Cytological Pattern of Cervical Papanicolaou Smear in Rajasthan: A Screening and Diagnostic Procedure

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ABSTRACT

BACKGROUND
The most common cancer among females of developing country like India is Cervical cancer and is one of the major causes of deaths among them. Papanicolaou(Pap) smear test is one of the best screening methods to detect early pathological changes in cervix.

OBJECTIVE
This study was conducted to determine the prevalence and clinico-pathological correlation with importance of conventional Pap smears for the diagnosis of inflammatory, premalignant and malignant lesions of the cervix.

MATERIALS AND METHODS
This cross-sectional study was conducted from Nov 2013 to August 2014, on 1200 women of aged between 20-70 years coming for a Pap smear examination in our hospital(tertiary care) in Udaipur, India. After staining with conventional Papanicolaou technique, all cases were classified as per Bethesda nomenclature(2001).

RESULTS
A total of 1200 cases of pap smears were received in our cytology department during this ten months period, 747 (62.2%) abnormal Pap smears, 422 (35.2%) cases were normal 31 (2.6%) cases were inadequate. Out of 747 cases, 628 (52.3%) cases were reported to have inflammatory/reactive changes, whereas abnormality in epithelial cell was reported in 80 (10.7%) cases and 39 (3.3%) cases were atrophic.

CONCLUSION
Pap smear examination is an effective screening and diagnostic procedure for diagnosing cervical abnormalities. Factors such as less awareness, poor hygiene, shyness, and old age could be responsible for abnormal Pap smears which needs special attention in prevention of cervical lesions.

KEYWORDS: Cervical Pattern, Clinico-pathological, Bethesda System, Pap Smear, Rajasthan

INTRODUCTION

The Papanicolaou (Pap) test is a screening test performed using cells from the uterine cervix. This test was introduced by George Papanicolaou as a cervical pathology screening test in 1941.¹ The procedure is very simple, quick and painless. Performed by woman lying on an examination table, the physician inserts a speculum into the vagina of patient to open it and then by inserting a wooden scraper, sample is taken from in and around the cervix. Then the material is placed on a glass slide and rinsed in a liquid fixative to send it to the laboratory for...
examination. The Papanicolaou test is capable of detecting cervical cancer at an early stage which is widely used in developed countries, by which there is a decrease in incidence and mortality of cervical cancer. Unfortunately, some developing countries lack this facility to carry out widespread Pap screening. Pap test can detect 75% endometrial cancers and 90% of cervical cancers.

Cancer Cervix is the commonest malignancy of women in India and second most common cancer in the world as a whole. Worldwide, especially in developing countries, cervical cancer still the major cause of mortality in women which accounts for an estimated 160,000 deaths every year. In developing countries, factors like early marriage, low socio-economic status, poor hygiene and sexually transmitted infections are the associated causes of cervical malignancy where it is the leading cancer in women. National Cancer Registry Programme (NCRP) has reported Cervix cancer as the most important cancer in India, over past two decades. According to NCRP estimated number of cervical cancers during 2007 in India was 90,708.

The Pap test is done by taking samples from the transformation zone, an area where physiologic transformation from columnar endocervical epithelium to squamous (ectocervical) epithelium takes place, where dysplasia and cancer arises.

**OBJECTIVE**

This study was conducted to determine the prevalence and clinico-pathological correlation with the importance of conventional Pap smears for the diagnosis of inflammatory, premalignant and malignant lesions of the cervix.

**MATERIALS AND METHODS**

This cross-sectional study was conducted at Geetanjali Medical College and Hospital (A tertiary care center). A total of 1200 patients age between 20-70 years (Table No.1) were screened in our department from the period of “November 2013 to August 2014”. In our department, we also took the proper history of the patient other than gynaecological history already written on their OPD cards. We asked their menopausal age, socioeconomic status and literacy level, history of early marriage, history of multiparity and sexual partners, history of used oral contraceptives, smoking history and history any of abortions. Only 8% of the subjects had a Pap test within the previous five years while 92% said, never had this test in the past.

<table>
<thead>
<tr>
<th>Age group (in years)</th>
<th>Number of subjects</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-30</td>
<td>362</td>
<td>30.1%</td>
</tr>
<tr>
<td>31-40</td>
<td>503</td>
<td>41.9%</td>
</tr>
<tr>
<td>41-50</td>
<td>219</td>
<td>18.3%</td>
</tr>
<tr>
<td>51-60</td>
<td>74</td>
<td>6.2%</td>
</tr>
<tr>
<td>61-70</td>
<td>42</td>
<td>3.5%</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>1200</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

Table No.1: Distribution of Subjects on the Basis of Age

The chief complaints of these patients were vaginal discharge, hypogastric pain, backache, dyspareunia, postcoital bleeding, dysuria, pruritus, infertility and menstrual irregularities. The material obtained by procedure was quickly smeared on two glass slides and put in the Coplin jar containing 95% ethyl alcohol. Then the smears were stained using the conventional Papanicolaou technique. Under the light microscope, unstained cells cannot be visualised. Cytoplasmic keratinisation was the
RESULTS

A total of 1200 cases of cervical pap smears were received in our cytology department during this ten month study period. Out of 1200 patients, maximum number of patients, i.e. 503 (41.9%) were in the age group of 31-40 years. Then, 362 (30.1%) were in second, 219 (18.3%) in fourth, 74 (6.1%) in fifth and 42 (3.5%) cases were in sixth decade respectively (Table 1).

There were 747 (62.2%) abnormal Pap smears (including abnormality in epithelial cell, reparative/ reactive cellular changes of inflammation, infections and atrophic smear). While 422 (35.2%) cases were normal and 31 (2.6%) cases were unsatisfactory (Table 2). Out of the 747 cases, 628 (52.3%) cases were reported to have inflammatory/reactive changes whereas epithelial cell abnormality were seen in 80 (10.7%) cases (Table 3). In this study Trichomonas vaginalis [Figure 1] infection was the most common specific etiology observed, in 29 cases while Candida infection was seen in 19 patients. There was no other fungal infection seen except candida. HSV infection having cytopathic changes were seen in 12 cases. These characteristic changes were large multinucleated cells with ground glass appearance having eosinophilic nuclear inclusions [Figure 2]. Cells with koilocytic change, suggestive of HPV infection were seen in 14 cases. No dysplastic or atypical features were seen in any of these cases. Atrophic vaginitis were seen in 39 subjects, in the age range of 40 to 70 years (Table 4) and all of them were postmenopausal.

**Table No.2: Distribution of subjects on the basis of cytolodiagnosis**

<table>
<thead>
<tr>
<th>Age-group</th>
<th>ASCUS</th>
<th>ASC-H</th>
<th>LSIL</th>
<th>HSIL</th>
<th>SCC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-30</td>
<td>1</td>
<td>Nil</td>
<td>03</td>
<td>Nil</td>
<td>04</td>
<td>31</td>
</tr>
<tr>
<td>31-40</td>
<td>11</td>
<td>06</td>
<td>06</td>
<td>07</td>
<td>Nil</td>
<td>30</td>
</tr>
<tr>
<td>41-50</td>
<td>08</td>
<td>04</td>
<td>06</td>
<td>11</td>
<td>03</td>
<td>32</td>
</tr>
<tr>
<td>51-60</td>
<td>03</td>
<td>01</td>
<td>02</td>
<td>03</td>
<td>04</td>
<td>13</td>
</tr>
<tr>
<td>61-70</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>01</td>
<td>01</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>11</td>
<td>17</td>
<td>21</td>
<td>08</td>
<td>80</td>
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</table>

**Table No.3: Distribution of cases with epithelial cell abnormalities on age wise**

<table>
<thead>
<tr>
<th>Age-group</th>
<th>Non-specific</th>
<th>Trichomonas</th>
<th>Candida</th>
<th>Atrophic vaginitis</th>
<th>HSV</th>
<th>HPV</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-30</td>
<td>183</td>
<td>08</td>
<td>06</td>
<td>Nil</td>
<td>02</td>
<td>Nil</td>
<td>199</td>
</tr>
<tr>
<td>31-40</td>
<td>234</td>
<td>11</td>
<td>07</td>
<td>Nil</td>
<td>07</td>
<td>07</td>
<td>266</td>
</tr>
<tr>
<td>41-50</td>
<td>89</td>
<td>06</td>
<td>03</td>
<td>04</td>
<td>03</td>
<td>05</td>
<td>110</td>
</tr>
<tr>
<td>51-60</td>
<td>30</td>
<td>03</td>
<td>02</td>
<td>17</td>
<td>Nil</td>
<td>01</td>
<td>53</td>
</tr>
<tr>
<td>61-70</td>
<td>18</td>
<td>01</td>
<td>01</td>
<td>18</td>
<td>Nil</td>
<td>01</td>
<td>39</td>
</tr>
<tr>
<td>Total</td>
<td>554</td>
<td>29</td>
<td>19</td>
<td>39</td>
<td>12</td>
<td>14</td>
<td>667</td>
</tr>
</tbody>
</table>

**Table No.4: Distribution of cases on the basis of inflammatory causes**

<table>
<thead>
<tr>
<th>Age-group</th>
<th>Non-specific</th>
<th>Trichomonas</th>
<th>Candida</th>
<th>Atrophic vaginitis</th>
<th>HSV</th>
<th>HPV</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-30</td>
<td>183</td>
<td>08</td>
<td>06</td>
<td>Nil</td>
<td>02</td>
<td>Nil</td>
<td>199</td>
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<tr>
<td>31-40</td>
<td>234</td>
<td>11</td>
<td>07</td>
<td>Nil</td>
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<td>51-60</td>
<td>30</td>
<td>03</td>
<td>02</td>
<td>17</td>
<td>Nil</td>
<td>01</td>
<td>53</td>
</tr>
<tr>
<td>61-70</td>
<td>18</td>
<td>01</td>
<td>01</td>
<td>18</td>
<td>Nil</td>
<td>01</td>
<td>39</td>
</tr>
<tr>
<td>Total</td>
<td>554</td>
<td>29</td>
<td>19</td>
<td>39</td>
<td>12</td>
<td>14</td>
<td>667</td>
</tr>
</tbody>
</table>
Abnormalities in epithelial cell were seen in 80 cases out of which the diagnosis in 23 cases were ASCUS (Atypical Squamous Cells of Undetermined Significance), 11 ASC-H (Atypical Squamous Cells cannot exclude HSIL) and 17 cases were LSIL (Low-grade Squamous Intraepithelial Lesion) [Figure-3]. While 21 cases were HSIL (High-grade Squamous Intraepithelial Lesion) [Figure-4] and 8 cases were Squamous cell carcinoma (SCC).

Among patients with abnormal Pap findings cervical erosion, cervicitis, vaginitis and cervical hypertrophy were the most common pathological conditions observed. According to symptoms, vaginal discharge was the most common presentation with hypogastric pain and backache were 2nd and 3rd common presentation (Table-5).

Table No.5: Distribution of patients on the basis of symptoms  
(Note:- Many patients had common symptoms)
DISCUSSION

Among various risk factors in these women, age at marriage less than 18 years, high parity, illiteracy leading to poor genital hygiene were observed to be the prominent risk factors associated with morbidity. These risk factors have also been reported as significant association with cancer cervix in few other studies. Low socio-economic status is also related with the neoplastic lesion of the cervix, as the study conducted in California had shown that low-income women were at higher risk of developing cervical cancer. Among the symptoms vaginal discharge, hypogastric pain is the two earliest presentations in the patients with abnormal cervical smears. Awareness to the Pap test was poor in our study population, with only 8% ever had a Pap test in the past five years.

Cytological smears presented with inflammatory lesion (52.3%) were having only neutrophils and bacilli on the slide with no atypia or dysplasia. Infection with candida seen along with a dense neutrophilic infiltrate on a necrotic background. Smears of Trichomonas showed degenerating squamous cells and neutrophil aggregate in the background with the characteristic pear shaped organisms showing slight cyanophilous tinge and faint eccentric nuclei. These were lying scattered or in small clusters. Many of the, Trichomonas infected patients presented clinically with complaints of a foul-smelling vaginal discharge. HSV infection at cervical cytology was observed with the higher incidence in the women of their third trimester of pregnancy. HPV infection showing koilocytosis without atypia, were present in fourteen cases. Schneider et al described the five important criteria i.e. mild koilocytosis, mild dyskeratosis, hyperchromic nuclei, bi and multinucleation and cleared cytoplasm to improve the sensitivity of cytologic diagnosis of HPV infections. HPV types 16 and 18 are the major genotypes in squamous lesions, but in glandular lesions type 18 is relatively more important.

Patients with atypical cervical epithelial lesion, pathologists denote ASCUS as cellular changes that are more marked than that attributable to reactive changes, but these changes are quantitatively or qualitatively insufficient for a definitive diagnosis of SIL.

Seventeen Pap smears revealed LSIL, having sheets or scattered atypical cells on an inflammatory background. Nuclear abnormalities were mostly confined to the mature squamous cells. The enlarged nucleus were at least three times the area of the normal intermediate nuclei, resulting in an increased nuclear/cytoplasmic ratio. There was mild to moderate variation in nuclear size and shape, many times with binucleation and multinucleation. The nucleoli were absent in these cells. Chromatin distribution was regular in the nuclear areas. Cell borders were distinct in LSIL, with well defined, clear cytoplasm. For the diagnosis of LSIL, perinuclear halos and nuclear abnormalities are important.

In HSIL, the nuclear changes were more cleared than in LSIL. The nuclear abnormalities were observed mainly in squamous cells with ‘immature’ lacy and delicate or a dense metaplastic cytoplasm. On occasions, the cytoplasm was ‘mature’ and densely keratinized. Nuclear enlargement with decreased area of cytoplasm resulted in marked increase in the nuclear/cytoplasmic ratio. The cell size of HSIL was smaller than in LSIL. Chromatin was fine or coarsely granular with hyperchromasia, and was more irregular in distribution than in LSIL. Nucleoli were evident in most of the HSIL cells which were not seen.
in LSIL. Two features were more clearer in HSIL cases i.e. irregularities in nuclear membrane and irregular distribution of coarse chromatin in the nuclear areas.

There was marked variation in the shape and size of the cell, with caudate and spindle-shaped cells that often contained dense orangeophilic cytoplasm. There was also marked variation in size and shape of nuclei, with numerous dense opaque nuclear forms. The chromatin, was coarsely granular and irregularly distributed. There were many macro-nucleoli, but these were less common than in non keratinizing SCC. Smears having the above features were diagnosed as SCC with keratinisation. SCC without keratinisation was seen scattered or in syncytium like aggregates and the cells showed almost all the features of HSIL but, in addition, they contained prominent macro-nucleoli and distributed chromatin was markedly irregular, with coarse chromatin clumping. The associated tumor diathesis were consisted of necrotic debris and old blood with macrocytic, multinucleated, fine chromatin, and hyperchromic or condensed macro or micronuclei with karyopyknotic or karyorhexic changes. These features on cytology features were consistent with the criteria described by Koss, Höffken, and Smith et al. 

**REFERENCES**


**CONCLUSION AND SUGGESTION**

From the above study we conclude that more than 50% of Pap Smears examined in the Pathology department of our institution were of abnormal pattern either of inflammatory or any atypical pattern.

As we know that Cervical cancer is the leading malignancies in Indian women. So every women by the age of 21 or within 3 years of sexual activity should undergo The Papanicolaou (Pap) test. This is a cost-effective cancer screening and diagnostic test and is a simple method to detect lesions in cervix at an early stage. There is also poor hygiene, lack of knowledge, awareness, old age and low socio-economic status are the responsible factors for the abnormal Pap smears which need special attention in prevention of cancer activity.
Shekhar H et al: Cytological Pattern of Cervical Papanicolaou Smear in Rajasthan


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