ANALYSIS OF CERVICAL PAP SMEARS AS PER BETHESDA SYSTEM IN A TERTIARY CARE CENTRE.

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Abstract
Background: The value of exfoliative cervical cytology is undisputed today. Most studies focus on the application of pap smears to detect precancerous and cancerous lesions but pap smears are also significant in detecting inflammatory lesions and etiology wherever possible which can help the clinician in the management and prevent further dysplasias.

Aim & Objectives: To find out the prevalence of various non-neoplastic and neoplastic lesions of cervix as per the Bethesda system 2014 and to find out the etiology of inflammation wherever possible.

Methodology: This study was conducted over a period of one year from January 2017 to January 2018. 1518 pap smears received in the Department of Cytology were stained with pap stain & reported according to 'The 2014 Bethesda System for reporting cervical cytology'.

Results: 1518 pap smears were screened in which 28 (1.8%) smears were unsatisfactory for evaluation and 1490 (98.2%) were satisfactory. NILM was the predominant finding seen in 1473 (98.86%) smears. Among the specific infections, the most common was bacterial vaginosis in 128 (8.59%) smears, trichomonas vaginalis in 10 (0.67%), candida infection in 7 (0.47%) smears and 26 (1.75%) smears showed reactive changes associated with inflammation. Atrophic changes were found in 50 (3.36%) cases. Epithelial cell abnormalities were seen in 17 (1.17%) cases in which 9 (0.6%) cases were reported as ASCUS, 2 (0.13%) cases as LSIL and HSIL was seen in 1 (0.07%) case. Squamous cell carcinoma (SCC) was diagnosed in 4 (0.27%) cases and Adenocarcinoma in 1 (0.07%) case.

Conclusion: Cervical cytology is a simple and cost effective test and should be established as a routine diagnostic aid.

Keywords: Atypical squamous cells of undetermined significance (ASCUS), Bethesda System, High grade squamous intraepithelial lesion (HSIL), Low grade squamous intraepithelial lesion (LSIL), Negative for intraepithelial lesion or malignancy (NILM), Papanicolaou smears.

Introduction
Pap smear is the microscopic examination of cells scraped and exfoliated from the cervix, to detect precancerous and cancerous lesions. Dr. George N. Papanicolaou first described it in 1928 and hence it was named after him.¹

The infections of the genital tract are not uncommon in reproductive-age women and the cost associated with it has considerable importance. It is a common finding that many women do not show any symptoms in the presence of vaginitis or cervicitis.²-³

The advantage of pap test is that it is a simple, quick, and painless procedure performed on the cells scraped from the uterine cervix and is widely used as a screening test for the prevention of the cancer of uterine cervix.⁴⁻⁵

Though pap test plays an important role in detection of carcinoma and pre cancer, its role in diagnosis of
infective conditions along with the causative organism such as trichomonas vaginalis, candida etc, hormone related changes seen in the epithelium which are usually benign and changes caused by therapeutic agents is also found to be successful.

As the pap test is a simple, effective and versatile test, it has been made an integral part of routine clinical examination and large amount of workload in gynaecological and pathological practice is due to this test.

The current cytological terminology “The Bethesda System” was the outcome of work of an expert panel meeting which was held in 1988.

The Bethesda System replaced mild dysplasia with 'Low grade squamous intra epithelial lesion' (LSIL) and moderate dysplasia, severe dysplasia and carcinoma in situ with 'High grade squamous intra epithelial lesion' (HSIL). Minor changes were incorporated in the 1991 Bethesda System Terminology which was further revised in 2001 and again in 2014.

This study was done with the aim to detect various infections and epithelial cell abnormalities of cervix using Bethesda system for reporting cervical cytology.

AIM & OBJECTIVES

To find out the prevalence of various non-neoplastic and neoplastic lesions of cervix as per "The 2014 Bethesda system for reporting cervical cytology" in a tertiary care centre.

MATERIALS AND METHODS

PLACE OF STUDY:

The present study was conducted after approval from institutional ethical committee in the Department of Pathology, Sri Aurobindo Medical College and Post Graduate Institute, Indore, M.P.

STUDY DESIGN AND DURATION:

The study design was cross sectional and included prospective cases from January 2017 to January 2018.

CASE SELECTION:

All the pap smears received in the Department of Cytology during study period were stained with pap stain. All the information related to patient was noted i.e. Name, Age, Registration number, complaints and clinical diagnosis.

The stained slides were studied under microscope along with clinical data.

The pap smears were reported according to 'The 2014 Bethesda System for Reporting Cervical Cytology'.

In our study, data was collected which was then distributed and tabulated according to age, presenting symptoms, clinical diagnosis and cytological diagnosis.

Prevalence of different lesions was shown in percentage.

RESULTS

The study included total of 1518 pap smears received during the period of one year from January 2017 to January 2018 in the Department of Cytology of Sri Aurobindo Medical College and P.G Institute, Indore.

The maximum number of cases i.e. 587 (39.4%) were seen in the age group of 21-30 years followed by 504 (33.8%) cases in the age group of 31-40 years. The youngest patient in our study was 18 years old and oldest patient was 85 years old.

A total of 1518 pap smears were screened in which 28 (1.8%) smears were unsatisfactory for evaluation and 1490 (98.2%) were satisfactory.

The major bulk of pap smears i.e. 874 (58.66%) smears came for routine screening. Only 616 (41.34%) women were symptomatic. Among the symptomatic women, the most common symptom was white discharge per vaginum seen in 288 (46.75%) women. The second most common symptom was pain in abdomen seen in 131(21.27%) women followed by menstrual irregularities seen in 101 (16.4%) women.

All the cases were then classified according to 'The 2014 Bethesda System for Reporting Cervical Cytology' as shown in Table 1.

The senile atrophic changes were most common above 40 years of age with maximum i.e. 22 (44%) at 41-50 years of age followed by 12 (24%) at 51-60 years of age and 11 (22%) at 61-70 years of age.
Table 1: Distribution of Pap smear reports as per The Bethesda System (2014)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Diagnosis</th>
<th>No. of cases</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Negative for Intraepithelial Lesion or Malignancy (NILM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>163</td>
<td>10.94</td>
</tr>
<tr>
<td></td>
<td>Organisms</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trichomonas vaginalis</td>
<td>10</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>Fungal organism</td>
<td>07</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>Bacterial vaginosis</td>
<td>128</td>
<td>8.59</td>
</tr>
<tr>
<td></td>
<td>Actinomyces species</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Non-specific inflammation</td>
<td>1089</td>
<td>73.09</td>
</tr>
<tr>
<td>3</td>
<td>Other non-neoplastic</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reactive cellular changes associated with inflammation (Squamous metaplasia)</td>
<td>26</td>
<td>1.75</td>
</tr>
<tr>
<td></td>
<td>Radiation</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>IUCD</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Atrophy</td>
<td>50</td>
<td>3.36</td>
</tr>
<tr>
<td>II</td>
<td>Epithelial cell abnormalities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Squamous cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ASCUS</td>
<td>09</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>LSIL</td>
<td>02</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>HSIL</td>
<td>01</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>SCC</td>
<td>04</td>
<td>0.27</td>
</tr>
<tr>
<td>2</td>
<td>Glandular cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AGUS</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adenocarcinoma</td>
<td>01</td>
<td>0.07</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1490</td>
<td>100</td>
</tr>
</tbody>
</table>

Graph 1: Distribution of cases diagnosed as "Negative for intraepithelial lesion or malignancy"

On distributing the cases as per 'The Bethesda System 2014' it was found that out of the 1490 satisfactory smears, 1473 (98.86%) smears were diagnosed as 'Negative for Intraepithelial Lesion or Malignancy' (NILM). Among these 163 (10.94%) smears were normal. 1089 (73.09%) smears showed non-specific inflammation, 128 (8.59%) smears showed bacterial vaginosis, 10 (0.67%) smears showed trichomonas vaginalis infection, 7 (0.47%) smears showed candida infection and 26 (1.75%) smears showed reactive changes associated with inflammation. Atrophic changes were found in 50 (3.36%) smears.

Graph 2: Distribution of cases diagnosed as "Epithelial cell abnormalities"

Out of the 1490 satisfactory smears, 17 (1.14%) cases were diagnosed as having epithelial cell abnormalities. Among these, 9 (0.6%) cases were reported as ASCUS (Atypical squamous cells of undetermined significance), 2 (0.13%) cases as LSIL (Low grade squamous intraepithelial lesion) and HSIL (High grade squamous intraepithelial lesion) was seen in 1 (0.07%) case. Suggestive of squamous cell carcinoma (SCC) was diagnosed in 4 (0.27%) cases and adenocarcinoma in 1 (0.07%) case.

PHOTOMICROGRAPHS

Figure 1: Photomicrograph of a pap smear showing shift in bacterial flora with clue cells and large number of coccobacilli suggestive of bacterial vaginosis

(Pap stain; 400X)
Figure 2: Photomicrograph of an inflammatory smear showing pear shaped *Trichomonas vaginalis* along with neutrophils and superficial and intermediate squamous cells (Pap stain; 400X)

Figure 3: Photomicrograph showing atypical squamous cells of undetermined significance (ASCUS) with increased nuclear hyperchromasia and high nuclear to cytoplasmic ratios (Pap stain; 400X)

Figure 4: Photomicrograph of pap smear showing LSIL having squamous cells with enlarged nuclei and variable chromatin. Few binucleate and multinucleate forms seen (Pap stain; 100X)

Figure 5: Photomicrograph of pap smear showing HSIL with dysplastic cells having high N:C ratio, hyperchromatic nuclei arranged in a syncytial cluster (Pap stain; 400X)

Figure 6: Photomicrograph of a pap smear showing SCC with atypical squamous cells having high N:C ratio, hyperchromatic nuclei and eosinophilic cytoplasm (Pap stain; 400X)

Figure 7: Photomicrograph of a pap smear showing adenocarcinoma with atypical round to oval cells having high N:C ratio, hyperchromatic with acini formation (Pap stain; 400X)
DISCUSSION

Conventional cervical cytology is the most widely used cervical cancer screening test in the world. This has been one of the most successful cancer screening techniques in the field of medicine. Cervical cytology screening programme in several developed countries have been associated with impressive reduction in cervical cancer burden.

Some of the cervical-vaginal infections can also be easily diagnosed from cervical cytology by identification of either the organism or cytological characteristic of the sample.

The index study group included women from different age groups. Maximum number of cases i.e. 587 (39.40%) were in the age group of 21-30 years followed by 504 (33.82%) cases in the age group of 31-40 years which is in concordance with the study done by Sharma P et al.\textsuperscript{10} and Bajpai M et al.\textsuperscript{11} The '2014 Bethesda System' maintains the 'satisfactory for evaluation'' and 'unsatisfactory for evaluation'' categories. A conventional cervical PAP smear should have minimum of approximately 8000-12000 well visualized and well-preserved squamous epithelial cells to be adequate. This includes squamous cells while endocervical cells and cells obscured completely with hemorrhage and inflammation are excluded from this estimate.

The inadequacy in PAP smear reporting is chiefly because of sampling error, improper fixation and non-co-operative patients. In the present study, 98.2% smears were satisfactory for evaluation which is in concordance with the studies of Philipose TR et al.\textsuperscript{12} with 99.2%, Abdullah L et al.\textsuperscript{13} with 97.2%, Burkadze G et al.\textsuperscript{14} with 98.7% and Saha D et al.\textsuperscript{15} with 96.2% satisfactory smears.

The most common symptom found in the present study is white discharge per vaginum seen in 46.72% cases which is in concordance with the study done by Maruf Raza AKM et al.\textsuperscript{16} 70.2% patients presented with chronic white discharge with itching.

The second most common symptom in the present study was pain in abdomen in 21.27% cases which was also the finding of studies by Ali SS et al.\textsuperscript{17} and Bhalerao A et al.\textsuperscript{18} The findings of the study of Selhi PK et al.\textsuperscript{19} also corroborates with the index study in which 70% of the patients presented with discharge per vaginum followed by lower abdominal pain.

In the index study, out of the total satisfactory smears, 98.86% smears were reported as NILM- a finding which is comparable with the studies of Selhi PK et al.\textsuperscript{19} (96.8%), Philipose TR\textsuperscript{12} et al. (95.88%), Pudasaini S et al.\textsuperscript{20} (98.6%), Kalyani R et al.\textsuperscript{21} (96.92%) and Swapnil D et al.\textsuperscript{22} (96.19%).

A comparative analysis of inflammatory smears between the present study and studies done by Malik SN et al.\textsuperscript{23}, Vaghela BK et al.\textsuperscript{24} and Hosamane S et al.\textsuperscript{25} was done.

Majority of the smears i.e. 1089 (73.09%) showed non specific inflammation which correlates with the study of Malik SN et al.\textsuperscript{23}, Vaghela BK et al.\textsuperscript{24} and Hosamane S et al.\textsuperscript{25} The second most common finding in the present study was Shift in vaginal flora i.e Bacterial Vaginosis seen in 128 (8.59%) smears. This finding was in concordance with the studies of Malik SN et al.\textsuperscript{23} and Hosamane S et al.\textsuperscript{25} While in study done by Vaghela BK et al.\textsuperscript{24}, bacterial vaginosis was the least common finding seen in only 1.6% cases.

Infection caused by candida was least common in the index study, seen in only 7 cases (0.47%). While in studies done by Malik SN et al.\textsuperscript{23} and Hosamane S et al.\textsuperscript{25} the least common cause of inflammation was trichomonas vaginalis seen in 1 (0.68%) case and 13 (0.51%) cases respectively. Candida infection was the second most common finding in study done by Vaghela BK et al.\textsuperscript{24} seen in 14 (2.8%) cases. Atrophic cervicitis was reported in 50 (3.36%) cases in the present study which is in concordance with study done by Vaghela BK et al.\textsuperscript{24} and Hosamane S et al.\textsuperscript{25} in which atrophic cervicitis was seen in 2.64% cases and 2.4% cases respectively. Pudasaini S et al.\textsuperscript{20} reported bacterial vaginosis in 5.3% cases, atrophic changes in 2.4% cases and infection caused by trichomonas vaginalis in 1.5% cases which corroborates with the present study.

In the present study, 68% of cases showing atrophic changes are in the age group of 41-60 years. This finding is in concordance with the study of Maheshwari U\textsuperscript{26} et al. in which 65.52% cases showing atrophic changes were in the age group of 41-60 years. Thus it can be concluded that atrophic changes are common in the perimenopausal and menopausal age group due to the decreased levels of oestrogen and progesterone.
Atrophic changes was also seen in few younger females (4%) which may be due to decreased levels of oestrogen in situations like postpartum period.

In the present study, 0.6% cases of ASCUS, 0.13% cases of LSI and 0.07% cases of HSIL were reported which correlates with the study of Mulay K et al.\(^7\) in which 0.64% cases of ASCUS, 0.22 % cases of LSI and 0.16% cases of HSIL were reported. In the study of Patel MM et al.\(^8\) 0.1% cases of LSI and 0.1% cases of HSIL were reported which is in concordance with the present study but the incidence of ASCUS was 4.1% which is higher than the index study. While the incidence of ASCUS, LSI and HSIL were higher in the studies of Verma I et al.\(^9\), Warpe BM et al.\(^10\), Maruf Raza AKM et al.\(^11\) and Ali SS et al.\(^12\). This could probably be due to the smaller sample size in these studies and selective screening of cases. However the present study comprised of non selective screening at a tertiary care centre.

The incidence of squamous cell carcinoma in the present study was found to be 0.27% which is in concordance with the studies of Verma I et al.\(^9\) with 0.8% cases, Patel MM et al.\(^8\) with 0.7% cases, Warpe BM et al.\(^10\) with 0.29% cases and Maruf Raza et al.\(^11\) with 0.7% cases of SCC. While in the study done by Mulay K et al.\(^7\) despite of a huge sample size (6010 cases), the incidence of SCC was 0.06% because this study was done only in the urban population and the rural population was not included which is at higher risk of developing cervical cancer.

**CONCLUSION**

Carcinoma of uterine cervix is the third most common cancer among women worldwide and is the major cause of cancer related morbidity and mortality. The incidence of cervical carcinoma is very high in developing countries owing to lack of proper knowledge and non-existent preventive programmes.

Studies have revealed that the problem of cervical cancer we know by hospital based registries is only the Iceberg. The actual disease burden resides in the rural areas where there is lack of availability of diagnostic facilities, illiteracy, absence of trained personnel, social and cultural taboo of sexually transmitted diseases and lack of good publicly funded screening programmes and more incidence of cervical infections due to poor hygiene.

The value of exfoliative cervical cytology is undisputed today. Most of the studies focus on the application of pap smears to detect precancerous and cancerous lesions. It can be concluded in the present study that the main bulk is of inflammatory lesions and pap smears are no less significant in detecting inflammatory lesions and etiology wherever possible which can help the clinician in drawing up the line of management and prevent further dysplasias.

In any case, since prevention is always better than cure, it is better to take preventive measures than face the consequences and hence more of screening programmes should be conducted to detect epithelial lesions in early stages.

Bethesda system used here for reporting provide good view of the patients in which preventive measures can be taken to herald the disease progression. So we can conclude that cervical cytology is a simple and cost effective test and should be established as a routine diagnostic aid.

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