### Spectrum of Cervical Lesions and Cytohistological Correlation: A Study in Tertiary Care Center

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#### ABSTRACT

**Introduction:** Cancer of the cervix is a global health problem and clinical cytopathology brings about detection and diagnosis of disease at stages earlier than possible before which can be further confirmed by histopathology technique. The objective of the present study was to evaluate accuracy of cervical cytology in the diagnosis of cervical lesions including both neoplastic and non neoplastic.

**Material and Methods:** The study comprised of 100 patients with abnormal Pap smear cytology. A detailed clinical history, general physical and systemic examination was conducted and cervical biopsies as well as pap smears received from department of Obstetrics and Gynaecology were processed and stained.

**Results:** A total of seven cases of squamous cell carcinoma were reported majority of which were seen in the advanced age group of greater than 50. In present study 61% cases were reported as NILM, 01% as ASCUS, 06% AGUS. LSIL was diagnosed on cytology in 20% patients, whereas 04% patients had HSIL and 07% patients revealed SCC, and remaining 01 patient (01%) with adenocarcinoma were diagnosed on cytological evaluation. The histopathological findings in 100 cases confirmed 72% cases of chronic cervicitis, 15% as CIN-1, 03% of CIN-2, 01% CIN-3, 01% adenocarcinoma and 08% were diagnosed as invasive squamous cell carcinoma. Cytohistological correlation of 100 cases revealed an overall sensitivity of 95.60% and a specificity of 77.78%.

**Conclusion:** Pap smear test was found to be equally sensitive to histopathological examination for the early detection of different cervical lesions.

**Keywords:** Cancer Cervix, Pap Smear, Histological Correlation, Early Diagnosis

#### **INTRODUCTION**

Disease and health are reflected accurately in both tissue and cellular patterns which can be studied under histopathology and cytopathology respectively. Clinical cytopathology brings about detection and diagnosis of disease at stages earlier than possible before.<sup>1</sup>

Cancer cervix is a global health problem. It is the second most common cancer among women in the world and ranks as the first most frequent cancer among women in India and in the other developing countries. The worldwide incidence of cervical cancer is approximately 510,000 new cases annually, with approximately 288,000 deaths worldwide.<sup>2</sup>

Cervical cancer occurs early and strikes at the reproductive period of a woman's life. The incidence rises in 30-34 years of age and peaks at 55-65 years with median age of 38 years. Early sexual activity, multiple sexual partners and low socioeconomic status are major risk determinants of cervical cancer, suggesting that sexually transmitted infections like HIV, Chlamydia, gonorrhea, and syphilis are of etiological importance in the disease. Potentially oncogenic venereal infections include Herpes simplex type 2, cytomegalovirus, human papilloma virus, HPV 16 and 18 account for more than 70% of all cervical cancers.<sup>3</sup>

As the cervix is relatively easily accessible organ, the logistics for screening cervical cancer are also simple. Screening programmes have reduced the incidence and mortality from cancer cervix in many developed countries. It is well accepted that Pap smear has been the most effective cancer screening test ever introduced.<sup>4</sup>

To date the cervical cancer prevention effort worldwide have focused on screening sexually active women using cytological smears and treating precancerous lesion thus by decreasing the incidence and mortality from cervical cancer. The diagnosis is made by screening an asymptomatic population. Tests in use are cervical cytology, and histopathological examination of the biopsy materials added by various techniques such as cervicography and assessment of HPV DNA typing.<sup>5</sup>

#### **MATERIAL AND METHODS**

This was a prospective study done over a period of one year. The present study was conducted in the Department of Pathology consisting of 100 cases with cervical lesions. Pap smears and biopsies were collected from all the patients for the study and correlation was done. A detailed clinical history, general physical and systemic examination was conducted and cervical biopsies as well as pap smears received from department of Obstetrics and Gynaecology were processed and stained. The results were recorded and compared.

The patients presenting with discharge per vaginum, bleed per vaginum and hypertrophied uterine cervix were included in the study. The cytological data obtained was compared with the histopathological diagnosis. Statistical data pertaining to the sensitivity, specificity and positive predictive evaluation of Pap smear and biopsies in diagnosing cervical lesions was

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**How to cite this article:** Anuradha Sharma, Sonia Singh. Spectrum of cervical lesions and cytohistological correlation: a study in tertiary care center. International Journal of Contemporary Medical Research 2019;6(6):F5-F10.

DOI: http://dx.doi.org/10.21276/ijcmr.2019.6.6.13

#### calculated.

#### STATISTICAL ANALYSIS

For statistical analysis, a report of NILM, ASCUS, AGUS, LSIL, HSIL, Adenocarcinoma and SCC is considered positive. Statistical analysis is shown in table 3. Descriptive statistics were used for the interpretation of data.

#### RESULTS

The present study was based upon the cytological and histological evaluation of cervical lesions from 100 patients.

#### Age distribution of the patients in the study

The age of the patients ranged from 21 to 70 years. The maximum numbers of the patients were in third decade of life, followed by fourth decade. The youngest patient in our study was 25 years of age and the oldest patient was 70 years of age. The mean age was 40.06 years.

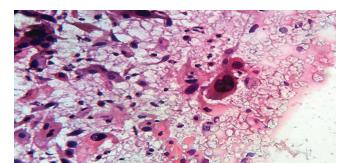
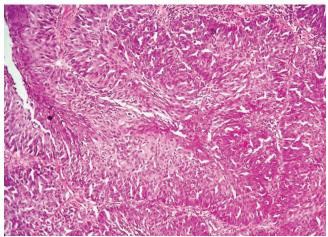


Figure-1: Squamous cell carcinoma (Pap, x 400).

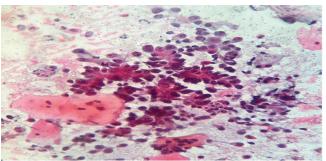
Cytological Diagnosis	No of cases			
NILM	61			
ASCUS	01			
AGUS	06			
LSIL	20			
HSIL	04			
SCC	07			
Table-1: Categorisation of cytodiagnosis.				

# Relationship of age with inflammatory lesions, sil and carcinoma

Maximum numbers of cases (26) reported as NILM, were in the reproductive age group of 31-40 years. LSIL was seen commonly in the age group of 31 to 40 years. Out of the total patients showing HSIL (4), maximum (2) were reported in the age group of more than 50 years whereas



**Figure-2:** Basaloid variant of squamous cell carcinoma (H and E, x 400)



**Figure-3:** Adenocarcinoma. Smear showing cells arranged in rosettes with, nuclear pleomorphism, hyperchromasia with coarse nuclear chromatin. (Pap, x400)

Cytological Diagnosis	No of cases	Histopathological Diagnosis							
		Chronic cervicitis	CIN-1	CIN-2	CIN-3	Adenocar-cinoma	SCC		
NILM	61	61	00	00	00	00	00		
ASCUS	01	01	00	00	00	00	00		
AGUS	07	06	00	00	00	01	00		
LSIL	20	05	15	00	00	00	00		
HSIL	04	00	00	03	01	00	00		
SCC	07	00	00	00	00	00	07		
Total	100	73	15	03	01	01	07		
Table-2: Cyto-histological correlation									

Statistical Analysis	Lesions (Percentage)						Overall Study	
	NILM	ASCUS	AGUS	LSIL	HSIL	ADENOCAR-CINOMA	SCC	
Sensitivity	83.33	00.00	00.00	93.75	66.00	100	85.71	95.60
Specificity	96.55	98.98	94.04	94.04	98.95	100	98.94	77.78
PPV	98.36	00.00	00.00	75.00	50.00	100	100	97.75
NPV	70.00	98.98	98.94	98.70	97.94	100	98.94	63.03
Diagnostic accuracy	88.00	93.00	93.94	94.00	95.00	100	99.00	94.00
Table-3: Analysis of the data								

 International Journal of Contemporary Medical Research

 Volume 6 | Issue 6 | June 2019 | ICV: 98.46 |
 ISSN (Online): 2393-915X; (Print): 2454-7379

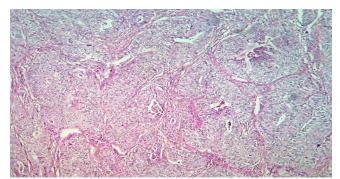


Figure-4: Adenocarcinoma (H and E, x 100).

others were reported between 21-40 years of age. One case of adenocarcinoma was reported at 40 years of age. A total of seven cases of squamous cell carcinoma reported on cytology, maximum (3) were seen in the advanced age group of >50 years of age.

### Relationship of parity with inflammatory lesions, sil, and carcinoma

The overall incidence of cervical cancer was found to increase with increasing parity. Maximum number of cases of HSIL, SCC and adenocarcinoma were seen in multiparous women, presenting mainly with post-menopausal bleeding.

## Relationship of presenting symptoms with inflammatory lesions, sil and invasive carcinoma.

Discharge per vaginum (DPV) was the most common complaint of total 48% of the cases. Majority of the cases, who presented with discharge per vaginum were inflammatory in nature (34 cases). Patients who were diagnosed as having squamous cell carcinoma mostly presented with post menopausal bleeding (03 cases), bleed per vaginum (02 cases) and contact bleeding(02). A single case of adenocarcinoma presented with discharge per vaginum.

## Gross appearance of cervix and its relationship with inflammatory lesions, sil and invasive carcinomas

Hypertrophied uterine cervisx (HUC) was observed in 41% of the patients maximum of which were reported as Negative for intraepithelial lesions on pap smears followed by LSIL. Other per speculum findings included endocervicitis with erosion (EE), erosion (Er), endocervicitis (E) and a visible growth arising from cervix. Hard, nodular growth was observed in 5% of the patients, out of which three turned out to be invasive squamous cell carcinoma.

#### Categorization of cytodiagnosis

In cytology, smears were examined and classified according to The Bethesda system into NILM, ASCUS (atypical squamous cells of undetermined significance), LSIL (low grade squamous cells of undetermined significance), HSIL (high grade squamous cells of undetermined significance), AGUS (atypical squamous cells of undetermined significance), SCC (squamous cell carcinoma) and adenocarcinoma, depicted in table-1.

The smears of NILM showed mainly superficial and intermediate cells along with marked inflammatory exudates consisting of neutrophils, lymphocytes and histiocytes. Degenerative changes like vacoulation of the cytoplasm, perinuclear halo etc. was observed. Reactive changes like hyperkeratosis and parakeratosis were also seen. The endocervical cells showed squamous metaplasia in few cases.

Cases reported as ASCUS showed borderline cytological changes like nuclear enlargement, 2.5-3 times a normal intermediate cell nucleus, mild nuclear hyperchromasia, smooth nuclear outline and mild variation in size and shape. Cases reported as LSIL had nuclear size 4-6 times the size of normal intermediate cell nucleus, occupying less than 1/3 rd of the total area of the cells. Chromatin was uniformly distributed, but coarsely granular. Koilocytes were observed as superficial or intermediate squamous cells showing perinuclear halo, with peripheral condensation of cytoplasm, wrinkled nuclear membrane and multinucleation.

Smears diagnosed as HSIL showed abnormal cells of variable size. In addition to superficial and intermediate cell types, parabasl cell were also found. Most of the cells were round to oval but spindle shaped elongated cells and cells with bizarre shapes were also seen. Cytoplasmic staining was usually cyanophilic but some showed eosinophillia of the cytoplasm. The cytoplasm was sparse in some cases forming a rim around the nucleus. The nuclei were large hyperchromatic with increased N: C ratio and nucleus occupying  $\frac{1}{2}-\frac{2}{3}$  rd of the total area of the cell.

Cytological smears reported as squamous cell carcinoma showed marked variation in size and shape with caudate and spindle cells. The cytoplasm of some of the cells stained eosinophillic. The nuclei were enlarged, irregular with hyperchromatic, coarsely granular and unevenly distributed nuclear chromatin. Most of the smears showed a dirty background due to excessive necrosis and tumor diathesis.

#### Histopathological findings

Histopathalogical evaluation of H and E stained paraffin sections of cervical biopsy revealed that 72 patients had chronic cervicitis. CIN-1 was diagnosed in 15 patients and 04 were diagnosed to have CIN-2, CIN-3. Squamous cell carcinoma was identified in 08 cases and 01 case was diagnosed as adenocarcinoma.

The histopathological findings in chronic cervicitis consisted of basal cell hyperplasia of the ectocervical lining. The endocervix also showed basal cell hyperplasia and squamous metaplasia of endocervical glands. The inflammatory exudates consisted mainly of mononuclear cells.

Cases reported as CIN-1 in cervical biopsy specimens showed relatively regular arrangement of cells with preserved stratification in upper two thirds of the epithelium. The dysplastic changes consisting of aberrations in nuclear morphology and disturbed arrangement and stratification were observed mostly in lower 1/3rd of the epithelial lining. Cases reported as CIN-2 the section showed immature basaloid cells occupying upto two-third of the epithelial thickness not extending into the upper third of the epithelian. The cases reported as CIN-3, evidence of stratification was seen only in the upper 1/3rd of the epithelium. Nuclear abnormalities consisting of increased nuclear size, hyperchromatic, coarsely granular nuclear chromatin was seen throughout the epithelium. Mitosis was observed in increased numbers.

Squamous cell carcinoma of the cervix showed two patterns – Keratinizing and Non keratinizing. In the keratinizing squamous cell carcinoma irregular infiltrating nests of cells with cytoplasmic keratinization and epithelial pearl formation was observed. Non keratinizing squamous cell carcinomas did not show epithelial pearl formation. Necrosis was a prominent feature.

#### Cyto-histological correlation

Correlation of cervical smear findings with histopathological diagnosis revealed the following:

Of the total 61 cases diagnosed as negative for intraepithelial lesion or malignancy (NILM) were confirmed histopathologically in cervical biopsies as depicted in table-2. 01 case of ASCUS was reported on pap smear turned out to be chronic cervicitis on histopathological examination.

Cytological diagnosis of LSIL was offered in 20 patients, 15 out of them were confirmed to have CIN-1 on histopathology, and rest of the 5 were found to have chronic cervicitis.

Cytological diagnosis of HSIL was offered in 04 patients. Histological conformation of CIN-3 was made in 01 patient along with CIN-2 in 03 patients. 07 cases of squamous cell carcinoma diagnosed on cytology were confirmed histologically.

#### DISCUSSION

The main objective of the present study was to evaluate the usefulness of cytology in detecting various preneoplastic and neoplastic lesions of cervix, to evaluate and to interpret the cases of epithelial lesions according to The Bethesda 2001 classification system and correlation of cytological findings with follow-up histology sections. Carcinoma uterine cervix is one of the leading causes of cancer death among women worldwide. To detect this widely prevalent cancer at an early stage, the simplest test has been a pap smear. To check the sensitivity and specificity of Bethesda system, the cytological findings have to be correlated with histopathology considering it as gold standard.

The Bethesda system for reporting cervical cytological diagnosis is a uniform system for reporting, and is useful to provide effective communication among cytopathologist and referring physician. It also facilitates cytological and histopathological correlation. The present study provides an analysis of patterns of Pap smear, age distribution, relationship of the lesion with different age groups, study of the clinical features.

#### Age distribution

In our study, 82% of the patients were in the reproductive age group. The age of the patients ranged from 21 to 70 years of age with a mean of 40.06 years. The maximum patients with cervical lesions presented in the age group of 21-50 years. Other studies have also shown a similar wide range of patients presenting with cervical lesions. Our study correlates

well with the study of Chhabra et al<sup>6</sup> where more than two third of the women were in the reproductive age group. It also collaborates the fact that sexually active women in the reproductive age group have a higher chance of having an unhealthy cervix. In a study conducted by Mulazim Hussain Bukhari et al<sup>7</sup> peak incidence of abnormal smears were found during age group of 30-39 years.

#### Negative for intraepithelial lesion or malignancy (NILM)

In present study, NILM accounted for maximum number of 61% of cases observed in the age group of 31-40 years. Similarly in the study of Musmar<sup>8</sup> inflammatory reactive smears were found to be commonest with frequency of 57.2%, whereas in the study of Pradhan B<sup>9</sup> et al number of inflammatory smears were 30%. In an another study conducted by Mulazim H Bukhari<sup>7</sup> the maximum number of inflammatory smears accounted for 38.3% which were observed in the age group of 30-39 years.

#### Low grade squamous intraepithelial lesion (LSIL)

It was observed that as age progresses there were sequential progression in the development of LSIL to HSIL and HSIL to squamous cell carcinoma. The result of our study correlates well with Pradhan B et al<sup>9</sup> who reported the mean age of LSIL as 31-40 and accounted for 31%, and Chhabra et al<sup>6</sup> who reported mean age of LSIL as 34.6 years. In present study the maximum number of patients presenting with LSIL were also in the age group of 31-40 years. The present observation has been supported by fact that Cervical Intraepithelial Neoplasia is continuous process which begins in its morphologically identifiable stage as LSIL and ends in invasive cancer.

#### High grade squamous intraepithelial lesion (HSIL)

HSIL is a cytological category that encompasses biologically different category smears, entities like moderate dysplasia and in situ squamous cell carcinoma, with potentially different outcome. Most of the cases of squamous cell carcinoma and high grade intraepithelial lesions in the present study were reported in the age group of >50 years and HSIL was observed in 4% of the cases. This correlates well with the study of Subhalakshmi Mukopadhayay<sup>10</sup>, where HSIL was encountered in about 9.04% of cases in the age group of 46-50 years.

#### Squamous cell carcinoma

Maximum cases of squamous cell cancer were seen in our study in patients of >50 years of age. This correlates with other studies which are given as under in table 9. Age for detection of SCC in studies conducted by Regan et al<sup>11</sup>, Domandia et al<sup>12</sup>, Chhabra et al<sup>6</sup> are 45-55 years, 39-48 years and 41-50 years respectively.

#### Parity

Another established risk factor for cervical cancer is high parity. Chhabra et al<sup>6</sup> observed most of the cases of squamous cell carcinoma in women who had four or more children. In their study, no case of squamous intraepithelial lesion or cancer was seen in nulliparaous women. Our study was also consistent with the same findings that maximum number of patients of squamous cell carcinoma were noted in women who had three or more children in 03 patients. In a study conducted by Mulazim Hussain Bukhari<sup>7</sup> premalignant and malignant lesions were most commonly observed with multipara women (67.6%). In our study also premalignant and malignant lesions were seen in multipara women that accounted a total of 66 patients.

#### Symptoms

The most common presenting symptom in our study was discharge per vaginum in 47 cases. Most of cases who presented with this complaint were inflammatory in nature, followed by bleed per vaginum which accounted for 20 cases. Patients who were diagnosed as having squamous cell carcinoma mostly presented with post menopausal bleeding. This correlated well with the study conducted by Pradhan B et al<sup>9</sup> where discharge per vaginum was the commonest complaint in different lesions of cervix and bleed per vaginum was found to be more specific for cervical malignancy. The same was observed in the study conducted by Mulazim Hussain Bukhari et al<sup>7</sup> where vaginal discharge and abnormal bleeding was found in 91.2%and 60.7% respectively, of the neoplastic lesions.

In our study the common per speculum finding was hypertrophied uterine cervix in 41% of the cases. On cytology 26% of these cases were of NILM, 10% were those of LSIL, 02% HSIL and 3% were diagnosed as SCC. Growth was also the common presenting symptom in patients with SCC.

#### Comparison of pattern of pap smear with various studies

In the present study, inflammatory smears accounted for maximum number of cases those were 61% similar to the study of Subhalakshmi Mukhopadhyay<sup>10</sup> where Negative for intraepithelial lesion or malignancy were found to be the commonest with the frequency of 64%.

#### CIN-1

In our study 100 patients were exposed to biopsy where 15 patients were diagnosed as CIN-1 on histology, on cytology total 20 patients were reported as LSIL, remaining 05 cases which were reported as LSIL on cytology those were reported as chronic cervicitis on histopathology.

For CIN-1 in our study the Sensitivity of 93.75%, Specificity 94.04%, Positive Predictive Value 75%, Negative Predictive Value 98.7% with a Diagnostic accuracy of 94% was obtained. As compared to the study conducted by Saha R and Thapa  $M^{13}$  sensitivity, specificity, positive predictive value, negative predictive value, diagnostic accuracy for CIN-1 was 60%, 93.9%, 75%, 88.6%, 86%, respectively. The correlation rate for CIN-1 in present study is 75% as compared to Saha<sup>13</sup> study having the same correlation rate of 75%.

#### CIN-2 and CIN-3

In our study of 100 patients 04 were reported as HSIL on smear cytology. In subsequent biopsy study these cases were reported as CIN-2 and CIN-3. So the correlation was established in 100% of the cases. The correlation rates in the study conducted by Nawaz<sup>14</sup>, Gupta and Sondhai<sup>15</sup> were 92% and 74%.

In the study by Gupta and Sondhai<sup>15</sup> 100 cases were reported as CIN-2 and CIN-3 on histology and on cytology they were correctly able to identify 74 HSIL cases whereas in 26 cases a diagnosis of LSIL or below was given. These cases were reviewed of which 16 cases were reclassified as HSIL on cytology while remaining 10 were the cases which showed persistent diagnosis of LSIL. 12/16 (75%) cases represented interpretative error. Sampling error was 7/10 and air drying 5/10 was found in under diagnosed cases.

The correlation rate for CIN-2 and CIN-3 in present study is 100% as compared to 92% in the study done by Nawaz et al.<sup>14</sup> In study by Gupta and Sondhai<sup>15</sup>, correlation was established in 74% of cases whereas that by Yeoh<sup>16</sup> it was74.6%.

In our study for CIN-2 and CIN-3 the Sensitivity was 66% with Specificity of 98.95%, Positive Predictive Value 50% and Negative Predictive Value of 97.94% was obtained. Compared to the study conducted by Vaishali Jain<sup>17</sup> the sensitivity was 42% with specificity of 36.8% and positive predictive value of 61.3% was observed.

#### Squamous cell carcinoma (SCC)

On cytology 07 patients were diagnosed as SCC and one was diagnosed as HSIL subsequently on histopathology all cases were diagnosed as SCC giving a correlation rate of 87.5% in our study. It is seen that correlation rate for squamous cell carcinoma in our study was 87.5% with sensitivity of 85.71%, the specificity was as high as 98.94% and with positive predictive value of 100%. Thus a negative predictive value of 98.94%, with over all diagnostic accuracy of 99.0% was obtained. As compared to the study conducted by Vaishali Jain<sup>17</sup> the correlation rate was 83.6% with sensitivity 84%. The specificity was 90.4% and positive predictive value of 95.3% in their study. The correlation rate for squamous cell carcinoma in our study is 87.5% compared to the studies by Saha<sup>13</sup>, Yeoh<sup>16</sup> and Nawaz<sup>14</sup> in which the rate was 100%, 60% and 97.33% respectively.

#### CONCLUSION

Pap smear test was found to be equally sensitive to histopathological examination for the early detection of different cervical lesions. However, it is advised to perform biopsy if any abnormalities are detected in Pap smear for correlation and confirmation. Carcinoma cervix is one of the most common cancers in women and it is the most researched disease. The regular screening of population by Pap smear is a cost effective method for early detection of premalignant and malignant cervical lesions. Correlation of cervical cytology with cervical biopsies has been a common component of continuous quality improvement programme for accreditation purposes.

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#### Source of Support: Nil; Conflict of Interest: None

Submitted: 09-04-2019; Accepted: 17-05-2019; Published: 16-06-2019