Society (2012), the Pap smear test is a routine cancer screening method that should be done every 3 years [9].

All cervical smears in our study were examined and reported based on the BETHESDA system for reporting cervical cytology 2014.15

Cytological smears were reported as unsatisfactory for evaluation because of:

1. **Inadequate cellularity**

For conventional Pap smears: 8,000-12,000 and for liquid based preparations 5,000 well visualised or well preserved squamous or metaplastic squamous cells are considered adequate.

and /or

2. Absence of endocervical / transformation zone component

and /or

3. Squamous cells obscured by blood, mucus or inflammatory cells.

Table 5: The Bethesda system 2014

Table 1. The 2014 Bethesda System

SPECIMEN TYPE:
Indicate conventional smear (Pap smear) vs. liquid-based preparation vs. other

SPECIMEN ADEQUACY

- ❑ Satisfactory for evaluation (*describe presence or absence of endocervical/transformation zone component and any other quality indicators, e.g., partially obscuring blood, inflammation, etc.*)
- ❑ Unsatisfactory for evaluation . . . (*specify reason*)
  - ❑ Specimen rejected/not processed (*specify reason*)
  - ❑ Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of (*specify reason*)

GENERAL CATEGORIZATION (*optional*)

- ❑ Negative for Intraepithelial Lesion or Malignancy
- ❑ Other: See Interpretation/Result (*e.g., endometrial cells in a woman ≥45 years of age*)
- ❑ Epithelial Cell Abnormality: See Interpretation/Result (*specify 'squamous' or 'glandular' as appropriate*)

INTERPRETATION/RESULT

NEGATIVE FOR INTRAEPITHELIAL LESION OR MALIGNANCY
(When there is no cellular evidence of neoplasia, state this in the General Categorization above and/or in the Interpretation/Result section of the report—whether or not there are organisms or other non-neoplastic findings)
Non-Neoplastic Findings (optional to report)

- ❑ Non-neoplastic cellular variations
  - o Squamous metaplasia
  - o Keratotic changes
  - o Tubal metaplasia
  - o Atrophy
  - o Pregnancy-associated changes
- ❑ Reactive cellular changes associated with:
  - ➢ Inflammation (includes typical repair)
    - o Lymphocytic (follicular) cervicitis
  - ➢ Radiation
  - ➢ Intrauterine contraceptive device (IUD)
- ❑ Glandular cells status post hysterectomy

Organisms

- ❑ *Trichomonas vaginalis*
- ❑ Fungal organisms morphologically consistent with *Candida* spp.
- ❑ Shift in flora suggestive of bacterial vaginosis
- ❑ Bacteria morphologically consistent with *Actinomyces* spp.
- ❑ Cellular changes consistent with herpes simplex virus
- ❑ Cellular changes consistent with cytomegalovirus

OTHER

- ➢ Endometrial cells (*in a woman ≥45 years of age*)

(Specify if "negative for squamous intraepithelial lesion")

esda 2014

Acta Cytologica 2015;59:121–132
DOI: 10.1159/000381842

Table 1 (continued)

EPITHELIAL CELL ABNORMALITIES

SQUAMOUS CELL

- ➢ Atypical squamous cells
  - of undetermined significance (ASC-US)
  - cannot exclude HSIL (ASC-H)
- ➢ Low-grade squamous intraepithelial lesion (LSIL)
  (*encompassing: HPV/mild dysplasia/CIN 1*)
- ➢ High-grade squamous intraepithelial lesion (HSIL)
  (*encompassing: moderate and severe dysplasia, CIS; CIN 2 and CIN 3*)
  - with features suspicious for invasion (*if invasion is suspected*)
- ➢ Squamous cell carcinoma

GLANDULAR CELL

- ➢ Atypical
  - endocervical cells (NOS *or specify in comments*)
  - endometrial cells (NOS *or specify in comments*)
  - glandular cells (NOS *or specify in comments*)
- ➢ Atypical
  - endocervical cells, favor neoplastic
  - glandular cells, favor neoplastic
- ➢ Endocervical adenocarcinoma in situ
- ➢ Adenocarcinoma
  - endocervical
  - endometrial
  - extrauterine
  - not otherwise specified (NOS)

OTHER MALIGNANT NEOPLASMS: *(specify)*

ADJUNCTIVE TESTING
Provide a brief description of the test method(s) and report the result so that it is easily understood by the clinician.

COMPUTER-ASSISTED INTERPRETATION OF CERVICAL CYTOLOGY
If case examined by an automated device, specify device and result.

EDUCATIONAL NOTES AND COMMENTS APPENDED TO CYTOLOGY REPORTS (*optional*)
Suggestions should be concise and consistent with clinical follow-up guidelines published by professional organizations (references to relevant publications may be included).

Based on these criteria, total 26 out of 766 cases were reported as unsatisfactory, out of which in majority of the cases inadequate cellularity was observed.

In our study, majority of patients were belonging to 31-40 years of age group(47.50%), which was compared to the studies done by Omna shaki *et al*.,Sujatha *et al*. and Parate *et al*. [4, 12, 13] as shown in table no.6.

Table 6: Age group wise comparision with other studies

Name of the study		Present study	Parate et al. (2017) %	Sujatha et al. (2016) %	Omna Shaki et al. (2013-2017) %
Age Group in Years	<20	-	0.86	-	-
	21-30	15.92	31.02	18	18.2
	31-40	47.50	40.25	46	44.4
	41-50	33.94	16.86	36	29.8
	>50	2.64	11.01	-	7.4
Total		100	100	100	100

Vast majority of the cases in our study were diagnosed as inflammatory lesions (49.86%). Among epithelial cell abnormalities, ASCUS was diagnosed in 5 cases (0.67%), LSIL in 6 cases (0.78%) and HSIL in 1 case (0.13%). Similar results were observed in studies conducted by Garima et al., [8] Akinfolarin AC et al. [10] and Karuna et al. [14] in which total 100 and 1793 cases were studied respectively. [Table no.7]

Table 7: Comparision of Pap smear results among the other studies

Name of the study		Present Study	Karuna et al. (2003)	Akinfolarin AC et al. (2017)	Garima et al. (2013-2015)
Diagnosis	Inflammatory lesions (%)	49.86	48	34.2	74.99
	ASCUS (%)	0.67	6	6.5	0.42
	LSIL (%)	0.78	7	11.7	0.57
	HSIL (%)	0.13	5	6.2	0.54
	Squamous cell Carcinoma	-	-	-	0.48
	Adenocarcinoma	-	-	-	0.03
Total no of cases		766	100	1793	7127

In the study done by Garima et al., [8] 34 cases were diagnosed as squamous cell carcinoma and 2 cases as adenocarcinoma, whereas in our study of 1.5 years duration, no any single case of malignant cervical lesion has been reported.

Among premalignant lesions, maximum cases were of LSIL- 6 cases (Figure no.6) showing presence of cluster of epithelial cells having nuclear enlargement with mildly increased N:C ratio, hyperchromasia and moderate amount of cytoplasm in the background of inflammation and haemorrhage. 5 cases were diagnosed as ASCUS (Figure no.5) which shows squamous cells with nuclear enlargement, anisonucleosis and moderate amount of cytoplasm. Single case diagnosed as HSIL (Figure no.7A and 7B) shows presence of cluster of squamous epithelial cells showing nuclear enlargement, high N:C ratio, pleomorphism, irregular nuclear membrane and scanty cytoplasm.

Conclusion

Pap smear is an easy, simple, safe and noninvasive method of cervical examination. It is useful screening test which helps in early detection of cervical premalignant and malignant lesions besides detection of non-neoplastic inflammatory and non- inflammatory conditions. In developing countries, due to lack of awareness about cervical cancer screening programme, cervical malignancies are commonly being detected at an advanced stage. So by public health education and encouraging the female population of reproductive age group for screening at an early age, we can reduce the rate of development of cervical carcinoma.

References

1. Mehta V, Vasanth V, Balachandran C. Pap smear. Indian J Dermatol Venereol Leprol 2009;75:214-6.
2. Mandakini Patel M, Amrish Pandya N, Jigna Modi. Cervical Pap smear study. National Journal of Community Medicine 2011, 2(1).
3. Ashtarian H, Mirzabeigi E, Mahmoodi E, Khezeli M. Knowledge about Cervical Cancer and Pap Smear and the Factors Influencing the Pap test Screening among Women. IJCBNM. 2017;5(2):188-195.
4. Shaki O, Chakrabarty BK, Nagaraja N. A study on cervical cancer screening in asymptomatic women using Papanicolaou smear in a tertiary care hospital in an urban area of Mumbai, India. J Family Med Prim Care 2018;7:652-7.
5. Vandana Patel, Piyush Solanki, Harshid Patel. Study of awareness about early detection of cervical cancer by pap smear. Indian Journal of Pathology and Oncology 2017;4(2):308-312.
6. Umarani MK, Gayathri MN, Madhu Kumar. Study of cervical cytology in Papanicolaou (Pap) smears in a tertiary care hospital. Indian Journal of Pathology and Oncology 2016;3(4):679-683.
7. Geethu G. Nair, Fatima Shamsuddin, Thidil Narayanan, Prejisha Balan. Cytopathological pattern of cervical pap smears - a study among population of North Malabar in Kerala. Indian Journal of Pathology and Oncology 2016;3(4);552-557.
8. Malpani G, Agrawal P, Varma AV, Khandelwal N, Tignath G. Cervical Pap smear study and detection of abnormal epithelial lesions and determination of its accuracy by cytohistological correlation in patients of tertiary care teaching hospital in central India. Int J Reprod Contracept Obstet Gynecol 2016;5:2312-6.
9. Sachan PL, Singh M, Patel ML, Sachan R. A study on cervical cancer screening using pap smear test and clinical correlation. Asia Pac J Oncol Nurs 2018;5:337-41.
10. Akinfolarin AC, OlusegunAK, Omoladun O, OmoniyiEsan GO, Onwundiegu U. Age and pattern of Pap smear abnormalities: implications for cervical cancer in a developing country. J Cytol 2017;34:208-11.
11. Chee Kai Chan, Gulzhanat Aimagambetova, Talshyn Ukybassova, Kuralay Kongrtay, Azliyati Azizan. Human Papillomavirus Infection and Cervical Cancer: Epidemiology, Screening, and Vaccination—Review of

Current Perspectives. Hindawi Journal of Oncology 2019, 11. Article ID 3257939.
12. Sujatha P, Indira V, Kandukuri Mahesh Kumar. Study of PAP smear examination in patients complaining of leucorrhoea – A 2 years prospective study in a teaching hospital. IAIM 2016;3(5):106-112.
13. Parate SN, Gupta A, Wadadekar A. Cytological Pattern of Cervical Smears in Leucorrhea. Int J Sci Stud 2017;4(10):85-89.
14. Karuna Gaspanal V, Van Dan Brule R. The clinical profile and cervical cytomorphology, Indian Journal of pathol. Microbiol 2003;46(2):78.
15. Ritu Nayar, David C. Wilbur. The Pap test and Bethesda 2014. Acta Cytologica 2015;59:121-132. Doi: 10.1159/000381842.
16. https://acsjournals.onlinelibrabry.wiley.com/doi/full/10.3322/caac.21628.
17. Fatima Ahmed AL-Hammadi et al. Cervical Cancer Screening in UAE. Asian Pacific Journal of Cancer Prevention 18. Doi:10.22034/APJCP.2017.18.12.3379.
18. Rezaie-Chamani et al. KAP study about Pap smear. Journal of Family and Reproductive Health 6(4).