Spectrum of Cervical Lesions in Pap Smear with Histopathological Concordance: A Cross-sectional Study

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ABSTRACT

Introduction: Cervical cancer is amongst the leading causes of death in females. According to the World Cancer Statistics, the global incidence of cervical cancer is 6.5% and mortality related to it is 7.5%. The most effective way to screen and thus treat in early stages is achieved by screening the patients with Papanicolaou (Pap) smear.

Aim: To assess the spectrum of cervical lesions in Pap smears, classify them as per the Bethesda System of reporting Cervical Cytology and compare the cytology results with histopathology where available.

Materials and Methods: The present study was a cross-sectional study carried out for a period of two months from August 2022 to September 2022 in Rohilkhand Medical College and Hospital, Bareilly, Uttar Pradesh, India. The slides of Pap smear were reported as per the 2014 Bethesda System of reporting cervical cytology. Comparison with the histopathological findings was done in cases whenever cervical biopsy or hysterectomy specimen was received. Data was collected, entered and compiled in Microsoft excel followed by analysis using software Statistical Package for the Social Sciences (SPSS) 23.0. The data was represented in frequency and validity was calculated in terms of sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and accuracy. The p-value was calculated using Chi-square test and significance was set at p<0.05.

Results: All cases received during the study duration of two months were included in the study which was 400 consecutive PAP smears. Epithelial cell abnormality was seen in 17 (4.25%) cases. Atypical Squamous Cells of Undetermined Significance (ASCUS) was seen in 8 (2%) cases, Low-grade Squamous Intraepithelial Lesion (LSIL) in 2 (0.5%) cases, High-grade Squamous Intraepithelial Lesion (HSIL) in 1 (0.25%) case, Atypical Squamous Cells- cannot exclude high-grade squamous intraepithelial lesion (ASC-H) in 4 (1%) cases and Squamous Cell Carcinoma (SQCC) in 2 (0.5%) cases. Concordance with histopathathology was seen in 33 of 37 cases. The overall sensitivity, specificity, PPV, NPV and diagnostic accuracy was 90%, 88.9%, 75%, 96% and 89%, respectively. The p-value was 0.00001.

Conclusion: Cases diagnosed on Pap smear as low-grade epithelial cell abnormality including ASCUS and LSIL should be kept in follow-up, whereas cases diagnosed with high-grade epithelial cell abnormality including ASC-H, HSIL or SQCC, appropriate treatment should be planned.

Keywords: Atypical squamous cells, Intraepithelial, Negative for intraepithelial lesion or malignancy

INTRODUCTION

Cervical cancer is one of the leading causes of death in females [1]. According to the World Cancer Statistics, the global incidence of cervical cancer is 6.5% and mortality related to cervical cancer is 7.5% [2]. In Asian region too, the cervical cancer is amongst the second most common cancers in incidence after breast cancer but has highest mortality rate in certain areas. The World Health Organisation (WHO) cervical cancer elimination campaign launched in November 2020 aims to achieve a world without cancer by large scale implementation of prevention and control measures, screening and treatment and to make these universally available. The most effective way to screen and thus treat in early stages is achieved by screening the patients with Pap smear [2]. Pap smear cytology is a safe, non invasive Out Patient Department (OPD) based screening method with quick diagnosis which involves scraping of the cervical epithelial cells, staining and analysing them for epithelial cell abnormalities [3-5].

The reporting of pap smears is done according to the 2014 Bethesda System for Reporting Cervical Cytology [6] which is an internationally accepted format of reporting pap smears thus making the reporting pattern more reliable and reproducible. Histopathological correlation of the cases where indeterminate results are obtained is helpful in making a final diagnosis [7,8]. This study intends to gain insight in identifying the common lesions of cervix and early detection of epithelial cell abnormality which will help prompt treatment of carcinoma cervix

and better prognosis of the disease. Study was aimed to assess the spectrum of cervical lesions in pap smears and classify them as per the Bethesda System of reporting cervical cytology. To compare the results of pap smear cytology with histopathology where available. Spectrum assessment of cervical lesions includes all the entities separately specially the benign and infective causes which are all clubbed into a single entity of Benign Reactive changes of Inflammation and Repair (BRIC), Negative for Intraepithelial Lesion or Malignancy (NILM) in the Bethesda classification.

MATERIALS AND METHODS

The present study was a cross-sectional study carried out for a period of two months from August 2022 to September 2022 after ethical clearance from the Institutional Ethics Committee (IEC) (IEC No-IEC/RMCH/82/2022/AUG).

Inclusion criteria: All the samples of Pap smear received in the Department of Pathology from the gynaecology department for routine investigation were included in the study.

Exclusion criteria: Cases where previous therapeutic interventions in cervix like cervical surgery were done were excluded from the study.

Procedure

The demographic details, symptoms, per speculum findings were noted from the case sheet. The slides were received in alcohol fixative and stained using the Pap stain. The detailed description of the slides

was noted and the cases were reported as per the 2014 Bethesda System of reporting cervical cytology [6]. For histopathology the fixative used was 10% formalin and the slides were stained using Haematoxylin and Eosin (H&E). Comparison with the histopathological findings was done in cases whenever cervical biopsy or hysterectomy specimen was received.

STATISTICAL ANALYSIS

Data was collected, entered and compiled in Microsoft excel followed by analysis using the software SPSS version 23.0. The data was represented in frequency. The validity was calculated in terms of sensitivity, specificity, PPV and NPV. The p-value was calculated using Chi-square test.

RESULTS

The most common age group of the females presenting in the OPD was 21-30 years with 158 (39.5%) of cases, followed by 31-40 years with 147 (36.75%) of cases. A total of 397 (99.25%) of the females were married [Table/Fig-1].

Variables	N (%)
Age range (years)	
11-20	06 (1.5)
21-30	158 (39.50)
31-40	147 (36.75)
41-50	58 (14.50)
51-60	16 (04)
61-70	12 (03)
71-80	03 (00.75)
Marital status	
Unmarried	03 (00.75)
Married	397 (99.25)
Total	400 (100)

[Table/Fig-1]: Demographic profile of patients.

The most common symptom at presentation was pain abdomen in 120 (30%) of patients followed by white discharge in 86 (21.5%) of patients, menstrual disturbance in 78 (19.5%) cases, infertility in 28 (7%) of cases. Postcoital bleeding and postmenopausal bleeding was seen in 4 (1%) of cases each, 40 (10%) were asymptomatic at presentation [Table/Fig-2].

Main symptom	N (%)
Asymptomatic	40 (10)
Dysmenorrhoea	6 (1.5)
Hypomenorrhoea	20 (5)
Amenorrhoea	12 (3)
Menorrhagia	20 (5)
Irregular cycles	20 (5)
Postcoital bleeding	4 (1)
Postmenopausal bleeding	4 (1)
White discharge	86 (21.5)
Something coming out per vagina	12 (3)
Pain abdomen	120 (30)
Fever	8 (2)
Infertility	28 (7)
Burning micturition	20 (5)
Total	400 (100)

[Table/Fig-2]: Distribution of patients as per main presenting symptom.

The per speculum examination findings are shown in [Table/Fig-3].
[Table/Fig-4] shows categorisation of cases as per age and the 2014 Bethesda system. Maximum number of PAP smears, 376 (94%)

were conventional smears. Liquid based cytology was done in 24 (6%) cases. A total of 389 (97.25%) of the smears received were adequate for reporting. Eleven (2.75%) cases were inadequate. The inadequate cases were of the conventional cytology. None of the liquid based cytology cases included in this study were inadequate. The inadequacy of smears was attributed to low cellularity of squamous cells, abundant neutrophils or haemorrhage obscuring the cellular details. Majority of pap smears 372 (93%) were NILM with additional non neoplastic findings of BRIC in 337 (84.25%) cases. Bacterial vaginosis was seen in eight cases. *Trichomonas vaginalis* {as shown in [Table/Fig-5]}, *Candida* and granulomatous inflammation (as shown in [Table/Fig-6]) were seen in one case each, 24 cases showed changes of atrophy.

Finding	N (%)
Healthy looking cervix	120 (30.00)
Discharge per vagina	129 (32.25)
Cervical erosion	82 (20.50)
Hypertrophied cervix	38 (9.50)
UVprolapse/cystocele	22 (5.50)
Cervix Bleeds on touch	9 (2.25)
Total	400 (100)

[Table/Fig-3]: Distribution of patients according to per speculum examination findings.

| Interpretation	Age of patients (years)							Total
	11-20	21-30	31-40	41-50	51-60	61-70	71-80	
Conventional smear	6	153	138	51	13	12	3	376
Liquid based preparation	-	5	9	7	3	-	-	24
Inadequate smear	-	4	2	4	-	1	-	11
NILM	6	152	141	50	11	10	2	372
ASCUS	-	2	1	2	1	1	1	8
LSIL	-	-	2	-	-	-	-	2
ASC-H	-	-	1	2	1	-	-	4
HSIL	-	-	-	-	1	-	-	1
SQCC	-	-	-	-	2	-	-	2
Total	6	158	147	58	16	12	3	400

[Table/Fig-4]: Categorisation of cases as per Bethesda System and age of patients.
*NILM: Negative for intraepithelial lesion or malignancy; ASCUS: Atypical squamous cells of undetermined significance; LSIL: Low-grade squamous intraepithelial lesion; ASC-H: Atypical squamous cells- cannot exclude high-grade squamous intraepithelial lesion; HSIL: High-grade squamous intraepithelial lesion; SQCC: Squamous cell carcinoma
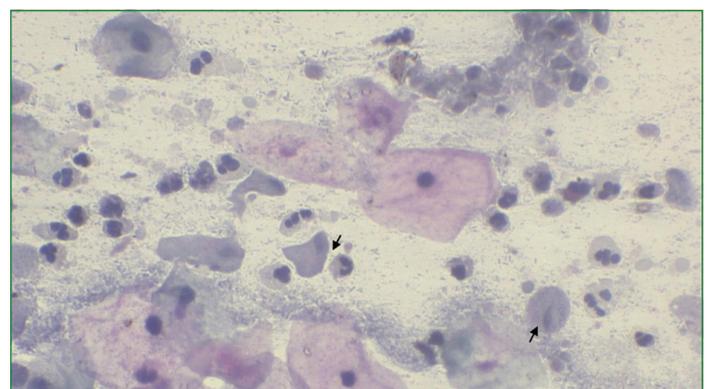

[Table/Fig-5]: Conventional smear-*Trichomonas vaginalis* (black arrow) (Pap stain, X400).

Epithelial cell abnormality was seen in 17 (4.25%) cases. ASCUS was seen in eight cases, LSIL (as shown in [Table/Fig-7]) in two cases, ASC-H (as shown in [Table/Fig-8]) in four cases, HSIL (as shown in [Table/Fig-9]) in one case, and SQCC (as shown in [Table/Fig-10]) in two cases. Distribution of the lesions as per the age of cases showed BRIC along with the infective aetiology was seen in younger age groups upto 40 years. The epithelial cell abnormality
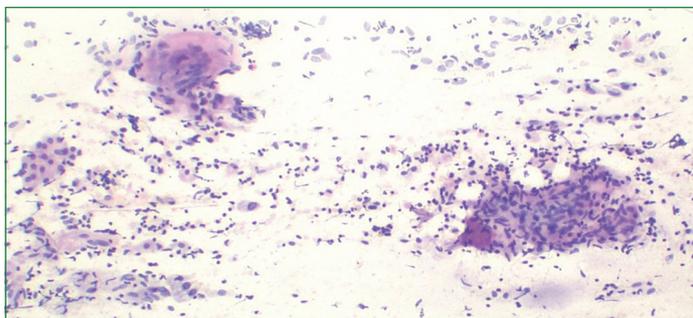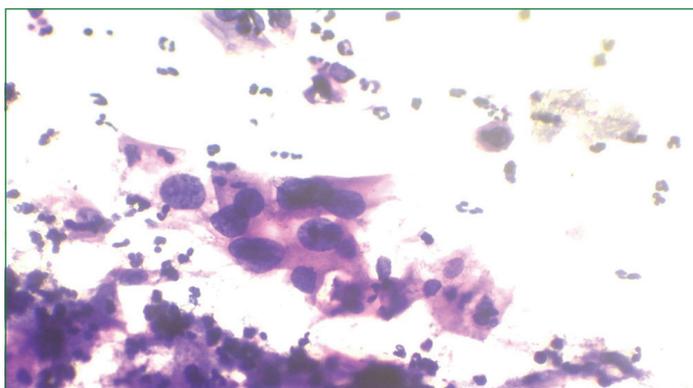
[Table/Fig-6]: Conventional smear showing a granuloma and a giant cell-granulomatous lesion (Pap stain, X100).

was mostly seen in older age groups above 40 years of age and the atrophic smear in more than 40 years of age.
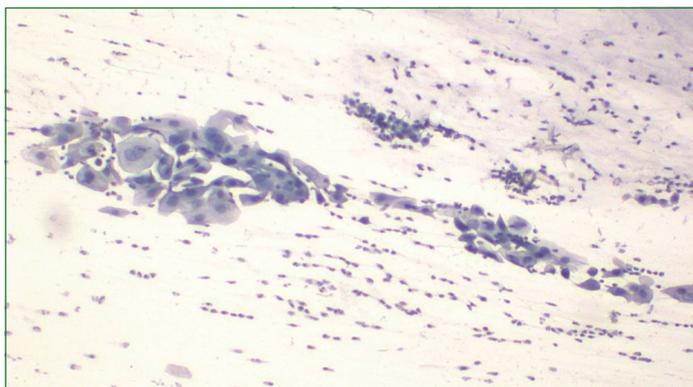
[Table/Fig-7]: LSIL Mature squamous cells with nuclear enlargement >3 times area of intermediate cell nuclei (Pap stain X100).


[Table/Fig-8]: ASC-H (Conventional Smear)- A group of atypical squamous cells with high N/C ratio, irregular nuclear membrane, dense cytoplasm. (Pap stain, X400).
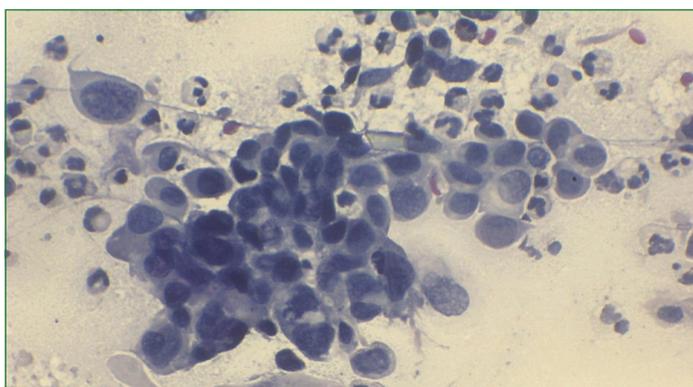
[Table/Fig-9]: HSIL- conventional Pap Smear Hyperchromatic crowded groups with immature cytoplasm and nuclear abnormalities (Pap stain X400).

There was good cytological-histological concordance as shown in [Table/Fig-11]. No gross misdiagnosis was seen on the pap smear cytology. Discordance was considered as detection of epithelial cell abnormality in one modality versus negative result in other modality. Out of 37 cases where histopathology was available, 33 cases showed concordance, four cases showed discordant results. One
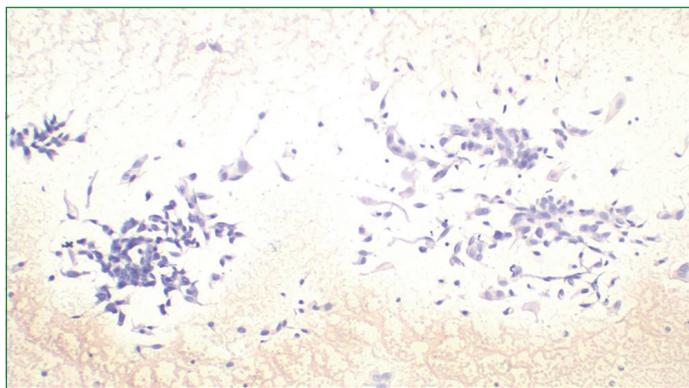
[Table/Fig-10]: Squamous Cell Carcinoma (SQCC)- conventional Pap Smear Atypical, singly scattered, spindle shaped squamous cells with tumour diathesis (Pap stain X100).

case which was reported as NILM on cytology showed nuclear atypia in lower third of the epithelium thus diagnosed as CIN-1 on histopathology. Amongst the four cases of ASCUS on cytology, two cases showed lymphocytic infiltration of the stroma and did not show any atypia on histopathology, thus were labeled as chronic cervicitis as shown in [Table/Fig-12a,b] whereas two cases showed mild atypia in lower third of the epithelium thus diagnosed as CIN-1. Two case which showed nuclear atypia and enlargement >3 times in mature looking squamous cells were diagnosed as LSIL on conventional cytology. On histopathology one case showed atypia in lower 1/3rd epithelium, thus labeled as CIN-1, whereas the other case did not show any atypia, only lymphocytic infiltration, thus diagnosed as chronic cervicitis. One case of ASC-H was CIN-3 (as shown in [Table/Fig-13]) on histopathology, whereas two cases were SQCC. One case of HSIL and two cases of SQCC turned out to be SQCC on histopathology as shown in [Table/Fig-14].

The overall sensitivity, specificity, PPV, NPV and diagnostic accuracy was 90%, 88.9%, 75%, 96% and 89%, respectively. The p-value was 0.00001.

DISCUSSION

India is a developing nation with low income, low literacy rates and gender inequality, due to which women face constrains in seeking health services, though reforms are been undertaken to increase the healthcare information and availability by the government. Cervical cancer is one of the leading causes of death in adult females. Thus, pap smear test has great importance in our country as it is cheaper, relatively simple test with good accuracy. The cervical cancer screening is offered to all the females attending the gynaecology OPD which includes clinical examination, per vaginal and per speculum examination. In present study, the age range was 18 to 75, majority of the females were in the age range of 21-40. This was comparable to other researchers like Saha R and Thapa M, who have shown a mean age of 40 years, Jain V and Vyas AS, and Atla BL et al., both of whom have shown an age distribution of 20 to 70 years in their studies [7,9,10].

The most common symptom in present study was pain abdomen in 30% cases followed by discharge per vaginum, present in 21.5% cases. Other studies like Sherwani RK et al., have also shown white discharge and pain abdomen as the most common presenting complaints in 42.5% of cases and 27.5% cases, respectively [11]. A young female who presented with infertility underwent a Pap smear test and was diagnosed as granulomatous inflammation suggestive of genital tuberculosis as the cause of infertility which can be diagnosed accurately if index of suspicion is kept high also shown by Seth A et al., [12]. Likewise postcoital bleeding was associated with high-grade squamous lesions in the present study as also shown by Sharif YH in their study [13]. The majority of pap smears were conventional smears, 94%. Liquid based cytology comprised of 6% of cases. Though the institute offers both the

| Bethesda classification	Histopathological impression							Concordant cases	Discordant cases
	Non neoplastic	CIN-1	CIN-2	CIN-3	CIS	SQCC	Total		
NILM	24 (96%)	1 (4%)	-	-	-	-	25 (100%)	24	1
ASCUS	2 (50%)	2 (50%)	-	-	-	-	4 (100%)	2	2
LSIL	1 (50%)	1 (50%)	-	-	-	-	2 (100%)	1	1
ASC-H	-	-	-	1 (33.33%)	-	2 (66.67%)	3 (100%)	3	0
HSIL	-	-	-	-	-	1 (100%)	1 (100%)	1*	0
SQCC	-	-	-	-	-	2 (100%)	2 (100%)	2	0
Total	27	4	0	1	0	5	37	33	4

[Table/Fig-11]: Cyto-histopathological concordance
*Discordance was considered as detection of epithelial cell abnormality in one modality verses negative result in other modality. Since this case showed epithelial cell abnormality in both cytology and histology, this case was considered to be concordant; **NILM: Negative for intraepithelial lesion or malignancy; ASCUS: Atypical squamous cells of undetermined significance; LSIL: Low-grade squamous intraepithelial lesion; ASC-H: Atypical squamous cells- cannot exclude high-grade squamous intraepithelial lesion; HSIL: High-grade squamous intraepithelial lesion; SQCC: Squamous cell carcinoma; CIN: Cervical intraepithelial neoplasia; CIS: Carcinoma in situ
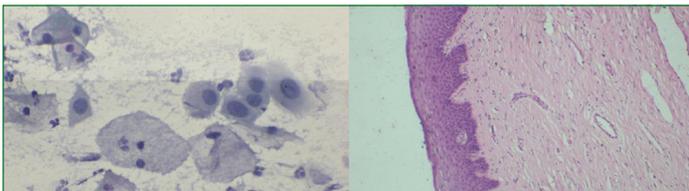

[Table/Fig-12a]: ASCUS shows intermediate squamous cells with enlarged nucleus 2 to 3 times the normal and slight nuclear membrane irregularity, even chromatin distribution (Pap stain X400).
[Table/Fig-12b]: Chronic cervicitis shows mild lymphocytic infiltration, no atypia seen (H&E, X100). (Images from left to right)
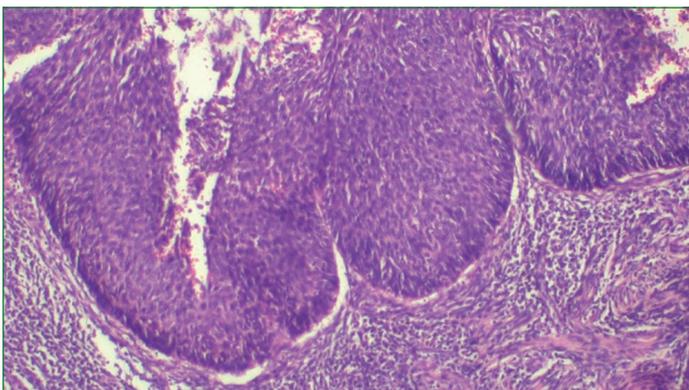
[Table/Fig-13]: CIN-3 Full thickness nuclear abnormality (H&E, X100).
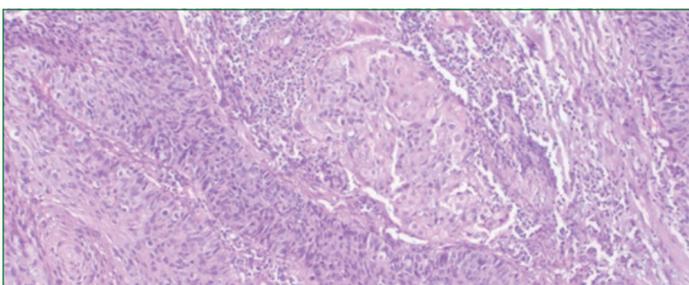
[Table/Fig-14]: Squamous Cell Carcinoma (SQCC) shows tumour cells having high N/C ratio, hyperchromatic nucleus, moderate to abundant eosinophilic cytoplasm infiltrating as anastomosing nests and single cells in the stroma. Focal keratinisation also seen (H&E, X100).

modalities, the cost of conventional pap smear is insignificant compared to the liquid based cytology cost.

The rate of inadequate smears was low in present study, 2.75%, lower to other studies like Sherwani RK et al., who have shown an inadequacy rate of 5% on conventional smears and markedly lower than those stated by Narasimha A et al., of 24%. 93% cases were NILM [11,14]. An 84.25% of cases showed BRIC. A 2.75% showed the infective aetiology including bacterial vaginosis, *Trichomonas vaginalis*, *Candida* and granuloma formation. This was similar to the study by Sherwani RK et al., who have shown infective aetiology in 3.1% of conventional pap smears [11]. Changes of atrophy were seen in 6% cases. In present study, epithelial cell abnormality on cytology was found to be 4.25%, which was comparable with other studies like Cherian DM et al., in Maharastra who found an epithelial cell abnormality of 4.76%, a study by Abdullah LS, showed a rate of 4.3% and 5% in a study by Sadan O et al., [15-17]. This was less as compared to studies by Sachan P et al., who have shown an epithelial cell abnormality of 8.48% [18].

The epithelial cell abnormality included the entities ASCUS, LSIL, ASC-H, HSIL and SQCC. The ASCUS and LSIL were seen in the age group of 21-40 years whereas ASC-H, HSIL and SQCC were seen in 41 to 60 years of age. Similar age distribution was seen in the study done by Banik U et al., who have shown incidence of low-grade lesions was in age group of 35-39 years and high-grade lesions in age group >45 years [19]. However, they have also shown occurrence of low-grade lesions too in the higher age group. Other studies like Bal MS et al., have also shown the occurrence of epithelial cell abnormalities in similar age group who have shown mean age of HSIL and invasive carcinoma as 40.5 years and 57 years, respectively [20]. In present study, two cases of ASC-H were seen in age group of 41-50 years similar to Bal MS et al., [20]. However, one case of ASC-H was also seen in the age group of 31-40 years.

The age group of 51-60 was most common in present study for the occurrence of high-grade epithelial cell abnormalities. One case of ASC-H, one case of HSIL and two cases of SQCC was seen in this age group. The overall sensitivity, specificity, PPV, NPV was 90%, 88.9%, 75%, 96%, respectively. These results were comparative to the findings of Atla BL et al., who have shown values of 94.1%, 64%, 82.75% and 85%, respectively and somewhat superior to the study of Nkwabong E et al., who have shown the values as 55%, 75%, 88.2% and 33%, respectively [10,21]. The diagnostic accuracy in present study was 89% which was slightly higher to other studies by Jain V et al., with values of 79% [9]. Major cytology-histology discordance, defined as HSIL or CIN-2 in one modality with negative result in the other test as described by Gupta R et al., [22]. No major discordance was seen. In present study, the main discrepancy was seen in the low-grade epithelial cell abnormalities which include ASCUS and LSIL. The discrepant cases were of the conventional cytology. The number of discrepant cases can be reduced by increasing the use of liquid based cytology for the pap smear cytology as also shown by Agarwal P et al., liquid based cytology being superior in detecting more ASCUS and HSIL cases when compared to conventional pap however, in high-risk females both methods were equally good [23]. In four cases of ASCUS where histopathology was available two showed CIN-1. These results are comparable with the study of Rad FS et al., who have shown ASCUS in pap smear results with a frequency of 51.9% was associated with CIN-1 in histopathology [24]. The atypia in squamous cells of these cases was attributed to the atypical metaplasia and reactive changes associated with marked inflammation. This highlights the fact that such cases should be followed. Srivastava AN and Misra JS, have postulated in their study that the women in rural areas

follow poor hygiene, and thus harbour persistent genital infections, which in long-term may lead to carcinoma cervix [25].

Limitation(s)
Main limitation of the study was that HPV genotyping was not done.

CONCLUSION(S)
In this study, cases of ASCUS and LSIL showed low-grade atypia. Based on the findings of this study, cases diagnosed on cytology as low-grade epithelial cell abnormality including ASCUS and LSIL should be kept in follow-up, whereas cases diagnosed with high-grade epithelial cell abnormality including ASC-H, HSIL or SQCC, appropriate treatment should be planned.

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