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CERVICAL CYTOLOGY (THE BETHESDA SYSTEM) AND COLPOSCOPIC GUIDED BIOPSY CORRELATION IN NEOPLASTIC CERVICAL LESIONS

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ABSTRACT: Cervical cancer is a significant health problem worldwide among women. Cancer cervix is the most common cancer in developing countries. It is considered as preventable cancer since there is availability of screening method and effective diagnostic and therapeutic procedures. Despite the accuracy and efficacy of cervical cytology in detecting and diagnosing cervical neoplasia it must remain a screening technique with further evaluation based on histologic diagnosis. Objectives of the study are to compare cytology and histopathology of neoplastic cervical lesions and to know the sensitivity and specificity of PAP smear. Present study is undertaken to evaluate the role of cytology and colposcopic guided biopsy in diagnosing neoplastic cervical lesions.

INTRODUCTION: Cancer cervix is one of the leading causes of cancer deaths in women in the world.[1] In India, cancer of the uterine cervix is the most frequent neoplasm among women, accounting for 20%-50% of all female cancers and 80% of all female genital cancers.[1] Dr. Papanicolaou recognized the importance of wet fixation of Cytological specimens and presented in his paper New Cancer Diagnosis in Jan 1928.[2] Dr. Aurel, in 1927-28 published a major article on the same subject in which the accurately described the appearance of cells of squamous cell carcinoma in scrapings of uterine cervix.[3] Publications of Papanicolaou and Traut in 1941 and 1943 launched the second era of cytopathology.[4] This era saw the advent of screening for cervical cancer, which has revealed that undetected cervical cancer exists in all populations and its detection by cytological screening is possible and practical.[5]

When cervical cytology has been applied in mass screening, there has been a reduction in the frequency and the death rate from cervical carcinoma.[4] It has been known that the squamocolumnar junction of cervix is the site of predilection for carcinoma of the cervix. It is possible to prevent the development of invasive carcinoma by identifying and treating pre invasive lesions.[6] Cervical cytology has been successful in reducing the incidence and mortality but several reviews of the efficacy of cervical cytology suggested a high false negative rate because of the error occurring at various stages like sampling preparation, interpretation of cervical smears.[7] Despite the accuracy and efficacy of cervical cytology in detecting and diagnosing cervical neoplasia it must remain a screening technique with further evaluation based on histologic diagnosis. Present study is undertaken to evaluate the role of cytology and colposcopic guided biopsy in diagnosing neoplastic lesions of uterine cervix.

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MATERIALS AND METHODS: A 2 year prospective study of 120 cases is done in the department of pathology from September 2006 to September 2008. The patients attending the outpatient department of Obstetrics and Gynecology in Vani Vilas Hospital are included for the study. Patients with history of white discharge, post-coital bleeding, bleeding pervaginum and suspicious looking (unhealthy) cervix on examination are selected. Patients are clinically examined after eliciting detailed clinical history, PAP smear and colposcopic directed biopsy obtained in all selected cases. PAP smear is taken using Ayre's spatula by rotating it 3600 clockwise at the transformation zone of cervix. The material is evenly spread on a clean glass slide and immediately fixed in 95% alcohol. The slides are stained with Papanicolaou's stain and various cytological changes noted using The Bethesda System of reporting. After obtaining PAP smears visualization of cervix is done using 2% acetic acid and colposcope. Cervical biopsy is taken from abnormal areas under colposcopic guidance and biopsy tissue fixed immediately in formalin solution and subjected for tissue processing. These sections obtained are stained with routine Haematoxylin and Eosin stain and examined under microscope. The histopathological changes occurring at various levels are noted.

STATISTICAL METHODS: Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean. SD and results on categorical measurements are presented in number (%). Chi square test /Fisher Exact test has been used to find the significance of association of cytology findings with histology. Diagnostic statistics such as sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value and diagnostic accuracy has been used to find the correlation of cervical cytology with histology.

RESULTS: 120 cases of cervical smears were studied using The Bethesda System of cytology and the cytologic findings were compared with the corresponding histologic diagnoses in all cases.

In the present study the age of patients ranged from 23 to 73 years, the youngest being 23 years and oldest was 70 years. The mean age was 36.7 years. Maximum number of cases was found to be in third decade i.e., 31-40 years of age comprising 35% of the study population. However, least number of patients were seen in seventh decade (61-70 yrs) comprising only 1.7% of study population. Majority of patients were married at the age of 18-19 years (46%). In the present study the mean age at marriage was 18.1 years. In the present study maximum numbers of patients were multiparous (73%) and parity ranged from 3 to 4. The major presenting complaint was white discharge per vaginum seen in 96 patients (80%). There were 14 cases who presented with post-menopausal bleeding forming 11.6%, 6 cases of irregular bleeding (5%) and 2 cases of post coital bleeding (1.7%) and mass per vaginum (1.7%) respectively.

Out of 120 cases of PAP smears there were 8 cases of Low grade Squamous Intra epithelial Lesion (LSIL), 8 cases of High grade Squamous Intra epithelial Lesion (HSIL), 8 cases of carcinoma and 96 cases belong to Negative for Intraepithelial Lesion/Malignancy (NILM) category. Ratio of NILM to neoplastic lesions on cytology was 4:1. Among the neoplastic lesions LSIL, HSIL and Squamous Cell Carcinoma constituted 33.3% each. Out of 120 Colposcopic

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biopsies studied on histology there were 10 cases of Cervical Intraepithelial Neoplasia 1(CIN)/LSIL, 6 cases of CIN 2 or 3/HSIL, 12 were squamous cell carcinomas and rest were nonspecific cervicitis.

Cytologic diagnosis	No of cases	Percentage
LSIL	8	33.3%
HSIL	8	33.3%
SCC	8	33.3%
TOTAL	24	100%
Table 1: Distribution of neoplastic lesions on cytology		

Histologic diagnosis	No. of cases	Percentage
LSIL	10	36%
HSIL	6	21%
SCC	12	43%
TOTAL	28	100%
Table 2: Distribution of neoplastic lesions on histopathology		

120 cases of cervical PAP smears were studied and diagnosis was offered in all cases. Out of 120 cervical smears, total 24(20%) smears were diagnosed as neoplastic lesions (6.7% each of LSIL, HSIL and carcinoma) and 96 (80%) smears were reported as NILM. The total percentage of premalignant and malignant lesions according to cytology was 18%. Among the neoplastic lesions, HSIL and LSIL and carcinoma constituted 33.3% each. Out of 120 colposcopic guided biopsy examined there were 92(76.6%) cases of chronic non-specific cervicitis (Inflammatory lesions) and total 28(23.4%) cases of premalignant and malignant lesions. The percentage of malignancy (SCC) was 10%andpremalignantlesion was 13.4% of total study population. There were 12 cases of squamous cell carcinomas forming 43% of neoplastic lesions and therewere6casesofHSILand10casesofLSIL forming 21% and 36% of neoplastic lesions. The table 1 and 2 shows distribution of neoplastic lesions among various cytologic and histologic categories.

Out of 96 NILM lesions diagnosed on cytology 92 cases (95.8%) correlated with histology and 4 cases were reported as CIN I. out of 8 cases of LSIL on cytology six cases (75%) correlated with histology and 2 cases were CIN II on histology. Among 8 HSIL cases diagnosed on cytology only four cases (50%) correlated with histology and rest 4 were reported as squamous cell carcinoma. All 8 cases of carcinoma on cytology (100%) correlated with histology (Table 3).

Cytologic diagnosis		Histological diagnosis			
Category	Number	NILM	CIN 1	CIN 2/3	SCC
NILM	96	92	4		
LSIL	8		6	2	

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HSIL	8			4	4
SCC	8				8
TOTAL	120				

Table 3: Correlation of cytological diagnosis with histologic diagnosis
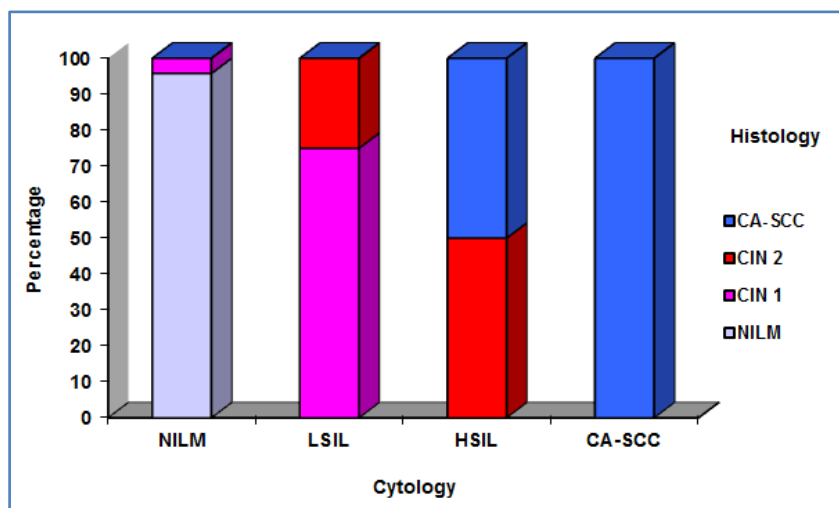
Fig. 1: Correlation of cytological and histological diagnosis

DIAGNOSIS	Sensitivity	Specificity	PPV	NPV	Accuracy	P value
NILM	100.0	85.7	95.8	100.0	96.7	<0.001
LSIL	60.0	98.2	75.0	96.4	95.0	<0.001
HSIL	66.7	96.5	50.0	98.2	95.0	<0.001
CA	66.7	100.0	100.0	96.4	96.7	<0.001

Table 4: Different statistical values for cervical cytology (PAP smear)

Sensitivity and specificity for cervical cytology was 66.7% and 100% respectively. There were 10 false positive and false negative diagnoses each, giving a false positive and negative rate of 8.3%. Positive predictive value and negative predictive value were 100% and 96.4% respectively. Sensitivity of cytology in LSIL, HSIL and carcinoma are 66.7%, 100% and 96.7% respectively. Specificity of cytology obtained in LSIL, HSIL and carcinoma is 85.7%, 98.2% and 100% respectively (Table 4).

DISCUSSION: Total 120 cases of cervical PAP smears were studied and the cytologic diagnoses were compared with the corresponding histologic diagnoses in all cases. TBS system used for reporting PAP smears. Cervical cytology (PAP smear) correlated with histopathology (Colposcopic guided biopsy) in 95.8% of NILM lesions, 75% of LSIL/CIN I cases, 50% of HSIL/CINII/III cases and 100% of malignant lesions.

The mean age in the present study was 36.7 years. The mean age in the study of Mostafa M. et al[8] was 41.66 years and 40.3 years in the study conducted by Saha R. et al.[9] The mean age at marriage was 21 years in the study by Saha R. et al.[9] It was found to be 18 years in the present study. The major presenting complaint was white discharge per vaginum in the study of Saha R. et al. 9(62.8%) and in the present study (80%), other symptoms being post-menopausal bleeding in 11.6%, irregular bleeding in 5%, two cases presented with post coital bleeding and mass per vaginum each. Saha R et al[9] reported a slightly higher rate of premalignant lesions (5.6:1). Mostafa et al.[8] had found a lower rate of premalignant lesions (3.2:1) which is comparable to the present study (2:1). Early detection of premalignant lesions in developed countries prevents the progress of premalignant lesions to malignant lesions and hence the lower rate of malignant lesions.

Study	LSIL	HSIL	Carcinoma
Mostafa M. et al.[2]	22.91%	53.46%	23.62%
Saha R et al.[55]	38%	42.8%	14.2%
Present study	33.3%	33.3%	33.3%
Table 5: Distribution of neoplastic lesions on cytology in different studies			

The distribution of neoplastic lesions in various studies is given in table 5. The present study the percentages of LSIL, HSIL and carcinoma (SCC) were found to be 33.3% each. This probably indicates a variation in individual interpretation of squamous intra epithelial lesion. Among 22 NILM lesions diagnosed on cytology 19 were proved to be inflammatory on histopathology by Saha R et al[9] and hence 86.36% of correlation. The present study detected 98 inflammatory lesions on cytology of which 96 were proved same on histopathology giving 95.8% of correlation. Saha R et al[9] found 75% of correlation for HSIL/CIN II, III which is similar to the present study. Saha R et al[9] found only 55% of correlation for HSIL/CIN II, III which is similar to the (55%) present study. Less correlation was due to sampling errors and air drying artifacts in the smears. Interpretation errors were categorized into over-diagnosis and under-diagnosis.

Among 22 inflammatory lesions diagnosed on cytology 19 were proved to be inflammatory on histopathology by Saha R et al 55 and hence 86.36% of correlation. The present study detected 96 inflammatory lesions on cytology of which 92 were proved same on histopathology giving 95.8% of correlation. Saha R et al 55 found 75% of correlation for HSIL/CIN II, III which is similar to the present study. Saha R et al 55 found only 55% of correlation for HSIL/CIN II, III which is similar to the (55%) present study. Less correlation was due to sampling errors and air drying artefacts in the smears.

Name of the study	Audit by Simon Rasbridge 1995	Yeoh study 1997	Saha's study 2005	Nawaz study 2005	Present study
Concordance Rate	81.2%	52%	60%	74 %	91%
Table 6: Various concordance rates among different studies.					

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In the present study overall concordance rate was 91% which is higher than other studies. The other studies like Saha's,[9] Yeoh,[13] Nawaz[14] & Rasbridge[15] has concordance rates as 60%, 52%, 74% and 81.2% respectively (Table 6).

Out of the 10 discordant cases, 4 cases were underdiagnosed on cytology as HSIL, 2 cases were reported as LSIL and 4 cases were called as negative/benign. The main factor for underreporting was less cellularity with hemorrhagic background.

There was100% correlation for carcinoma (SCC) in both present study and study by Saha R et al.[9] Sensitivity of cervical cytology was highest in study by Saha R et al[9] 100%). In the present study it is 66.3% which is comparable to the study by Singh SL et al 10(63%). Specificity of cervical cytology in the study by Saha R et al[9] is 100% which is similar in the present study (100%). According to Saba AS[11] specificity was 99.3%. The various statistical parameters in the study by Saha R et al[9] are comparable to the present study in all parameters except in sensitivity which is comparatively lower (66% vs 100%) in present study (Table 6). This is attributed to ten false negative cases on cytology.

Study	LSIL	HSIL	SCC
Saha R et al[55]	86%	90.7%%	100%
Mostafa et al.[2]	77.7%	77.77	68%
Present study	95%	95%	96.7%
Table 7: Diagnostic accuracy for malignancy in different studies			

The diagnostic accuracy of cytology for all neoplastic lesions are given in the table 8. Diagnostic accuracy of carcinoma in the study of Saha Retal.[9] is comparable with the present study i.e. 96.7% (Table 8). Mostafa etal,[8] in their study, found a lower accuracy rate (68%), which could beat tribute to under diagnosis or due to errors in interpretation. Saha R et al[9] reported a high diagnostic accuracy of 90.7% for HSIL which is comparable with the present study (95%). Lozowskietal. found a diagnostic accuracy rate of 76.2%. This could be again attributed to either under diagnosis or sampling error. Mostafa et al. also reported similar findings in their study (77.7%). The diagnostic accuracy for LSIL is higher in study by Saha R et al9detected higher (86%) among others. However the present study noted the accuracy rate of 95% for LSIL. Under diagnosis by cytology was an important reason for inaccurate diagnosis. Under diagnosis by cytology was noted in 8.3% of cases in the present study which is comparable with 6.9% by Saha R et al.[9] Therate was higher in study by Mostafa et al. (27%).

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Authors	Sensitivity	Specificity
Saha R et al[55]	100%	100%
Saba AS et al[16]	75%	99.3%
Singh SL et al[20]	63%	74%
Present study	66.7%	100%
Table 8: Comparison of Sensitivity and specificity of cervical cytology in various studies		

Mostafa et al.[8] reported 41cases (5.6%) adenocarcinoma. There were no cases of adenocarcinoma detected in the present study which was comparable to the study by Saha R et al.[9] The cervical cancer screening for adenocarcinoma was less effective because of sampling difficulties.

CONCLUSION: The regular screening of population by Pap smear is a cost-effective method for early detection of premalignant and malignant cervical lesions and down staging of carcinoma cervix. The procedure is simple, in expensive and can be performed in the outpatient department. Hence, it should be recommended routine lyasa method of improving reproductive health. Cervical cytology is more specific but less sensitive. As with all screening tests, cervical cytology is also limited by both false negative and false positives. The sensitivity for cervical intraepithelial neoplasia is low but it can be increased by adequate sampling and avoiding technical errors like air drying and fixation artifacts. The discrepancy can be minimized by following the Bethesda system for adequacy of sampling. To bring down false negative and false positive rates use of complementary methods like colposcopic biopsy aids in early detection of cervical neoplasia. Cytology and histopathological correlation can be used for better evaluation and as part of quality improvement.

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