

Diagnosing Abnormal Intraepithelial Lesion of Cervix to Screen for Cervical Cancer

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Abstract: *Introduction: cervical cancer is the most common genital cancer among women in India. Cervical intraepithelial neoplasia (CIN) is a premalignant transformation and abnormal changes in squamous cells on the surface of the cervix. The precursor can be readily detected by a painless, reproducible, and reasonably accurate screening test, the Pap smear, which has been the basis for the significant decrease in cervical cancer mortality. Aim of the study: The role of pap smear in detecting premalignant and malignant lesions of the cervix and to determine the prevalence of various lesions. Materials and methods: this was a retrospective study done in a private clinic and pathology lab in district Shivpuri (MP). The study was done during the period from 1st October 2018 to 30th September 2020. Result: In this study out of 500 cases, 2.8 % of cases were reported as unsatisfactory. Non-neoplastic smears were reported in (87.2) %, out of which, Inflammatory smear was 77.2 % normal smear were 6.8 % and atrophic smear was 0.4 %. Epithelial cell abnormalities were found in 10 % of cases. The most common epithelial abnormalities was ASCUS 4.8 % followed by LSIL 3 %, ASCH 1.2 %, AGUS 0.4 %, HSIL 0.4 % and SCC 0.2 %. IN our study the epithelial abnormalities were most commonly found in the patient of 41-50 years of age group. Conclusion: Premalignant lesions of the lower genital tract can be picked up in routine screening. Early diagnosis of cancer in the pre-invasive state has a better prognosis and advanced-stage morbidity is prevented. Pap smear is cheaper as compared to cancer treatment in the long run.*

Keywords: Epithelial cell abnormalities, cervical cancer, Papanicolaou Pap smears

1. Introduction

Cervical cancer is the second most common cancer in women worldwide after breast cancer. ¹ Every year in India, 122,844 women are diagnosed with cervical cancer and 67,477 die from the disease²

Cervical cancer is responsible for 25.9% of all cancer cases and 23.3% of cancer deaths among Indian women³. Cervical cancer can be prevented as it is easy to identify and treat its precursor lesions in most cases.

Cervical intraepithelial neoplasia (CIN) is a premalignant transformation and abnormal changes in squamous cells on the surface of cervix⁴ CIN is believed to originate in transformation CIN. The Transformation zone is susceptible to Oncogenic Factors especially during menarche or after pregnancy when the metaplasia is most active. Oncogenic factors are introduced at sexual intercourse. The main etiological agent is human papillomavirus (HPV) (16, 18, 31, 33) infection. Other agents may play & secondary role and are 1. Infections: HIV infection, Herpes simplex, Chlamydia, Trichomoniasis and others, 2. Early sexually intercourse (<16years), 3. Early age of first pregnancy, 4. Multiple sex partners, 5. Multiparity, 6. Dietary deficiency of Vit A, C, E, folic acid, 7. Oral contraceptives users, 8. Smoking, 9. Low socioeconomic conditions, 10. Immunosuppressed individuals.⁵

Precancerous changes of the cervix usually do not cause the pain or any other symptoms and are not detected unless a woman undergoes Screening, Symptoms generally do not appear until abnormal cervical cells become cancerous and invade nearby tissue.⁶ the most common symptoms are Abnormal vaginal bleeding or Bleeding or spotting between periods, Vaginal bleeding after menopause, Vaginal bleeding after sex, Longer or heavier menstrual periods than usual, abnormal vaginal discharge, pain during sexual

intercourse, urinary symptoms or backache. Various methods are available for screening of cervical cancer.

Cytology based screening programme is the mainstay of early detection and prevention of cervical cancer.

Papanicolaou Papanicolaou [Pap] test developed by George Papanicolaou in 1950. Pap test is used primarily as a tool for screening healthy women for pre-invasive cervical cancer (CIN) and early invasive cancer. Screening for cervical cancer using the paps test was successful in reducing the incidence of cervical cancer by 79% and mortality by 70%. According to different studies, cytology has a sensitivity that varies from 47 to 62% and specificity between 60 to 95% for the detection of high-grade cervical intraepithelial neoplasia (CIN2/CIN3), Errors of sampling, fixation, interpretation and follows up may be responsible for missed cases.⁷

Liquid-based cytology is a thin layer slide preparation technology and thus gives better morphological assessment. This will alleviate errors in sampling and preparation and increase the sensitivity to the original goal of 80%.⁵

Automated computerized image processor: It eliminates 25% of most likely negative smears and 75% are selected for cyto technician screening.

Cervicography: this technique was described in the 1990s where a photograph of the cervix is taken and sent for evaluation

Visual inspection with acetic acid (3-5%) (VIA) and Visual inspection with Lugol's iodine- (VILI): In Indian scenario VIA and VILI test for screening of cancer cervix and pickup of pre-invasive disease is proving to be highly sensitive but less specific and very cost-effective as compared to Pap smear. The sensitivity of VIA ranges 66 and 96% and specificity between 64 and 98%⁸. This is as good as or better than conventional cytology the sensitivity

and specificity of VILI was 87.2% and 84.7%, respectively as noted in a cross-sectional study involving 4444 women.⁹

Colposcopy was introduced by Hinselmann in 1972. The purpose of colposcopy is to detect abnormal areas so that selective biopsy can be done. It is not done routinely in all patients. Only patients with positive cervical cytology for malignant cells or suspicious cells with a normal looking cervix need a colposcopy.¹⁰ conservative surgery and its follow up can be done under colposcopic guidance. Abnormal areas appear under colposcopy as acetowhite areas, mosaics, punctuation and abnormal vessels. For final diagnosis, any one of the biopsy techniques e.g. cervical punch biopsy, wedge biopsy, loop biopsy or cone biopsy can be used.

Cone Biopsy is both diagnostic and therapeutic which can be conventional wedge biopsy or loop electro-surgical excision procedure (LEEP).¹¹

An endocervical curettage (ECC) is indicated when the lesion extends up the endocervical canal or if the upper extent of the lesion cannot be visualized.

HPV Testing

HPV Testing is an ideal primary screening modality for cervical cancer. This is attributed to its high sensitivity coupled with a high negative predictive value.¹²

Co-Testing With HPV and Cytology

Most countries with established cytology screening programs first introduced HPV testing as a co-test along with cytology.

Viral Oncoproteins and Biomarkers

Viral E6 and E7 oncoproteins are necessary for malignant conversion the abilities of high-risk HPV E6 and E7 associated with the tumour suppressors p53 and pRB, respectively have been suggested as a mechanism by which these viral proteins induce tumours.¹³

P16 and Ki-67 have emerged as important biomarkers for the detection of high-risk human papillomavirus and in confirming the histopathological diagnosis¹⁴. AgNOR is a new molecular tumour marker which stands for silver-stained nucleolar organizer regions.¹⁵

Screening Protocol: ^(11,15,16)

- When to start : >21 years, Repeat every – 3 yearly, if co-testing (pap smear + HPV DNA testing) – 5 yearly (HPV DNA testing should begin >30 years of age)
- When to stop screening – 65 years (if regular screening in the past 10 years)
- If the total hysterectomy was done for benign causes other than CIN / Cervical cancer – no Screening
- Women who have had a subtotal hysterectomy still have a cervix – Screened regularly.
- HIV positive women – Screened annually
- Vaccinated women – Screened regularly.

The terminology has been changed over the years. The terminology, carcinoma in situ (CIS) was introduced by Rubin (1910), ‘Dysplasia’ by Walters and Regan (1956) and cervical intraepithelial neoplasia (CIN) by Richart (1967). WHO (1975) redefined CIN into three categories.⁽¹⁶⁾

Persistent infection with high-risk HPV types may lead to precursor lesions of the cervix, referred to as CIN, which is epithelial cellular change, where the ratio nucleus to the size of the cell is increased. CIN is graded as CIN 1 (mild), CIN 2 (moderate), or CIN 3 (severe) depending on the proportion of the thickness of the epithelium showing mature, differentiated and undifferentiated cells. Most of the CIN 1 and 2 lesions regress spontaneously; however, untreated in which 4% reach the invasive stage by 1 year, 11% by 3 years, 22 % by 5 years and 30% by 10 years. CIN is now replaced with the low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL)⁷

The Bethesda System (TBS) in 1988 and modified in 2014, introduced a uniform terminology for reporting Pap test results (Table 1)¹⁷

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 the reason)
- A specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of (specify the 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

Squamous metaplasia
 Keratotic changes
 Tubal metaplasia
 Atrophy
 Pregnancy-associated changes
 Reactive cellular changes associated with:
 Inflammation (Includes typical repair)
 Lymphocytic (follicular) cervicitis
 Radiation
 Intrauterine contraceptive device (IUD)
 Glandular cells status post hysterectomy
Organisms
 Trichomonas vaginalis.
 Fungal organisms were morphologically consistent with Candida spp.
 A shift in flora suggestive of bacterial vaginosis.
 Bacteria were morphologically consistent with herpes simplex virus.
 Cellular changes were consistent with cytomegalovirus.
Other
 Endometrial cells (in a woman ≥ 45 years of age)
 (Specify if "negative for squamous intraepithelial lesion")
EPITHELIAL CELL ABNORMALITIES
SQUAMOUS CELL
 Atypical squamous cells

- Of undetermined significance (ASC-US)
- Cannot exclude HSIL (ASC-H)

 Low- grade squamous intraepithelial lesion (HSIL)
 (encompassing: HPV/mild dysplasia/CIN1)
 High-grade squamous intraepithelial lesion (HSIL)
 (encompassing: moderate and severe dysplasia, CIS; CIN 2 and CIN 3)

- With features suspicious for invasion (if an invasion is suspected)

 Squamous cell carcinoma
GLANDULAR CELL
 Atypical

- Endocervical cell (NOS or specify in comments)
- Endometrial cells (NOS or specify in comments)
- Glandular cells (NOS or specify in comments)

 Atypical

- Endocervical cells, favour neoplastic
- Glandular cells, favour 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 on 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).

For Prevention of cancer of cervix regular screening to find out any pre-cancerous lesion and treat them, and to get the HPV vaccine if the patient is eligible is a must. According to CDC recommended age for HPV vaccination is 11-12 years (before sexual activity) and can be given between 9 to 26 years.

2. Materials and Methods

This was a retrospective study done in a private clinic and pathology lab in district Shivpuri (MP) the study was done during the period from 1st October 2018 30th September 2020.

Inclusion criteria:- Patients complaining of vaginal discharge, post-coital bleeding dyspareunia intermenstrual bleeding postmenopausal bleeding were included in this study the age of the patients was between from 21 to 65 years.

Exclusion criteria: Women with invasive cervical cancer and those who are not giving consent for pap smear were excluded from the study.

In this study, the results of pap smear collected from 500 women were analyzed. Data were collected from the records and reports from the clinic and pathology lab. Data were

searched for detailed history, personal information for each case, literacy, socio-economic status, parity, various complaints, any associated high-risk factor, clinical finding and other relevant information Reporting was according to the guideline of 2014 Bethesda system.

Technique

No vaginal examination was performed before taking the smear. Women were advised to abstain from intercourse, douching and any medicinal creams for at least 24 hours before the test, the patient is placed in the dorsal position and a Cusco speculum is inserted, Sample was taken with Ayre’s spatula and cytobrush from transformation zone and end cervix, Slides are made, spray fixation was done and send to the pathology lab.

A total of 500 cases were analyzed during a period of two years. Different cases were studied and all the data were analyzed using IBM SPSS ver.20 software. Frequency distribution and cross-tabulation was used to prepare tables, data is expressed as a percentage.

3. Results

Table 2: Socio-demographic characteristics the age of women ranged from 21 to 75 years

Age Group (years)	No. of cases	Total (%)
<21-30	42	8.4
31-40	209	41.8
41-50	153	30.6
51-60	72	14.4
>61	24	4.8
Total	500	100
Residence		0
Rural	416	83.2
Urban	84	16.8
Total	500	100
Marital Status		0
Married	497	99.4
Unmarried	3	0.6
Total	500	100
Parity		0
Nulliparous	18	3.6
Primiparous	54	10.8
Multiparous	428	85.6
Total	500	100
Educational level		0
Illiterate	23	4.6
Primary	59	11.8
Middle	57	11.4
HSC	258	51.6
Graduation	77	15.4
Post Graduation	26	5.2
Total	500	100

Table 2 that shows in our study most of the women were of 31 to 40 year 41.8% age group, most of them were rural 83.2 and married 99.4% of women were multiparous 85.6%.

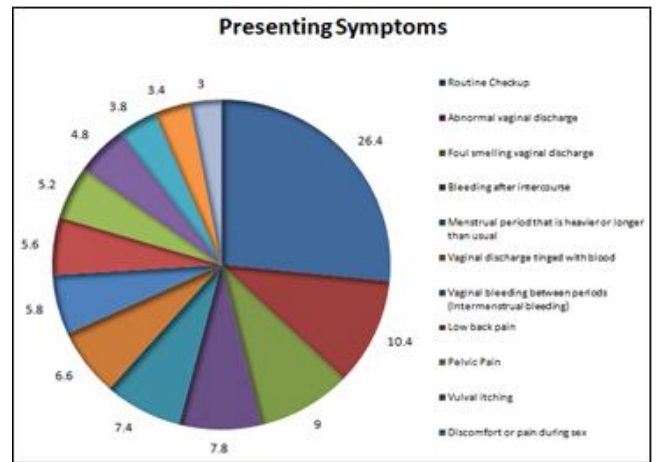


Figure 1: Presenting Symptoms

Fig. 1 shows that the most common presenting symptoms were abnormal vaginal discharge. 26.4%

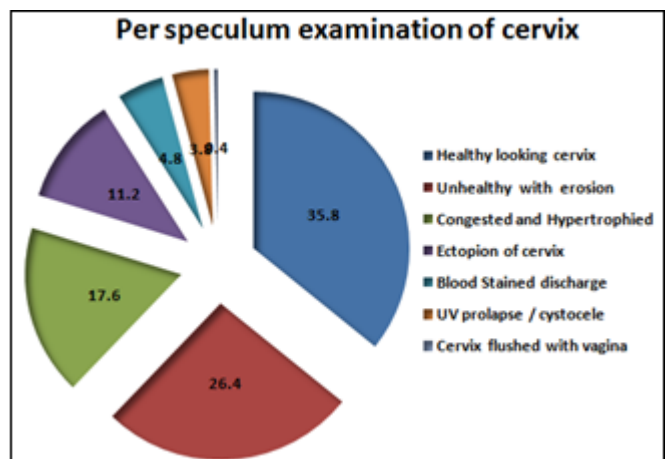


Figure 2: Per speculum examination findings of the cervix

The most common finding on per speculum examination was healthy-looking cervix 35.8% followed by unhealthy with erosion 26.4%.

Table 3: Non-neoplastic cytological diagnosis in pap smear (436 cases)

Non-neoplastic cytological diagnosis	Number	Percentage
NILM Normal	34	6.8%
Atrophy	2	0.4%
Inflammatory	386	77.2%
Nonspecific inflammation	261	52.2%
Chronic erosive cervicitis	125	25%
Others	16	2%
Squamous metaplasia	4	0.8%
Gardnerella	6	1.2%
Trichomonas	2	0.4%
Candida	2	0.4%
Total	436	87.2%

Out of 500 cases, 87.2% (436) patients were reported as negative for the intraepithelial lesion. 6.8% were reported as normal (NILM) The most common non-neoplastic cytological finding was inflammatory 77.2% out of which nonspecific inflammation 52.2%, chronic erosive cervicitis 25% and other inflammatory lesion were 2.8%.

Table 4: Categorization of epithelial abnormalities based on Cyto diagnosis

Cytopathology	No. of cases	Total (%)
Unsatisfactory	14	2.8
Epithelial cell abnormalities		
ASC-US	24	4.8
ASC-H	6	1.2
AGUS	2	0.4
LSIL	15	3
HSIL	2	0.4
SCC	1	0.2
Total Epithelial cell abnormalities	50	10

ASC-US: Atypical squamous cells of undetermined significance; ASC –H: Atypical squamous cells; that cannot rule out high- grade lesion; AGUS Atypical glandular cell of undetermined significance LSIL: Low – grade squamous intraepithelial lesion HSIL: High grade squamous intraepithelial lesion; SCC: Squamous cell carcinoma;

In our study, 2.8% of cases were reported as unsatisfactory. 10% of reports showed epithelial abnormalities. The most common epithelial abnormalities was ASCUS 4.8% followed by LSIL 3%, ASCH 1.2%, AGUS 0.4%, HSIL 0.4% and SCC 0.2%.

Table 5: Age-wise distribution of epithelial cell abnormalities

Epithelial abnormalities	21-30	31-40	41-50	51-60	>60	Total	%
ASCUS	8	7	6	3	0	24	4.8
ASC – H	0	1	4	1	0	6	1.2
AGUS	--	1	1	--	--	2	0.4
LSIL	0	7	5	2	1	15	3
HSIL	--	0	1	1	0	2	0.4
SCC	--	--	0	0	1	1	0.2

In our study, the epithelial abnormalities were most commonly found in Perimenopausal (41-50) age group. The most common age of the patient in our study for ASCUS 21-30 years, LSIL was 31- 40 years, HSIL was 41-60 years, SSC more than 60 years.

Table 6: Various parameters of pap smear in the present study compared with other studies.

Cytopathology	Present Study	Tejaswini et al	Kalyani et al ²⁵	Chandni B Patel	Sarvani P	Ekta Rani ²⁶	Shaki O ²⁷	Bal MS et al ²⁸
Unsatisfactory	2.8%	0.95%	17.80%	8.9%	6.50%	1.73%	1%	-
ASCUS	4.8%	2.38%	1.46%	1.7%	2.62%	0.86%	4%	0.3%
AGUS	0.4	0.48%	0.24%	0.4%	0.10%	00%	-	-
LSIL	3	0.95%	0.24%	0.6%	2.43%	00%	6.8%	2.7%
HSIL	0.4	1.43%	0.41%	1.2	1.26%	00%	1%	0.7%
SCC	0.2	0.48%	0.41%	0.1%	0.48%	0.43%	2.3%	1.0%

5. Conclusion

Several tests can be used for screening of cervical cancer. The Pap smear (cytology) is the only test that has been used extensively and has been pivotal in reducing cervical cancer mortality and incidence. Other tests like VIA, VILI and HPV also hold immense potential but there isn't significant evidence on their effectiveness. Some researchers are trying to study other tests in depth.

4. Discussion

In our study the most of the women were of 31 to 40 year 41.8% age group. Most of the women were rural 83.2%, married 99.4% and multiparous 85.6%.

The most common presenting symptoms were abnormal vaginal discharge. 26.4%

The most common finding on per speculum examination was healthy-looking cervix 35.8%. Cervical erosions were present in 26.4%, which was consisted of another study in which they found the same. ¹⁸

Out of 500 cases, 87.2% patient was reported as negative for intraepithelial lesion the most common non-neoplastic cytological finding was inflammatory 77.2% which was consisted of the study of Aruna lahari. ¹⁹

In our study 2.8% cases were reported as unsatisfactory, 50 cases (10%) showed epithelial abnormalities. The most common epithelial abnormalities were ASCUS 4.8 Which was consistent with the result of the study of Tejaswini et al ²⁰ 2.38%, Sarvani²¹ P 2.62%, But not consistent with the result of the study of Geethu G. Nair ²² in their study the most frequent epithelial abnormalities was LSIL 2.7%.

In our study, the epithelial abnormalities were most commonly found in the age group of (41-50) which was consistent with the study of J Mishra ²³ in which Cytopathological changes were mostly associated with the onset of menopause But our study was not consistent with the study of Geethu G. Nair epithelial abnormalities were most common in post-menopausal age group (51-60 years) with a mean age of 50.89 and in the study of Bukhari et al ²⁴ the 44.9% cases of epithelial abnormalities were also present in between (51-60yrs) age group.

The most common age of the patient in our study for ASCUS 21-30 years, LSIL was 31- 40 years, HSIL was 41-60 years, SSC more than 60 years. This is consistent with the study of Saravni P et al in their study. ASCUS and LSIL were seen predominantly in 21-30 years age group, HSIL in 31-40 years age group and SCC in the sixth decade

Cervical cancer should be diagnosed and treated at the pre-invasive phase at it lasts several years before it becomes invasive and incurable. Early diagnosis of cancer in the pre-invasive state has a better prognosis and advanced-stage morbidity is prevented. Cytology based screening program is still the mainstay in the prevention of cervical cancer. Pap test is the most effective, very simple, easy to perform, painless, cost-effective, reproducible and reasonably accurate screening test to detect cervical epithelial lesions

and thus helpful in reducing the morbidity and mortality of cervical cancer.

Medical Doctors Nursing Staff ANM Asha Anganwadi Worker and other health Workers Should be made aware about importance of Paps smear. Health Checkup camps should be conducted in order to raise awareness about Pap smear test and cervical cancer prevention.

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