

PAP Smear versus Colposcopy in the Absence of HPV-DNA Testing for the Screening of Pre-malignant and Malignant Cervical Lesions

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ABSTRACT:

Introduction: The incidence of cervical cancer is very high in developing countries. The extensive use of cervical screening with PAP smear and colposcopy has considerably increased the detection of premalignant and malignant lesions of the cervix. **Methods:** This was a hospital-based study conducted over a period of three months. All the patients underwent gynecological examination, PAP smear and colposcopy. In case of abnormal findings in colposcopy or follow-up cytology, patients were advised for cervical biopsy. With reference to the histopathological reports, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of PAP smear and colposcopy were evaluated. **Results:** The most common cytological result was Negative for Intraepithelial Lesion or Malignancy (NILM, 60%) followed by Atypical Squamous Cells of Undetermined Significance (ASCUS, 10%), Atypical Squamous Cells- cannot exclude High grade lesion (ASC-H, 6%), Low grade Squamous Intraepithelial Lesion (LSIL, 10%) and High grade Squamous Intraepithelial Lesion (HSIL, 11%). In colposcopy, 60% of the patients were normal and 40% showed atypical transition zone. Histo-pathological findings among 53 patients were NILM (13.2%), cervicitis (13.2%), Cervical Intraepithelial Neoplasia I (CIN I, 28.3%), CIN II and CIN III (39.6%) and invasive carcinoma (5.66%). The sensitivity, specificity, PPV and NPV of PAP smear were 63.6%, 66.6%, 33.3%, and 87.5% respectively. Similarly sensitivity, specificity, PPV, NPV of colposcopy for abnormal cervical lesions were 87.56%, 15.38%, 76.09% and 28.57% respectively. **Conclusion:** In the absence of HPV-DNA testing, the combined use of PAP smear and colposcopy to detect premalignant and malignant cervical lesions increases early detection and treatment.

Keywords: Colposcopy, Cytology, PAP smear, Pre-malignant, Invasive carcinoma.

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INTRODUCTION:

Cervical cancer is one of the most common gynecological malignancies. Worldwide, it ranks fourth after breast, colorectal and lung cancers with significant mortality.[1,2] It is the only preventable gynecological cancer. Yet, Nepal has one of the highest incidence

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and mortality rates due to cervical cancer in South East Asian region.[1]

World Health Organization (WHO) advises screening strategy with cytology by PAP smear followed by colposcopy examination, when available, to decrease the major public health problem.[2,3] Pap smear is a simple, safe, non-invasive and effective method of detection of precancerous and non-cancerous changes in the cervix. Colposcopy provides a unique method to study the benign and malignant lesions. It is a simple, non-invasive procedure that helps in determining the location, size and extent of abnormal cervical lesions thereby serving in detecting the sites for biopsies.[4,5] Colposcopy is complementary to cytology. The final diagnosis must be made on histo-pathological examination.[6]

Various cervical cancer screening tools and confirmatory tests are currently available which require different levels of expertise, cost and time. They have a fair level of accuracy and have to be customized based on their prerequisites. But there is not a single screening test with 100 % sensitivity and specificity and there remains scope to identify a better method for the screening and diagnosis of this common condition.[2,7] Hence, this study aimed to compare the efficacy of PAP smear and colposcopy in detecting the pre-malignant and malignant lesions of cervix.

METHODS:

It was a hospital-based observational study conducted in the department of Obstetrics and Gynecology, Lumbini Medical College and Teaching Hospital over a period of three months from March 2022 to May 2022.

All the married women aged 21 to 65 years having National Health Insurance presenting

to gynecology out-patient department (OPD) with complaints of vaginal discharge, post-coital bleeding, post-menopausal bleeding and persistent leucorrhoea were included in the study. The patients who were unmarried, pregnant and those with frank lesions and active infection were excluded.

Ethical approval was obtained from the Institutional Review Committee prior to commencement of the study (IRC-LMC-04/R-022). A total of 100 patients satisfying inclusion criteria were included. An informed consent was taken from all the patients. Confidentiality was maintained throughout the study.

The patients were interviewed using standard questionnaires regarding age, age at marriage, age at first pregnancy, parity, menstrual history, present symptoms, and smoking history. After a verbal explanation of the procedure, cervical examination was done using a Cusco's speculum and findings as abnormal discharge, erosions, hypertrophic cervix, suspicious mass or ulcerative appearance at the cervix were noted.

Through speculum examination, the smear was taken with Ayre's spatula and cytobrush, spread thinly on the slide and fixed with 95% ethyl alcohol. The slides were stained with Papanicolaou staining protocol in the pathology lab. PAP smear results were reported based on 2021 Bethesda system.[8] Cytology was considered positive if it revealed any of the following lesions- Atypical squamous cells of Undetermined Significance (ASC-US), Atypical squamous cell- cannot exclude high grade lesions (ASC-H), Low grade squamous intraepithelial lesion (LSIL), High grade squamous intraepithelial lesion (HSIL) or cells suspicious for malignancy. Negative smear included those with normal and inflammatory reports.

A colposcopic examination was done in all the patients using B'ORZE colposcope. If the complete transformation zone was not visualized; colposcopy was termed as "unsatisfactory" and not included in our study. Inspection was done using a green filter followed by 5% acetic acid and Lugol's iodine application. The margin and color of the lesion along with the appearance of blood vessels were noted. Abnormal colposcopy findings like acetowhite area, punctate lesions, mosaic pattern and abnormal iodine staining were noted.

Cervical punch biopsy was taken from the suspicious/abnormal areas at the same setting if colposcopy finding was abnormal. The specimen was then fixed in 40% formalin, labelled and sent for histopathological examination. All the patients after Pap smear were asked for follow-up with a cytology report. If any abnormal findings were noted in the cytology report, a cervical biopsy was taken in the second setting. Biopsy was not taken in whom Pap smear and colposcopy were normal. The histopathological terminologies used to describe the grade of the disease were: Cervical intraepithelial neoplasia (CIN) grades I, II and III, and invasive carcinoma.

All the data thus collected were entered into a Microsoft excel spreadsheet version 2016. It was then imported to Statistical Package for Social Sciences software version 23.0 (SPSS Inc., Chicago, IL, USA). Frequencies with percentages were used for descriptive statistics. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of cytology and colposcopy in comparison to histopathology were calculated.

RESULTS:

Of the 100 patients enrolled in the study, 53 patients whose colposcopic findings or cytological report or both were abnormal were selected for histopathological examination.

Table 1 represents the socio-demographic profile of the patients. A majority (40%) belonged to the age group of 31 to 40 years. The mean age of the patients was 41.86 ± 10.53 years, the minimum age being 21 years and the maximum, 65 years. The lowest age at marriage was 14 years. Most (48%) got married at 20-30 years with the mean age at marriage being 21.24 ± 4.33 years. Majority of them were Hindu (85%). The majority of the patients were Brahmin (39%) followed by Janjati, Chhetri and Dalit.

Whitish discharge was the most common clinical symptom (49%) among the patients followed by pain abdomen (35%), and post coital bleeding (6%) (Fig.1).

Among all the patients examined by colposcopy, 60% of the patients findings were normal and 40% patients showed abnormal transformation zone. Among 40% abnormal transformation zone 10% patients had additional atypical vessels and 3% of patients were suspected of invasive carcinoma.

The majority of the patients' cytology finding was NILM (60%) followed by HSIL (11%), ASCUS (10%), and LSIL (10%) (Fig. 2).

Table 2 summarizes the histopathological findings of the patients who underwent cervical biopsy.

Table 3 describes the correlation between the cytological and histopathological findings of the study.

Table 1: Socio-demographic profile of the study population (N=100)

Characteristics	Frequency	
Ethnicity	Brahmin	39
	Chhetri	17
	Dalit	14
	Janajati	29
	Muslim	1
Religion	Hindu	85
	Buddhist	14
	Muslim	1
Parity	Nullipara	3
	Primipara	40
	Multipara	57

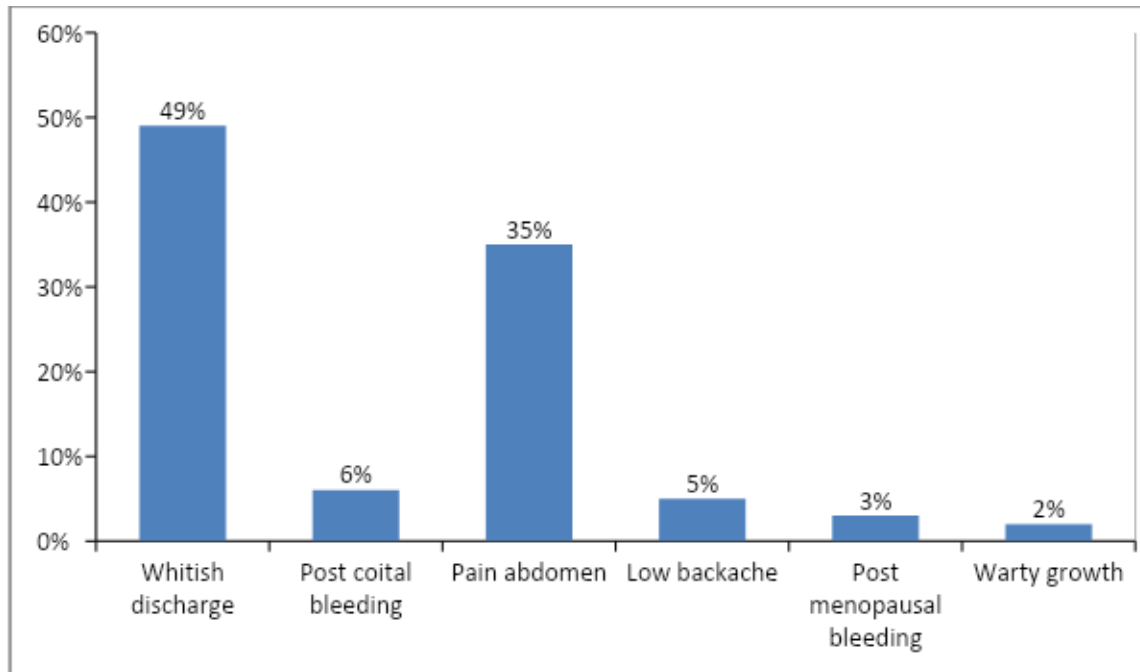


Fig.1. Distribution of symptoms in the study population (N=100).

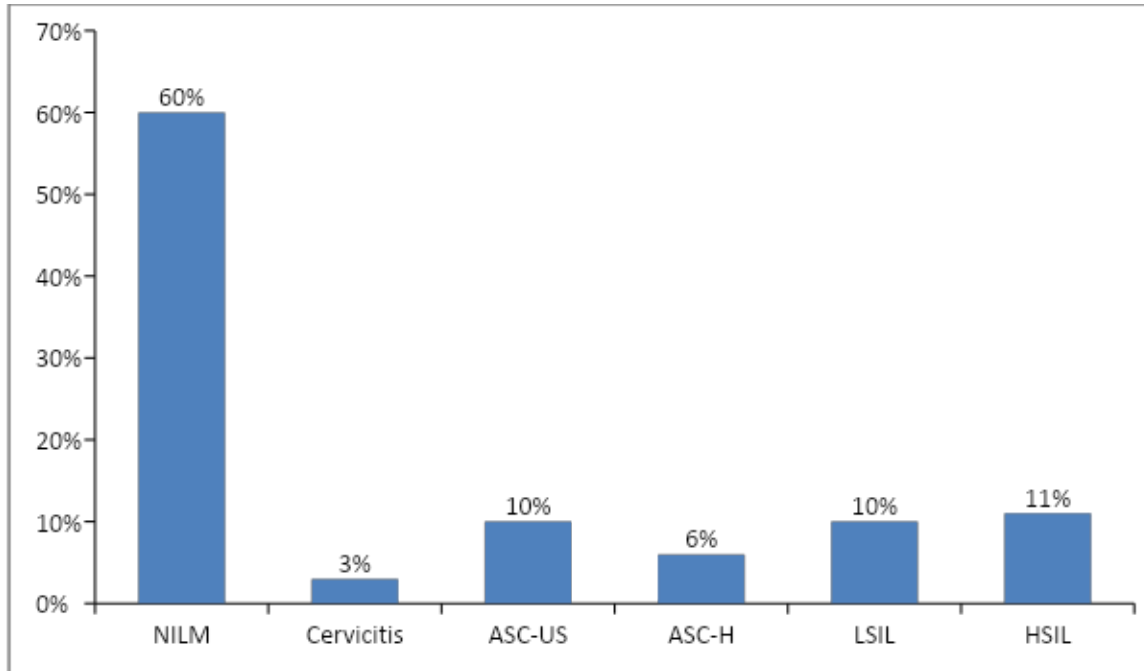


Fig.2. Distribution of cytological (PAP smear) reports of the study population (N=100)

*NILM- Negative for Intraepithelial Lesion or Malignancy; ASCUS- Atypical Squamous Cell of Undetermined Significance; ASC-H- Atypical Squamous Cell - cannot exclude High grade lesion; LSIL- Low grade Squamous Intraepithelial Lesion, HSIL- High grade Squamous Intraepithelial Lesion

Table 2: Distribution of histopathological findings (N=53).

Histopathological findings	Frequency (%)
Negative for intraepithelial lesion	7 (13.2%)
Cervicitis	7 (13.2%)
Cervical intraepithelial neoplasia I (CIN I)	15 (28.3%)
CIN II and CIN III	21 (39.6%)
Invasive carcinoma	3 (5.66%)

Table 4 summarizes the correlation between colposcopic findings with histopathological findings of our study.

The sensitivity, specificity, PPV and NPV of PAP smear in diagnosing various cervical pathologies are depicted in Table 5.

Table 3: Correlation between cytology with histopathology (N=53).

Cytology/ HPE	NILM	Cervicitis	CIN I	CIN II and CIN III	Invasive Ca	Total
NILM	4 (7.55%)	4 (7.55%)	1 (1.88%)	4 (7.55%)		13
Cervicitis	1 (1.88%)	1 (1.88%)		1(1.88%)		3
ASCUS	2 (3.77%)	2 (3.77%)	3 (5.66%)	3 (5.66%)		10
ASC-H			1 (1.88%)	5 (9.43%)		6
LSIL			9 (16.9%)	1 (1.88%)		10
HSIL			1 (1.88%)	7 (13.2%)	3 (5.66%)	11
Total	7 (13.20%)	7 (13.20%)	15 (28.30%)	21 (39.6%)	3 (5.66%)	53

*NILM- Negative for Intraepithelial Lesion or Malignancy; ASCUS- Atypical Squamous Cell of Undetermined Significance; ASC-H- Atypical Squamous Cell - cannot exclude High grade lesion; LSIL- Low grade Squamous Intraepithelial Lesion, HSIL- High grade Squamous Intraepithelial Lesion

The sensitivity, specificity, PPV and NPV of colposcopy in diagnosing abnormal cervical pathologies in this study were found to be

87.5%, 15.38%, 76.09% and 28.57% respectively.

Table 4: Correlation of colposcopy with histopathology (N=53)

Colposcopic findings	Normal findings	Abnormal findings			
	NILM	Cervicitis	CIN I	CIN II and III	Invasive Ca
Abnormal	5(9.43%)	4 (7.55%)	11(20.7%)	17(32%)	3(5.66)
Normal	2(3.77%)	2(3.77%)	4 (7.55%)	5(9.43%)	

Table 5: Sensitivity, specificity, PPV and NPV of PAP smear in diagnosing CIN I, CIN II and III, and invasive carcinoma.

Parameters	CIN I	CIN II and III	Invasive Ca
Sensitivity	90%	63.63%	---
Specificity	86%	66.67%	94.33%
PPV	60%	33.33%	0%
NPV	97.37%	87.5%	1%

DISCUSSION:

Invasive cancer of cervix is considered to be a preventable condition as it has a long pre-invasive state, making it amenable to screening and treatment.[7] The incidence of cervical cancer can be reduced by as much as 80% if the quality, coverage and follow up of screening methods are of high standard.[9] The major public health problems in female of low- and middle income countries (LMIC) have become rare entity in high income countries because of structured screening program by frequent Pap smear examination.[2] However, in a country like Nepal where there is a lack of awareness in the population about cervical cancer and the

necessity and availability of frequent screening, women still present in advanced stage of cancer.[2]

The necessity of frequent screening over a wide age range, requirement of skilled human resources and the low sensitivity of PAP smear have led the clinicians of LMICs to search for a better screening program to catch the preventable disease as early as possible and to reduce mortality and morbidity by this disease.

In the present study of 100 patients, the most common age group was 31 to 40 years (40%), which was comparable to the study done by Subedi K et al. (52%), and Kalyankar VY et

al. (39.7%).[2,4] Whereas Natsu N et al. found majority of the patients were aged 41 to 50 years (32.5%).[10] Majority of our patients were Hindu (85%) which might be due to the majority population of study area being Hindu and also due to different study found a significantly lower incidence of cancer in muslim.[4] This finding was comparable to the studies by Kalyankar VY et al. and Mohanty J et al.[4,11]

Early age at marriage, multiple number of sexual partners and sex with high risk males whose wives died due to cervical cancer also increase the risk of developing cervical cancer.[12] Mayavati et al. in her study showed association of cervical cancer with early marriage.[13] And also in the studies done by Nair R et al. and Dadkhah Fet al. 52% and 94% patients respectively were married before 20 years.[12,14] In our study 48% patients were married at the age of 20 to 30 years and 38% before 20 years.

Since the study area is a hilly region where majority of the population are usually Brahmins and Chhetris, the most common ethnicity in our study was Brahmins too (39%).

The majority of our patients were multiparous (57%) comparable to findings by Subedi K et al. (53%), Prasad D et al. (52%), and Kalyankar VY et al.(38.2%).[2,3,4] However, in the study by Shaki O et al. only 16.4% were multiparous.[15]

In different studies patients had various symptoms at the time of presentation. Excessive vaginal discharge for long duration also be proven to be risk factor for development of cervical cancer.[12] In our study majority of patients presented with whitish discharge per vaginum (48%) followed by pain abdomen (35%) which was comparable to that reported by Chaudhary RD et al. and Gandavaram J et al.[6,16] However,

Subedi K et al. found majority had the symptom of pain abdomen (49%).[2]

In the present study, 60% of cytology findings was NILM followed by HSIL (11%), ASC-US (10%), LSIL (10%), ASC-H (6%) and cervicitis (3%). These findings correlated with those of Natsu N et al. and Gandavaram J et al.[10,16] Contradictory to our findings, Sachen et al. found that 48% had normal smear, 42% had inflammatory smear, 5% had LSIL and only 0.48% had HSIL.[17] The differences in Pap smear reporting's are due to differences in sampling, staining, fixation techniques and reporting errors. In the present study, majority (60%) of patients had normal colposcopic findings and 40% showed abnormal transformation zone. Among abnormal findings, 3% were suspicious for invasive carcinoma. Our findings were similar to those of Joshi C et al. and Oglak S C et al.[5,18] Histopathological study was done in 53 cases, out of which 39.6% was CIN II and CIN III followed by CIN I (28.3%), cervicitis (13.2%), NILM (13.2%) and invasive carcinoma (5.66%).

The sensitivity and specificity of PAP smear have been reported differently in different studies. In a meta-analysis of 62 studies, the PAP smear sensitivity ranged from 11 to 99% and specificity, 14 to 97%. [18] In the present study, the sensitivity, specificity, PPV, NPV of PAP smear in diagnosing CIN I were 90%, 86%, 60%, and 97.3% respectively and for CIN II and CIN III were 63.6%, 66.67%, 33.33% and 87.5% respectively. The sensitivity and specificity of our study are comparable to the findings of Natsu N et al. and Cheraghi F et al.[10,19] In contrast to our findings Oglak S C et al. noted the sensitivity, specificity, PPV, and NPV for CIN I as 28.5%, 74%, 88.1% and 13.3% respectively.[18] These differences were thought to be related to the risk group of the patient population, the number of cases, different features and deficiencies likely to

occur at each stage of cytological examination.

In the present study, Sensitivity, Specificity, PPV, NPV of colposcopy were noted 87.5%, 15.38%, 76.09% and 97% respectively. Sensitivity was comparable to Kalyankar VY et al. and Natu N et al. 96% 82.57%.[4,10] Specificity was comparable to Cheraghi F et al. [19] PPV was comparable to Natu N et al. and NPV was comparable to Oglak S C et al.[10,18]

HPV DNA testing in cervical cancer screening is not always possible. Limitation and inadequacy of other screening and diagnostic methods such as PAP smear and colposcopy should also be known. Thus, whenever feasible, it is possible to increase the sensitivity and specificity with the sequential and combined use of these methods.

Limitations of the study:

This study did not use a calculated sample size which limits the generalizability of the findings. Additionally, evaluation of sensitivity, specificity, PPV and NPV of colposcopy in terms of CIN I, CIN II and III, and invasive carcinoma was not done.

CONCLUSION:

Cervical cancer screening is quickly becoming an urgent public health problem which needs immediate attention from all concerned parties. One of the immediate strategies should be sustained health education and awareness creation to promote early detection of cases through appropriate screening strategies. Hence, our study suggested the detection of cervical abnormalities was higher when cytology, colposcopy, colposcopic guided biopsies are used in combination in diagnosis of abnormal cervical lesions.

Conflict of Interest: The authors declare that no competing interests exist.

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