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Research Article

COMPARATIVE EVALUATION OF PAP SMEAR WITH HISTOPATHOLOGY IN CERVICAL LESION ACCORDING TO THE 2014 BETHESDA SYSTEM: AN INSTITUTIONAL STUDY

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ABSTRACT

Objectives: The objective of this study is to evaluate the efficacy of a pap smear using The 2014 Bethesda System (TBS) for the diagnosis of premalignant and malignant lesions of the uterine cervix.

Methods: A prospective study was conducted in the Department of Pathology at the institution from November 2015 to October 2017. Fifty patients with an unhealthy cervix have undergone conventional Pap smears and histopathological biopsies with the suspicion of cervical malignancy or premalignancy attending the Obstetrics and Gynecology Outpatient Department. The obtained smears were arranged according to TBS. The tissue biopsies have been processed routinely and they are stained with hematoxylin and eosin for histopathological diagnosis. Furthermore, cases such as non-neoplastic, metastatic lesions, and recurrent cases were excluded from this study.

Results: The common age group has been identified as between 41 and 60 years and patients had low educational levels. Sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy observed in this study were 89.28%, 63.63%, 64.1%, 72.72%, and 72% respectively.

Conclusion: Pap smear is a simple, effective, inexpensive procedure that can be routinely used as a screening tool for screening cervical cancer. TBSs also provide a standard approach for cytological evaluation that helps in the early detection of premalignant and malignant cervical lesions.

Keywords: The 2014 Bethesda System, Cervical pap smear, Cytology, Histopathology, Diagnostic accuracy.

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INTRODUCTION

Cervical cancer is considered the fourth most common cancer affecting women. There are over 6,60,000 new cases in the year 2022. There were also over 94% of 3,50,000 deaths caused by cervical cancer in 2022 in low-income and middle-income countries. Cervical cancer affects women irrespective of their age and it has resulted in 20% of children losing their mother to cervical cancer. This can be cured if diagnosed early and treated immediately. Various countries are working to eliminate cervical cancer in the forthcoming decade [1].

Usually, cervical cancer is diagnosed in an advanced stage. One of the simple and noninvasive methods for the screening of cervical abnormalities is the Pap smear test. This method helps in early detection of preinvasive cervical cancer. This method was developed by George Papanicolaou in the late 1940s for the prevention of cervical cancer [2].

Even if the Papanicolaou cytology test is considered an effective method for the prevention and detection of precancerous conditions of the uterine cervix, there are also some limitations like false negative results because of the sampling and interpretation errors by the technicians, which is one of the concerns. However, the final diagnosis must be made using the histological examination for the assessment of the accuracy of cervical cytology. One of the recommended European guidelines for quality assurance for the development of cytology laboratory performance is cytohistopathology and its relation with the pap smear method. This combination can help in the reduction of false positive results [3]. The report regarding Pap smear can be made by The Bethesda System 2001 before that there were various classification systems. This study aims to evaluate the efficacy of a Pap smear using The 2014 Bethesda System (TBS) for the diagnosis of premalignant and malignant lesions of the uterine cervix. The cytological findings have been assessed, and the accuracy has been measured using 2014 TBS and histopathological findings from biopsies in women with abnormal cervical smears.

METHODS

This was a prospective study conducted in the Department of Pathology of the institute on the conventional Pap smears obtained from women with an unhealthy cervix attending Obstetrics and Gynecology Outpatient Department during the period between November 2015 and October 2017. All women with unhealthy cervix with clinical suspicion of premalignant and malignant lesions were included in this study. A total of 50 women were included in this study. The cervical smears were reported according to the 2014 Bethesda Reporting System. The cases such as non-neoplastic, metastatic lesions, and recurrent cases were not included in this study. Informed consent was taken from participants of the study. This study was approved by the institutional ethical committee (No.2015/P-1-RP/154).

Each patient was positioned in the dorsal position. As the cervix was exposed, the squamocolumnar junction was scraped by rotating Ayer's spatula, and endocervical brushing was done from the endocervical canal. The scraping was spread evenly onto the glass slide, and it was fixed immediately by dipping the slide in a couplin jar that has equal amounts of ether and 95% ethyl alcohol. The cervical smears were stained with Papanocolaou stain and reported according to the 2014 Bethesda Reporting classification system.

All the cytological smears showing any evidence of malignancy were advised to undergo a biopsy. The tissue was routinely processed

and stained with routine H&E and histopathological findings were noted.

RESULTS

The maximum numbers of patients of 16 cases (32%) were between the ages of 51–60 and the age group between 41 and 50 had 15 cases (28%). Detailed information regarding the age and the number of patients has been given in Table 1. It has been identified that among the patients between the age group of 41–50 years, 46 patients (92%) were from rural areas. In the total of 50 cases, 62% of them are illiterate and 38% of them had their primary school education. In this study, it has been observed that the premalignant and malignant cervical lesions were 2% in 5-year married women, 8% for 5–10 years married women, 32% for 11–10 years of married women, and 58% for above 20 years of married women. It has resulted in consideration that the high chance of premalignant and malignant cervical lesions occurs with increased duration of sexual intercourse. The obstetric history of all the patients has been evaluated and identified that para 2 has 21 cases (42%), para 3 has 17 cases (34%), para 4 and above has 10 cases (20%), and para 1 has 2 cases (4%) Table 2. The menstrual cycle of all the cases has been assessed as regular (38%), irregular (34%), and menopause (28%) Table 1. The chief complaint has been noted for all the 50 cases. Bleeding per vagina was the common complaint that has been presented (56%) that was followed by pain in the abdomen (42%). The chief complaints are given in Table 1. The analysis per speculum examination has been carried out, and the details are given in Table 1.

The Pap smear findings and histopathological findings are given in Tables 3 and 4. The Pap smear test results have been categorized into various groups, namely, atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells-high grade (ASC-H), low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL), squamous cell carcinoma (SCC), and adenocarcinoma. ASC-H and ASC-US are atypical squamous cells, the results obtained can be considered benign or normal. LSIL, HSIL, SCC, and adenocarcinoma are considered abnormal types of cells. In the histopathological findings, cervical intraepithelial neoplasia (CIN) 1, CIN 2, and CIN 3 were cervical intraepithelial neoplasia. CIN3 was the most severe, whereas CIN1 was the least severe. Pap smear was analyzed for all cases. It showed HSIL in 36% of cases followed by LSIL (26%) and squamous cell carcinoma in 22% of cases. Pap smear has evaluated CIN in 52% of total cases.

Table 1: Demographic variables and patients history

Age distribution		
Age (Years)	**Number of patients**	**Percentages**
21–30	05	10
31–40	05	10
41–50	14	28
51–60	16	32
>60	10	20
Total	50	100
Obstetric history		
Para 1	02	04
Para 2	21	42
Para 3	17	34
Para 4 and above	10	20
Total	50	100
Menstrual cycle		
Regular	19	38
Irregular	17	34
Menopause	14	28
Total	50	100
Chief complaint		
White discharge per vagina	13	26
Pain abdomen	21	42
Bleeding per vagina	28	56
Mass per vagina	09	18
Burning micturation	06	12
Dyspareunia	05	10
Backache	08	16

The comparison of cytohistological findings is provided in Table 5. The cytology and histology for 50 patients have been compared in this Figs. 1-3 show the Pap stain and H&E stains on different types of cells.

The diagnostic accuracy of the cytology and histopathology of this study were sensitivity (89.28%), specificity (63.63%), positive predictive value (PPV) (64.10%), negative predictive value (NPV) (72.72%), and diagnostic accuracy (72%). Pap smear sensitivity was found to be 89.28% compared to its specificity which was 63.63%. This was attributed to a high false negative smear.

DISCUSSION

A Pap smear examination is vital for detecting pre-invasive and invasive cervical epithelial abnormalities. It is a simple screening test that is a simple, non-invasive, low cost, and reliable method. This method reduces the morbidity and mortality related to cervical carcinoma, if it has been detected early in the pre-invasive stage.

This study mainly aims to assess the diagnostic accuracy in the cytology of the cervix in cervical neoplasia diagnosis. In the present study, the Pap test has been compared with histological examinations, and the medical details and other symptoms were noted and analyzed. The findings from this study were compared with the results of other study findings.

The mean age observed in the present study was 48.92 years, and this was comparable to the mean age assessed by Alazzam *et al*. [4], which is 43 years. The parity in the present study was 42%, whereas

Table 2: Examination of per speculum in cervix

Examination of per speculum	**Number of patients**	**Percentage**
Hypertrophied, erosion	12	24
Cervix flushed with vagina	05	10
Friable growth	21	42
Bleeds on touch	31	62

Table 3: Pap smear findings

Cervical pap smear	**Number of patients**	**Percentages**
Atypical squamous cells of undetermined significance	02	04
Atypical squamous cells-high grade	04	08
Low-grade squamous intraepithelial lesions	13	26
High-grade squamous intraepithelial lesions	18	36
Squamous cell carcinoma	11	22
Adenocarcinoma	02	04
Total	50	100

Table 4: Histopathological findings

Histopathological findings	**Number of patients**	**Percentages**
CIN-1	18	36
CIN-2	06	12
CIN-3	14	28
Squamous cell carcinoma	10	20
Adenocarcinoma	02	04
Total	50	100

CIN: Cervical intraepithelial neoplasia

Table 5: Cytohistological comparison

Cytology	Histopathology				
	CIN-1	CIN-2	CIN-3	SCC	Adeno-carcinoma
Atypical squamous cells of undetermined significance (02)	02	00	00	00	00
Atypical squamous cells-high grade (04)	00	02	02	00	00
Low-grade squamous intraepithelial lesions (13)	07	03	03	00	00
High-grade squamous intraepithelial lesions (18)	00	05	10	03	00
Squamous cell carcinoma (11)	00	00	03	08	00
Adenocarcinoma (02)	00	00	00	00	02
Total (50)	09	10	18	11	02

CIN: Cervical intraepithelial neoplasia

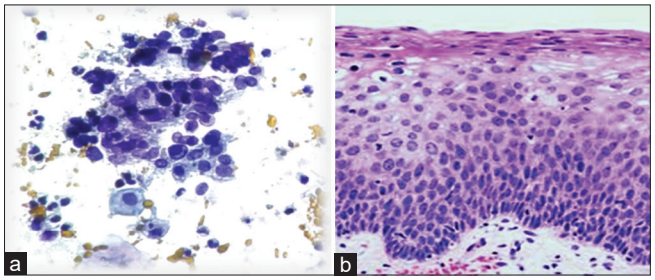

Fig. 1: (a) Cytosmear showing features of high-grade squamous intraepithelial lesions (b) cervical intraepithelial neoplasia -2 (H&E stain, ×400) (PAP stain ×400)

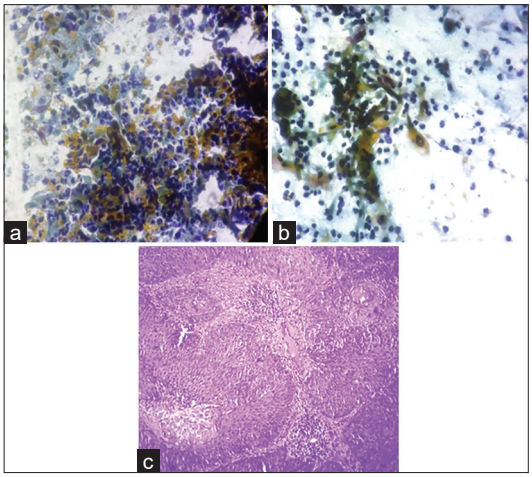

Fig. 2: (a) (PAP stain, ×400): Cytosmear from a friable growth in cervix showing features of squamous cell carcinoma (b) (PAP stain, ×400): Cytosmear from the same lesion with tadpole cells (c) (H&E stain, ×400): Biopsy showing features of squamous cell carcinoma (well-differentiate)

in the Thapa [5] study, the parity was 23%, and in Moss *et al*. [6], the parity was 65%. Most of the patients in this study were found to be from middle socioeconomic status (72%), and some belonged to poor socioeconomic status (28%). In the study by Thapa [5], 100% were from middle socioeconomic status, and in Moss *et al*. [6], 85% were from middle socioeconomic status. In this study, the mean marriage age was 26.18 which can be compared with Thapa [5] study, in which the mean age is 21 years.

The severity of the premalignant and malignant cervix lesions has been increased in the increased duration of marriage because of the increase in sexual intercourse demonstrated by Gonzalez-Bosquet *et al*. [9]; in the present study, the complaints by the patients have been assessed, in that most of them complained of bleeding per vagina (56%) and abdominal pain (42%). In the same way in the study by Thapa [5],

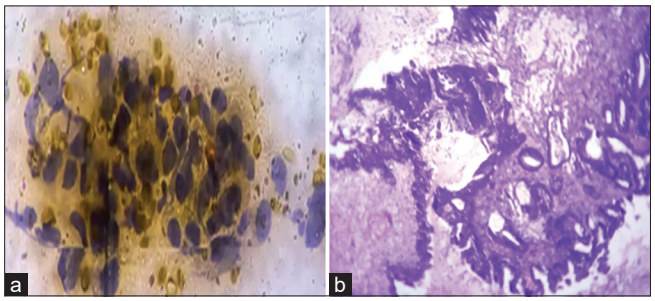

Fig. 3: (a) (PAP stain, ×400): Cytosmear showing features suggestive of adenocarcinoma, (b) (H&E stain, ×100): Biopsy showing features of adenocarcinoma

62.8% of patients observed white discharge per vagina, and 10% have post-coital bleeding. The relationship between the CIN and post-coital bleeding has been shown in the study by Rajaram *et al*. [10], and it was comparable with the present study. It has been observed that there is no correlation between the bleeding duration and pathology. In patients with intermenstrual bleeding, 22% (2/9) had CIN. The study by Erbil-Iraq and Muhammad [11] has identified that the chief complaints of women were post-coital bleeding and intermenstrual bleeding at 34.5% and 7%, respectively. In the present study, it has been identified that the bleeding in the vagina was 56%.

The sensitivity and specificity of the present study were 89.28% and 63.63%, respectively, and they were comparable to the studies by Saha *et al*. [12] and Thapa [5], here sensitivity was 100% and specificity was 60% for both studies. Furthermore, the sensitivity and specificity have been observed in the studies by Jain and Vyas [8], (Sensitivity (78%), specificity (26.9%)) and Rajaram *et al*., [10] (Sensitivity (56%), specificity (90%)). The PPV, NPV, and diagnostic accuracy obtained in this study are similar to the results obtained by Saha *et al*. [12]. Furthermore, the specificity and NPV observed in a Turkish study by Barut *et al*. [13] were 76% and 92%, respectively. Furthermore, in the study by Erbil-Iraq and Muhammad [11], the sensitivity (45.6%), accuracy (97.7%), NPV (89.9%), and PPV (80%) have been noted. Furthermore, the identified that most of the women affected were married women of age between 40 and 49 years old. Mostly they were married at the age of 15–24 years old. The specificity (89.47) in the study by Bamanikar *et al*. [14], was similar to the present study (89.28%).

In the present study, all 50 cases have undergone biopsy for Pap smear and histopathology analysis. Most of the cases in Pap smear were HSIL and LSIL followed by SCC, whereas in the study by Thapa [5], most of the cases were HSIL. The comparison of both Pap smear and histopathological studies with results observed in Thapa [5] has been given in Table 7. Furthermore, in the study conducted by Tailor *et al*. [15], the common epithelial cell abnormality (ASCUS (0.77%)) has been identified in the age group of 30–40 years. Furthermore, ASCUS has been observed on 10 women in a series by Nishimura *et al*. [16], most of the cases in histopathology were CIN 2 and CIN

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Table 6: Comparison of mean age in different studies

Study	Age range	Mean age
Thapa [5]	20–75	40.3
Tuon *et al.* [7]	20–70	30.2
Moss *et al.* [6]	20–67	37.5
Alazzam *et al.* [4]	23–79	43
Jain and Vyas [8]	20–70	32.6
Present study	20–>65	48.92

Table 7: Pap smear and histopathological findings comparison with other studies

Variables	Present study		Thapa [5]	
	No of cases	%	No of cases	%
PAP smear				
Atypical squamous cells of undetermined significance	2	4	1	2
Atypical squamous cells-high grade	4	8	----	------
Low-grade squamous intraepithelial lesions	13	26	8	18.6
High-grade squamous intraepithelial lesions	18	36	9	21
SCC	11	22	3	7
Adenocarcinoma	2	4	-----	-------
Histopathology				
CIN-1 (LSIL)	18	36	11	25
CIN-2 and 3 (HSIL)	20	40	05	12
SCC	10	20	03	07
Adenocarcinoma	02	04	00	00

SCC: Squamous cell carcinoma, CIN: Cervical intraepithelial neoplasia

3 followed by CIN 1, whereas in the studies by Saha *et al.* [11] and Thapa [5] most of the cases were CIN 1.

Cervical cancer normally develops slowly for years. Precancerous lesions such as intraepithelial neoplasia can appear before 5–10 years of cancer development. Early detection of these lesions can help in the treatment of abnormal lesions and the prevention of cancer development. Hence, it is recommended that women should at least take one Pap smear test before the age of 45 [17,18]**.** Furthermore, women with persistent inflammation have an increased risk of cervical intraepithelial lesion development. Repeated Pap smear must be taken after the treatment with antibiotics in such cases [19,20]. This study highlights the importance of Pap smear for cervical abnormalities detection.

Limitations

The main limitation of this study was the limited amount of samples. The other limitation was that not all the cases had been detected using Pap smear. This is because Pap smear has detected HSIL and LSIL, some cases that have precancerous lesions have not been detected. There is a need for more cases and studies to analyze the generalizability of the findings. In addition, there is a need to investigate the factors affecting the accuracy such as human eros and also HPV testing can be done along with Pap smear.

CONCLUSION

Pap smear method can be a cost-effective method for screening regularly for the early detection and prevention of malignant and premalignant cervical lesions, which lead to cervical cancer. This study has assessed the efficacy of a Pap smear using TBS for the cervical lesions diagnosis. It mainly involves the comparison of Pap smart results with histopathological results for biopsies of 50 women. Pap smear sensitivity was 89.28% and specificity was 63.63%. Furthermore, increased false-negative rates were observed in Pap smears. The common Pap smear findings were HSIL and LSIL which were 36% and 26%, respectively, and the common histopathological findings were CIN 2 and CIN 3 which were 36% and 26%, respectively. These findings are comparable to Pap smear accuracy. The sensitivity, diagnostic accuracy, and specificity were 89.28%, 72%, and 63.63%, respectively. The Pap smears using TBS are a valuable tool that can be used for early cervical cancer screening.

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AUTHORSHIP CONTRIBUTIONS

Dr. Gouranga Charan Prusty, Dr. Sunanda Nayak, and Dr. Tapas Ranjan Mishra – Design and Data collection or processing, interpretation of results, editing the manuscript. Dr. Ranjan Kumar Mallick, Dr. Goutami Das Nayak, and Dr. Chandraprava Mishra – analysis or interpretation, literature search, manuscript writing, and submission.

CONFLICT OF INTEREST

Nil.

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Nil.

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