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Original Article

Pathology Section

Cervical Cytology-Histopathology Concordance and Role of Dual Immunomarkers in Biopsy Samples of Uterine Cervix: A Cross-sectional Study from a Tertiary Care Hospital of West Bengal, India

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ABSTRACT

Introduction: Cervical cancer is one of the leading causes of cancer deaths. Liquid-based cervical cytology enables the detection and diagnosis of the disease at an early stage. p40 is a specific immunomarker that distinguishes squamous cell carcinomas from other cervical carcinomas with glandular and neuroendocrine differentiation.

Aim: To determine the role of Liquid-based Cytology (LBC) in the early detection of premalignant and malignant lesions of the uterine cervix and its concordance with histopathological findings.

Materials and Methods: This cross-sectional study was conducted at the Institute of Post Graduate Medical Education and Research (IPGME&R), Kolkata, West Bengal, India, over a period of one year and six months (from October 2020 to March 2022). Cervical samples were collected from a total of 200 females, and the materials were processed using the BD SurePath™ LBC method. The cytologically confirmed cases were biopsied, and histopathological concordance was established. Immunohistochemical (IHC) staining for p40 and p63 was performed using the peroxidase-antiperoxidase method to differentiate between premalignant, malignant squamous, and glandular lesions of the uterine cervix. For quantitative analysis, all cells were counted in 10 random fields at 400x magnification, and p63 and p40 were expressed as a percentage of positive cells per the total number of counted cells. Cases were considered positive if 5% or more of the tumour cell nuclei showed brown nuclear staining. The mean percentage positivity of p63 and p40 nuclear staining of all the cases in each category was calculated for non neoplastic and precursor/neoplastic lesions of the cervix.

Results: Among the 200 cases evaluated by LBC, 169 (84.5%) were non neoplastic, and the remaining 31 (15.5%) were precursor/neoplastic lesions. The vast majority (126 cases, 63%) were inflammatory smears, followed by 20 cases (10%) that were Negative for Intraepithelial Lesion or Malignancy (NILM). Total of 40 cases were followed-up with biopsy and histopathology, and concordance with the cytological diagnosis was evaluated. Among 40 cases, 23 were precursor/neoplastic on histopathology, and seven cases that were non neoplastic on Histopathological Examination (HPE) had been correctly diagnosed on LBC, giving a concordance rate of 75%. The overall sensitivity, specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) of LBC were 92%, 46.6%, 74.1%, and 77.7%, respectively, considering histological diagnosis as the gold standard. Regarding IHC expression on histopathology, the mean positivity of p40 and p63 for non neoplastic lesions was 9.40% and 10.06%, and for precursor/neoplastic lesions, 47.44% and 46.4%, respectively. Adenocarcinoma-in-situ and adenocarcinoma were negative for both p40 and p63.

Conclusion: Cervical cytology is a less invasive, cost-effective, and simple procedure to diagnose cervical epithelial cell abnormalities. Cytological-histopathological concordance revealed that LBC is a sensitive diagnostic method. The comparison between non neoplastic and neoplastic lesions of the cervix revealed a statistically significant difference with respect to the mean percent positivity of p40 and p63 IHC staining. Both of these markers can be used to differentiate squamous cell carcinoma from adenocarcinoma of the cervix.

Keywords: Immunohistochemistry, Liquid based cytology, p40, p63, Uterine cervical neoplasm

INTRODUCTION

Cervical cancer is the fourth most frequently diagnosed cancer and the fourth leading cause of cancer death in women, with an estimated 342,000 deaths worldwide in 2020. The main cause of cervical carcinogenesis is Human Papilloma Virus (HPV) infection [1]. Cervical cytology for cancer screening has significantly reduced mortality from cervical cancer as it detects precursor lesions effectively [2]. Currently, The Bethesda System (TBS) of reporting cervical cytology is the worldwide accepted reporting format. TBS is a two-tiered system that classifies Squamous Intraepithelial Lesions (SIL) as Low-grade Squamous Intraepithelial Lesions (LSIL), which includes HPV-induced changes and grade-1 Cervical Intraepithelial Neoplasia (CIN-I), and High-grade Squamous Intraepithelial Lesions (HSIL), which includes CIN-II and CIN-III [3].

In the female genital tract, p63 is expressed in the basal and parabasal cells of cervical epithelium, and also in reserve cells at the transformation zone [4]. p63 promotes squamous differentiation and is expressed in squamous cell carcinoma [5,6]. p40 is a specific immunomarker that distinguishes squamous cell carcinomas from adenocarcinoma, with a specificity of about 100% in lung carcinomas [7,8]. It has also been used for the detection of squamous differentiation as well as the exclusion of glandular and neuroendocrine differentiation in cervical carcinomas [9,10].

LBC for cervical cancer screening has been shown to increase the detection rate for preneoplastic SIL equal to or greater than the

conventional Papanicolaou smear method. Liquid-based collection and processing provide more representative cervical sampling than the conventional method. Liquid-based Pap test significantly reduces the unsatisfactory rate of Pap test slides and detects a significantly higher number of low- and high-grade squamous lesions when compared with the conventional Pap smear technique [11].

The aim of the present study was to determine the role of LBC in the early detection of premalignant and malignant lesions of the uterine cervix and its concordance with histopathological findings. Also, to know the role of p40 and p63 dual markers in histopathological sections in differentiating cervical squamous cell carcinoma, adenocarcinoma, and the precancerous lesions.

MATERIALS AND METHODS

The prospective cross-sectional study was conducted in the Department of Pathology in collaboration with the Department of Gynaecology and Obstetrics at a tertiary care hospital in West Bengal, India, over a period of one year and six months (from October 2020 to March 2022). All procedures performed in the current study were approved by the Institutional Ethics Committee in accordance with the 1964 Helsinki Declaration and its later amendments (IEC Memo No. IPGME&R/IEC/2021/012, Date: 02.02.2021).

Inclusion criteria: Women in the reproductive age group attending the Gynaecology and Obstetrics Outpatient Department (OPD) with complaints of abnormal vaginal bleeding, vaginal discharge, and dyspareunia were included in this study.

Exclusion criteria: Pregnant women were excluded from the study.

Sample size: A total of 200 women were selected.

Study Procedure

Following a detailed clinical and radiological examination, a cervical sample was collected with a cytobrush, and the materials were processed using the BD SurePath™ LBC method. Slides labeled with the respective numbers of the vial were stained with the PrepStain machine. A provisional cytological diagnosis was made following TBS of reporting, which is the worldwide accepted reporting format for cervical smears. Cytologically suspicious lesions were biopsied, and histopathological diagnosis was made following the World Health Organisation (WHO) 2020 classification of neoplasms of the uterine cervix [12].

Cytological and histopathological diagnosis were associated and followed by IHC staining. IHC staining was performed on formalin-fixed paraffin-embedded sections using a detection system based on the peroxidase-antiperoxidase method. Heat-induced epitope retrieval and enzymatic activation of the chromogen were conducted to visualise the antigen-antibody reaction product. The p63 mouse monoclonal antibody (4A4 clone, Lot no- R07105OA, PathnSitu, USA) and the p40 rabbit polyclonal antibody (Lot no- R07123TA, PathnSitu, USA) were used. Positive tissue controls for p63 were benign prostatic hyperplasia and for p40 were basal cell carcinoma of the skin. The same tissue was used for the internal negative control for each case. All slides were examined under light microscopy by two independent observers. For quantitative analysis, all cells were counted in 10 random fields at 400x magnification, and the p63 and p40 were expressed as a percentage of positive cells per total number of counted cells [13]. Cases were considered positive if 5% or more of the tumour cells showed strong brown nuclear staining. Less than 5% staining or no areas of brown staining were regarded as negative [14]. The percentages of immunoreactive cells in each case were recorded, and the mean percentage positivity of p63 and p40 nuclear staining for each category was calculated in non neoplastic and precursor/neoplastic lesions of the cervix.

STATISTICAL ANALYSIS

Data analysis was performed using the Statistical Package for Social Sciences (SPSS) software program version 20.0. The Pearson Chi-square test was used to assess the association between the positivity of both markers in each category of preneoplastic and neoplastic lesions of the cervix, and p-values <0.05 were considered significant.

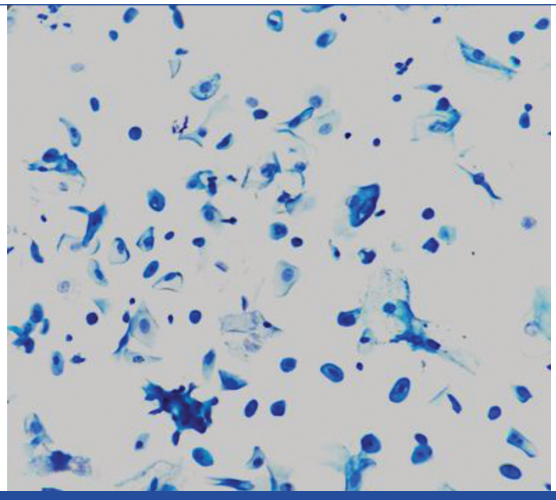
RESULTS

In the present study, the mean age of presentation was 44 years, and the most common presenting complaint was white discharge per vagina (82 cases, 41%). In LBC out of a total of 200 cases, cytological diagnosis of neoplastic and non neoplastic is shown in [Table/Fig-1].

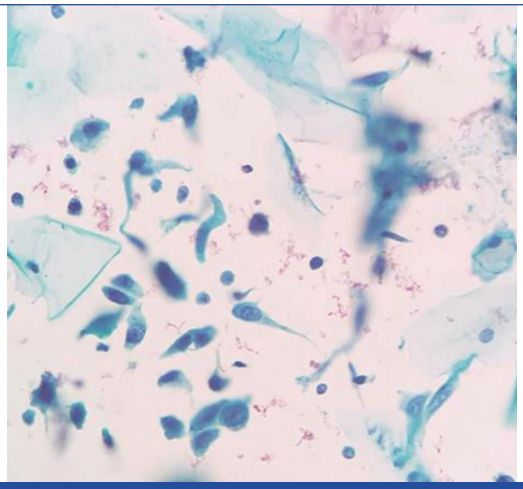
Cytological diagnosis	n (%)
Non neoplatic lesion	
NILM	20 (10)
Inflammation	126 (63)
Inflammation and necrotic debris	1 (0.5)
Atrophy	7 (3.5)
Candida infection	7 (3.5)
Trichomonas	8 (4)
Neoplatic lesion	
ASC-US	2 (1)
ASC-H	2 (1)
LSIL	9 (4.5)
HSIL	7 (3.5)
Squamous cell carcinoma	6 (3)
AGC	5 (2.5)

[Table/Fig-1]: Distribution of patients (n=200) according to cervical cytological findings. All cases of precursor/neoplastic lesions further underwent biopsy and histopathological examination. Moreover, 6 of the 126 cases of inflammation, the one case which reported as inflammation with necrotic debris and two of the seven cases of atrophy (all diagnosed as such on cytology) had unresolving symptoms despite conservative management, and were also chosen for histopathological follow-up.
NILM: Negative for intraepithelial lesion or malignancy; ASC-US: Atypical squamous cell of undetermined significance; ASC-H: Atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion; LSIL: Low-grade squamous intraepithelial lesion; HSIL: High-grade squamous intraepithelial lesion; AGC: Atypical glandular cell

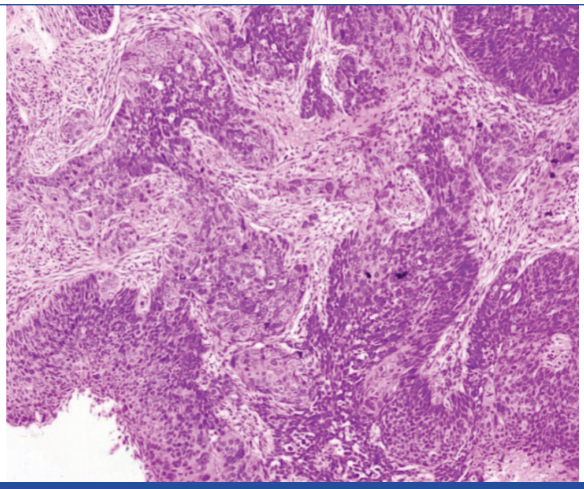
A total of 40 cases were cytologically diagnosed, including ASC-US, ASC-H, LSIL [Table/Fig-2], HSIL [Table/Fig-3], SCC, AGC, inflammation with necrosis, and atrophic smear with necrosis; these cases underwent cervical biopsy or hysterectomy and were evaluated histologically. Among these 40 cases, in which cytological-histological concordance was done, 25 had precancerous or neoplastic lesions, and their mean age was 49.6 years. The minimum and maximum ages found were 35 years and 72 years, respectively. The most common premalignant lesion on histology was CIN-1 (6 out of 40 cases), and the most common malignant lesion was squamous cell carcinoma (6 out of 40 cases) [Table/Fig-4].



[Table/Fig-2]: Microphotograph shows features of LSIL in a cytological smear (LBC, X400).



[Table/Fig-3]: Microphotograph shows features of HSIL in a cytological smear, a few spindle shaped tadpole cells present (LBC, X400).



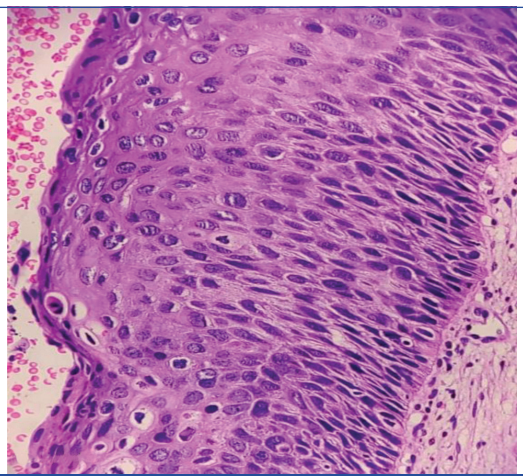
[Table/Fig-6]: Histopathology shows invasive squamous cell carcinoma, tumour cells infiltrating as irregular anastomosing nests within desmoplastic stroma (H&E, X100).

Cytological diagnosis	Histopathological diagnosis												
	Atrophy	Chronic cervicitis	Squamous meta-plasia	Endo-metriosis	Polyp	CINI	CIN II	CINIII/ Carcinoma-in-situ	Squamous Cell carcinoma	Adenocarcino-ma-in-situ	Adenocarcinoma	Adenosquamous carcinoma	Total
Inflammation		4			1				1		1		7
Atrophy	2												2
ASC-US			1			1							2
ASC-H				1			1						2
LSIL		2	1			4	2						9
HSIL	1	1				1	1	2	1				7
Squamous cell carcinoma									4			2	6
Atypical Glandular Cell (AGC)		1								2	2		5
Total	3	8	2	1	1	6	4	2	6	2	3	2	40

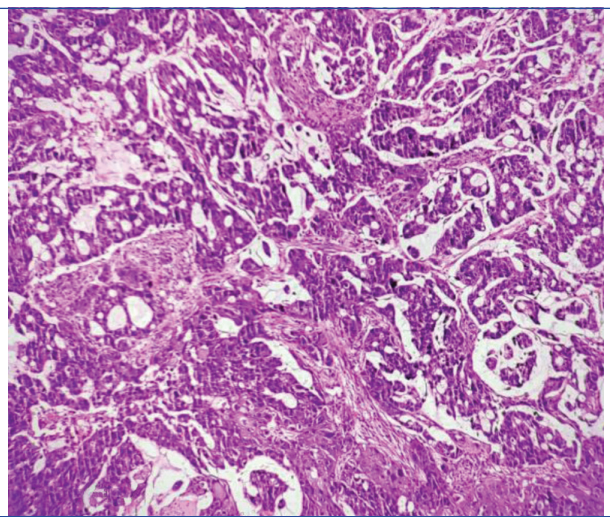
[Table/Fig-4]: Cytological and histopathological concordance in 40 patients who underwent cervical biopsy or hysterectomy.
N.B. Seven cases that were provisionally diagnosed as inflammation on cytology had persistent symptoms non responsive to conservative management. These cases were followed-up with biopsy and histopathology

On histopathology, 15 patients (37.5%) had non neoplastic lesions, which included chronic non specific cervicitis, atrophic cervicitis, squamous metaplasia, cervical endometriosis, and endocervical polyps. Neoplastic or precursor lesions were seen in 25 patients (62.5%), which included CIN I (15%), CIN II (10%), CIN III (5%) [Table/Fig-5], squamous cell carcinoma (15%) [Table/Fig-6], adenocarcinoma-in-situ (5%), adenocarcinoma (7.5%) [Table/Fig-7], and adeno-squamous carcinoma (5%) [Table/Fig-4].

In this study, seven cases were cytologically diagnosed as inflammatory. On histopathology, four were confirmed to be chronic non specific cervicitis, one was a cervical polyp, one was squamous cell carcinoma, and one was adenocarcinoma. Two cytological diagnosis of atrophic smears were proven to be atrophic on histopathological examination. Out of the two cases diagnosed as ASC-US, one was squamous metaplasia, and the other was CIN-I on histology. Among the two cases diagnosed as ASC-H, one was a case of cervical endometriosis, and the other was CIN-II on histology. In this study, there were five cases of AGC, out of which one was confirmed to be chronic non specific cervicitis on final



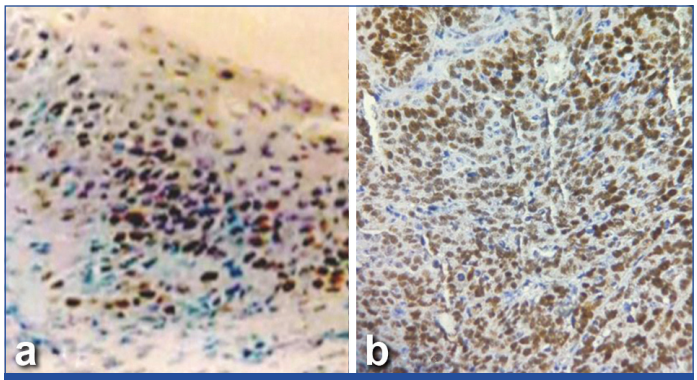
[Table/Fig-5]: Histopathology shows full thickness squamous dysplasia, cervical intraepithelial Neoplasia (CIN)- III (H&E, X400).



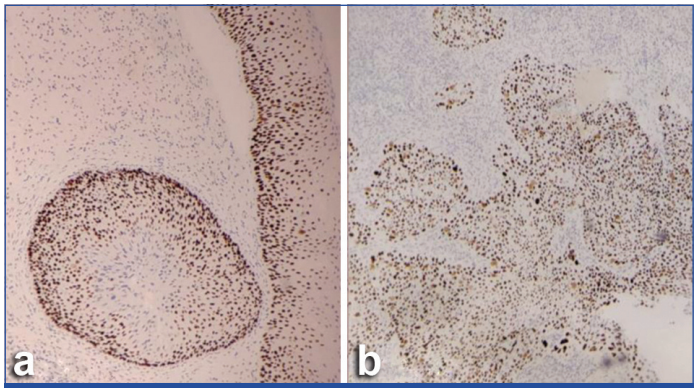
[Table/Fig-7]: Histopathology shows invasive adenocarcinoma of cervix (H&E, X100).

histopathological examination, two were Adenocarcinoma In-Situ (AIS), and the other two were adenocarcinoma. Twenty-three out of the 40 cases that were precursor/neoplastic on histopathology, and seven cases that were non neoplastic on HPE, had been correctly diagnosed on LBC, giving a concordance rate of 75%. The overall sensitivity, specificity, PPV, and NPV of LBC were 92%, 46.6%, 74.1%, and 77.7%, respectively, considering histological diagnosis as the gold standard.

The percentage positivity of p40 and p63 was calculated in non neoplastic, precancerous, and neoplastic lesions of the cervix using IHC stain. In non neoplastic lesions of the cervix, the positivity of p40 ranged from 1% to 50%, with a mean of 9.40%. In precursor/neoplastic lesions, the mean positivity for p40 was 47.44%. For p63 in non neoplastic lesions of the cervix, positivity ranged from 2% to 54%, with a mean of 10.06%. In precursor/neoplastic lesions, the mean positivity for p63 was 46.4%. AIS and adenocarcinoma showed no expression of p40 and p63; however, CIN-III and squamous cell carcinoma [Table/Fig-8a,b,9a,b] displayed 100% immunoexpression [Table/Fig-10]. The difference in the expression of p40 between non neoplastic and precursor/neoplastic lesions of the cervix was found to be statistically significant (p-value=0.003). Similarly, p63 expression also showed a statistically significant difference between these two groups (p-value=0.005).



[Table/Fig-8]: a) Immunohistochemical stain shows p40 positive nuclei in CIN-II (X400); b) Immunohistochemical stain shows p40 positive nuclei in invasive squamous cell carcinoma (X400).



[Table/Fig-9]: a) Immunohistochemical stain shows p63 positivity in CIN-III (X400); b) Immunohistochemical stain shows p63 expression in squamous cell carcinoma of cervix (X400).

DISCUSSION

Cervical cancer is common in developing countries like India. With the implementation of cervical screening and diagnostic programs, its mortality has decreased over the past 50 years. In the present study, most of the patients were in the age group 41-50 years (28%), which was similar to the study done by Bindroo S et al., who found the maximum number of patients in the age group of 41-50 years (32%) [15]. Inflammatory smears (63%) and NILM (10%) were the most common cytological diagnosis, which were consistent with studies done by Chandru C and Sheela SR [16]. NILM was the predominant category (49% of cases) in a study done by Singal P et al., [17]. In the present study, inflammatory smear was the most common cytological diagnosis due to the small sample size resulting from COVID-19 restrictions, and the fact that patients mostly presented with specific complaints rather than for routine screening.

Histopathological diagnosis (Precursor/Neoplastic)	Mean% positivity of p40	Mean% positivity of p63
CIN I	4.3	4
CIN II	78.75	77.5
CIN III/Carcinoma-in-situ	98	95
SCC	100	100
AIS	0	0
Adenocarcinoma	0	0
Adeno-squamous carcinoma	22.5	13

[Table/Fig-10]: Mean percent positivity of p40 and p63 immunomarkers in precursor/neoplastic lesions of cervix (N=25).
CIN: Cervical intraepithelial lesions; SCC: Squamous cell carcinoma; AIS: Adenocarcinoma in-situ

A cytology smear of ASC-US shows predominantly atypia in the superficial and intermediate groups of cells with a mild increase in nuclear size and nucleocytoplasmic ratio. The ASC-H cases closely resemble HSIL, but the cytological criteria are not sufficient to interpret them as HSIL. Cells in LSIL [Table/Fig-2] are usually present singly or in small clusters, with mild nuclear enlargement, irregular nuclear contour, and a low nucleocytoplasmic ratio. Cells in HSIL [Table/Fig-3] are usually round with scanty cytoplasm and a high nucleocytoplasmic ratio, along with enlarged, pleomorphic-hyperchromatic nuclei. A smear of SCC shows discrete and small groups of malignant cells with moderately pleomorphic, hyperchromatic nuclei and irregularly clumped chromatin. Spindle-shaped fiber cells with elongated hyperchromatic nuclei are noted. Discrete and small groups of cells with crowded nuclei are seen in a smear of AGC. In these cases, the cytoplasm is vacuolated with a distinct cell border, and the nuclei are mildly pleomorphic [3].

Histopathological findings of chronic cervicitis and chronic cervicitis with squamous metaplasia were seen in the majority of the women (52.9%), followed by CIN I in 16.8% of the women in a study done by Vidyadhar S et al., which was very close to present study findings [18]. Another study done by Dhakal R et al., found 78.7% of cases of chronic cervicitis, 8% with cervical dysplasia, and 5.3% with malignancy [19].

In this study, the concordance between cytology and histopathology was evaluated in the 40 patients who underwent cervical biopsy/hysterectomy. True positives were 23, where both the cytological and histopathological diagnosis was premalignant and malignant. True negatives were seven, where both the cytological and histopathological diagnosis was non neoplastic. False positives were eight, where the cytological diagnosis was premalignant and malignant, but on histopathology, lesions turned out to be non neoplastic. False negatives were two, where the cytological diagnosis was non neoplastic, but the histopathological diagnosis was neoplastic lesions [Table/Fig-4].

The sensitivity, specificity, PPV, and NPV of the present study were 92%, 46.6%, 74.1%, and 77.7%, respectively, which was close to a study done by Sirasagi AK et al., who found 90.57% sensitivity, 62.50% specificity, and 95.41% PPV [20]. In a study done by Sinha A et al., the sensitivity, specificity, and PPV were 90.9%, 89.5%, and 83.3% for cervical cytology [21]. The overall sensitivity, specificity, PPV, and NPV of Pap smear in diagnosing cervical dysplasia and malignancy were 86.61%, 73.33%, 96.49%, and 39.29%, respectively, in a study done by Malpani G et al., whereas Mellonie P et al., found 81.3% sensitivity and

92% specificity [22,23]. The sensitivity of cervical PAP smear was 29.7%, specificity was 94.4%, PPV was 70.4%, and NPV was 75.1% for diagnosing premalignant lesions of the cervix in a study done by Vidyadhar S et al., [18]. Cytohistological correlation of 100 cases done by Sharma A and Singh S revealed an overall sensitivity of 95.60%, specificity of 77.78%, PPV of 97.75%, and NPV of 63.03% [24]. A correlative study of Pap smear and histopathological examination of the cervix by Selvanayaki KM and Archana A revealed a sensitivity of 97%, specificity of 74% [25], and the sensitivity, specificity, PPV, and NPV were 69.7%, 79.9%, 60.5%, and 85.6% in a study done by Sandhya DS et al., [26].

The diagnostic accuracy of cytology in present study to determine non neoplastic and precursors or neoplastic lesions of the cervix was 75%, which was close to the findings of Sandhya DS et al., who found 76.6%, and Singhal A et al., who found 79.4% of diagnostic accuracy [26,27]. The Chandru C and Sheela SR was 81.71% and Sharma A and Singh S was 94% [16,24].

Present study investigated the expression of p40 and p63 in non neoplastic, precursor, and neoplastic lesions of the cervix. In non neoplastic lesions of the cervix, the mean positivity of p40 was 9.40%, while in precursor/neoplastic lesions, the positivity was 47.44%. Meanwhile, the mean positivity for p63 was 10.06% in non neoplastic lesions and 46.4% in precursor or neoplastic lesions.

According to the study, AIS and adenocarcinoma showed no expression of p40 and p63, whereas CIN III and squamous cell carcinoma exhibited 100% expression. Limited studies have been conducted on the expression of p40 and p63 in cervical lesions. One such study by Jacob AA and Sundaram A found p63 to be a useful marker in determining the progression of a lesion. They concluded that p63 could differentiate between benign and malignant cervical lesions [28]. Vosmik M et al., analysed 70 patients with cervical squamous cell carcinoma and discovered that 94.29% (66/70) had positive expression of p63 [29]. Das L et al., found that the percentage of p63-positive cells was highest in carcinomas, followed by dysplasia. They suggested that the malignant potential of dysplastic conditions might be correlated with the upregulation of p63 protein [30]. In a study conducted by Li H et al., the sensitivity of p40 and p63 was reported as 85.15% and 89.11%, respectively, while the specificity was 97.22% and 93.52%, respectively, in distinguishing between squamous cell carcinoma and adenocarcinoma [31].

Limitation(s)

The present study was single-institute-based, and the number of cases was limited due to COVID-19 restrictions. The short duration and the fact that patients mostly visited with definite complaints were the major limitations of this study.

CONCLUSION(S)

Cervical cytology is a less invasive, cost-effective, and simple procedure to diagnose cervical epithelial cell abnormalities. Timely intervention and appropriate treatment can reduce the burden of disease. LBC is a sensitive method for the early detection of neoplasms of the uterine cervix. When cytological diagnosis were correlated with histopathological findings, they were found to be more sensitive, with an accuracy rate of about 75%. Non neoplastic, precursor, or neoplastic lesions of the cervix can be distinguished by p40 and p63 immunostaining. These markers can also be used to differentiate poorly differentiated squamous cell carcinoma from adenocarcinoma of the cervix on histopathology. However, further studies in routine health screening patients would be required to establish whether adding p63 and p40 staining has a role in asymptomatic patients with clearly negative cytology. This study might be reviewed as a component of a large multicentre study to reach a definite conclusion in the future.

REFERENCES

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-49. https://doi.org/10.3322/caac.21660.

[2] Ellenson LH, Pirog EC. The Female Genital Tract. In: Kumar V, editor. Robbins And Cortan Pathologic Basis Of Disease. South Asia Edition: Elsevier; 2020:1000.

[3] Dey P. Cervical Carcinogenesis, Preneoplastic and Neoplastic Condition. In: Dey P, editor. Diagnostic Cytology. New Delhi: Jaypee; 2022:121-34.

[4] Ranade AV, Chougule MV, Kadam N, Kulkarni SH, Khatavkar PR. Expression of p63 among cervical carcinoma patients. J Adv Med Dent Scie Res. 2021;9(5):66-68.

[5] Truong AB, Kretz M, Ridky TW, Kimmel R, Khavari PA. p63 regulates proliferation and differentiation of developmentally mature keratinocytes. Genes Dev. 2006;20(22):3185-97. Available from: https://doi.org/10.1101/gad.1463206.

[6] Barbieri CE, Tang LJ, Brown KA, Pietenpol JA. Loss of p63 leads to increased cell migration and up-regulation of genes involved in invasion and metastasis. Cancer Res. 2006;66(15):7589-97. Available from: https://doi.org/10.1158/0008-5472.

[7] Downey P, Cummins R, Moran M, Gulmann C. If it's not CK5/6 positive, TTF-1 negative it's not a squamous cell carcinoma of lung. APMIS. 2008;116(6):526-29. Available from: https://doi.org/10.1111/j.1600-0463.2008.00932.x.

[8] Nobre AR, Albergaria A, Schmitt F. p40: A p63 isoform useful for lung cancer diagnosis - A review of the physiological and pathological role of p63. Acta Cytol. 2013;57(1):01-08. Available from: https://doi.org/10.1159/000345245.

[9] Bishop JA, Teruya-Feldstein J, Westra WH, Pelosi G, Travis WD, Rekhtman N. p40 (ΔNp63) is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma. Mod Pathol. 2012;25:405-15. Available from: https://doi.org/10.1038/modpathol.2011.173.

[10] Lin Z, Liu M, Li Z, Kim C, Lee E, Kim I. DeltaNp63 protein expression in uterine cervical and endometrial cancers. J Cancer Res Clin Oncol. 2006;132(12):811-16. Available from: https://doi.org/10.1007/s00432-006-0130-8.

[11] Bentz JS. Liquid based cytology for cervical cancer screening. Expert Rev Mol Diagn. 2005;5(6):857-71. Doi: 10.1586/14737159.5.6.857. PMID: 16255628.

[12] Herrington CS, Kim KR, Kong CS. Tumours of the Uterine Cervix. In Female Genital Tumours. 5th edition, Lyon: World Health Organization; 2020:8.

[13] Romus I, Triningsih FE, Mangunsudirdjo S, Harijadi A. Clinicopathology significance of p53 and p63 expression in Indonesian cervical squamous cell carcinomas. Asian Pac J Cancer Prev. 2013;14(12):7737-41. Available from: https://doi.org/10.7314/apjcp.2013.14.12.7737.

[14] Affandi KA, Tizen NMS, Mustangin M, Zin RRMRM. p40 Immunohistochemistry is an excellent marker in primary lung squamous cell carcinoma. J Pathol Transl Med. 2018;52(5):283-89. Doi: 10.4132/jptm.2018.08.14. PMID: 30235512; PMCID: PMC6166010.

[15] Bindroo S, Garg M, Gitika. Correlation of cervical pap smear with histopathological diagnosis in cervical lesions: A 2 years retrospective study. Int J Contemp Med. 2019;6(7):17-20.

[16] Chandru C, Sheela SR. Screening for carcinoma cervix by comparing pap smear cytology, histopathology with colposcopy in unhealthy cervix. J Clin of Diagn Res. 2021;15(12):QC01-QC05.

[17] Singal P, Kumar N, Kundal D, Singh K, Kumar R, Kaur M. Cytological, HPV genome, and histopathological correlation of cervical smears in females 30 years and above. Asian J Pharm Clin Res. 2022;15(5):41-45. Available from: https://doi.org/10.22159/ajpcr.2022.v15i5.44328.

[18] Vidyadhar S, Bhattacharya AB, Bohara S, Dwivedi AD, Agarwal A, Gangwar D. Comparison and correlation of cytology, colposcopy and histopathology of premalignant lesions of cervix in rural women of Barabanki district. IOSR J Dent Med Sci. 2017;16:13-18.

[19] Dhakal R, Makaju R, Sharma S, Bhandari S, Shrestha S, Bastakoti R. Correlation of cervical pap smear with biopsy in the lesion of cervix. Kathmandu Univ Med J. 2016;55(3):254-57.

[20] Sirasagi AK, Arpitha K, Neha S, Pattar PM. A two year retrospective study of cytohistopathological correlation of cervical smear in a tertiary care hospital. Asian J Med Sci. 2021;12(8):144-48.

[21] Sinha A, Kafle SU, Singh M, Kafle N. Variation of cervical cytology by pap smear and its correlation with cervical biopsy in patients attending Birat Medical College teaching hospital, Morang, Nepal. Birat J Health Sci. 2021;6(3):1615-20.

[22] 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;5:2312-16.

[23] Mellonie P, Sharon MP, Richie AJ. Correlation of PAP smear with cervical biopsy in malignant and non malignant lesions of cervix. Saudi J Pathol Microbiol. 2019;4(2):116-19.

[24] Sharma A, Singh S. Spectrum of cervical lesions and cytohistological correlation: A study in tertiary care center. Int J Contemp Med. 2019;6(6):F5-F10.

[25] Selvanayaki KM, Archana A. A comparative study of pap smear cytology and histopathology of cervix biopsy. Trop J Pathol Microbiol. 2020;6(3):230-37.

[26] Sandhya DS, Sumaya, Nagesha KR. Cytodiagnostic study of papanicolaou smears with histopathological correlation- An emphasis on 2014 Bethesda system for reporting cervical cytology. Saudi J Pathol Microbiol. 2019; 4(6): 443-449.
[27] Singhal A, Raina RK, Verma S, Verma A. Predictive accuracy of cervical cytology and colposcopy in diagnosing premalignant and malignant cervical lesions: A hospital-based study from the sub-Himalayan region of Indian subcontinent. CHRISMED J Health Res. 2019;6:39-43.
[28] Jacob AA, Sundaram A. P16, Ki67 and P63 staining pattern in squamous metaplasia, CIN and cervical cancer. Int J Res Med Sci. 2018;6:882-88.
[29] Vosmik M, Laco J, Sirak I, Beranek M, Hovorkova E, Vosmikova H, et al. Prognostic significance of human papillomavirus (HPV) status and expression of selected markers (HER2/neu, EGFR, VEGF, CD34, p63, p53 and Ki67/MIB-1) on outcome after (chemo-) radiotherapy in patients with squamous cell carcinoma of uterine cervix. Pathol Oncol Res. 2014;20(1):131-37.
[30] Das L, Naskar S, Sarkar T, Maiti AK, Das S, Chatterjee J. Immunohistochemical evaluation of prime molecules in cervical lesions towards assessment of malignant potentiality. J Can Res Ther. 2018;14:377-81. Available from: https://doi.org/10.4103/0973-1482.158029.
[31] Li H, Jing X, Yu J, Liu J, Zhang T, Chen S, et al. A combination of cytokeratin 5/6, p63, p40 and MUC5AC are useful for distinguishing squamous cell carcinoma from adenocarcinoma of the cervix. Diagn Pathol. 2020;15(1):104.

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