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## Original Research Article

## Cytomorphological study of cervical Pap smear based on the Bethesda system 2014

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## ABSTRACT

**Introduction:** The Papanicolaou (Pap) smear used worldwide for cervical screening was developed by Dr George Papanicolaou in 1940s. The Pap smear is a simple, safe and effective test to detect cervical cancer at an early stage.

**Objectives:** To study the spectrum of lesions in cervical Pap smear based on The Bethesda System (TBS) 2014 and to determine frequency of premalignant and malignant lesions.

**Materials and Methods:** The present prospective study included 200 cases above 20 years of age. The cytological interpretation of smears were made according to TBS 2014. When there was difficulty in differentiating HSIL from other lesions p16<sup>INK4a</sup> immunostaining was done and evaluated according to the criteria proposed by Wentzensen et al.

**Statistical Analysis Used:** Chi – square test was used to see the association between different attributes. Statistical analysis was performed using SPSS version 22. P value <0.05 was considered significant.

**Results:** The most common lesion was NILM with 175 cases (87.5%) followed by 7(3.5%) SCC, 4(2%) HSIL, 2(1%) cases each of ASCUS, AGC-NOS, LSIL and 1(0.5%) case of ASC-H. Among 200, 7(3.5%) cases were unsatisfactory for evaluation. The subjects were not uniformly distributed (P <0.001). The p16<sup>INK4a</sup> marker was used in 6(3%) doubtful cases of HSIL among which 2(33.33%) cases were positive.

**Conclusion:** Cervical Pap smear is a useful technique to detect the inflammatory, premalignant and malignant lesions of cervix which helps the clinician to plan further management.

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## 1. Introduction

The Papanicolaou (Pap) smear used worldwide for cervical screening was developed by Dr. George Papanicolaou in 1940s when he described how vaginal cells could be collected and stained as a means of detecting cytologic abnormalities of uterine cervix. The Pap smear is a simple, safe and effective test to detect cervical cancer at an early stage.<sup>1</sup> Cervical cancer is the commonest cancer cause of death among women in developing countries and it is associated with persistent HPV infection.<sup>2,3</sup> It is estimated that in India 1,26,000 new cases occur each year.<sup>1,4</sup> Pap

smear also has a greater role in diagnosis of inflammatory lesions including the identification of causative organisms and atrophic changes.

Papanicolaou test results are routinely reported according to The Bethesda System (TBS). This was introduced in 1988 and revised in 1991, 2001 and the most recent update was done in 2014. TBS provided the framework necessary for the development of systematic, evidence-based cervical cancer screening and management guidelines.<sup>5,6</sup>

Very few studies have been done on interpretation of cervical cytology according to TBS 2014. Hence this study was undertaken to study the spectrum of lesions in cervical Pap smear based on The Bethesda System 2014 and to

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determine the frequency of premalignant and malignant lesions.

## 2. Materials and Methods

The present prospective study was undertaken for a period of 18 months in the Department of Pathology after obtaining Ethical committee clearance. This study included 200 patients above 20 years of age attending obstetrics and gynaecology (OBG) outpatient department (OPD). Women during postpartum period, during menstruation and women with intravaginal medication were excluded from the study. In this study 200 patients who were willing to participate were enrolled after obtaining written informed consent. Demographic details and brief clinical history were taken.

Pap smears were taken with the help of wooden spatula by placing in the cervix and rotating it through 360°, sampling both ectocervix and endocervix. The material obtained was quickly smeared on a two clean glass slides and immediately fixed in 95% ethanol then the smears were stained by Papanicolaou technique and additional material was kept for immunocytochemical analysis. The smears were interpreted according to The Bethesda System 2014.

When there was difficulty in differentiating 6 doubtful cases of HSIL, p16<sup>INK4a</sup> immunostaining was done. These slides were processed manually by supersensitive polymer HRP detection system using peroxidase-antiperoxidase technique (Biogenex) along with positive control (Squamous cell carcinoma cytology slides). ICC staining was done by mouse monoclonal antihuman p16<sup>INK4a</sup> (G175-405) antibody. Slides were evaluated using microscope at 400x magnification and scored according to semiquantitative scoring system. Basic data was presented in terms of percentage or proportion and diagrams wherever necessary. Chi – square test was used to see the association between different variables.

## 3. Results

The mean age of the 200 participants who were included in the study was 41.4 years ranging from 20 to 75 years. The predominant participants were in the age group of 30-39 with 60 cases(30%) and more than 50% of participants screened were in age group of 20-49 years (72%) (Table 1). The most common presenting complaint was white discharge per vagina (63.5%) followed by intermenstrual bleeding in 18% cases. Postmenopausal bleeding was seen in 8% of the cases among (Table 2). In this study the majority of the cases had normal examination findings. On per speculum and per vaginal examinations, there were no gross pathology in 52% of the cases, whereas cervical growth was found in 2.5% of the cases. (Table 3)

Out of 200 cases, 175 (87.5%) were negative for intraepithelial lesion or malignancy, while 7 cases (3.5%) were unsatisfactory and 18 cases(9%) had abnormal lesions

which are categorised according to The Bethesda system 2014, squamous epithelial abnormality like HSIL was interpreted in 2% of cases and squamous cell carcinoma in 3.5% of cases. 2 cases(1%) were interpreted as atypical glandular cells – not otherwise specific). In the current study 139 cases were inflammatory smears and organisms like *Trichomonas vaginalis* was seen in 9 cases and 11 cases showed shift in flora suggestive of Bacterial vaginosis (Table 5). p16<sup>INK4a</sup> Immunocytochemistry was done on 6 doubtful cases of HSIL. Positive staining was observed in dysplastic cells in one case each of squamous metaplasia/HSIL and Atrophy/HSIL (Table 6).

**Table 1:** Age wise distribution of cases

Age Group (in years)	No. of cases	Percentage (%)
20-29	38	19
30-39	60	30
40-49	46	23
50-59	26	13
60-69	26	13
70-79	04	02
Total	200	100

**Table 2:** Distribution of cases based on clinical presentation in relation to different age groups

Age group (in years)	WDPV	IMB	PMB	MPV
20-29	37	01	-	-
30-39	42	13	-	05
40-49	26	16	01	03
50-59	13	05	05	03
60-69	08	01	07	10
70-79	01	-	03	-
Total	127	36	16	22
Percentage (%)	63.5	18	8	10.5

**Table 3:** Distribution of cases based on per speculum examination findings

Appearance	No. of cases	Percentage (%)
No gross pathology	104	52
Hypertrophied cervix	25	12.5
Cervical erosion	25	12.5
Cervical growth	05	2.5
Uterus prolapse	24	12
Vault growth	01	0.5
Congested cervix	16	08
Total	200	100

## 4. Discussion

This prospective study was done to interpret the cervical Pap smears based on the recently updated reporting system The

**Table 4:** Distribution of cytomorphological features in conventional Pap smears

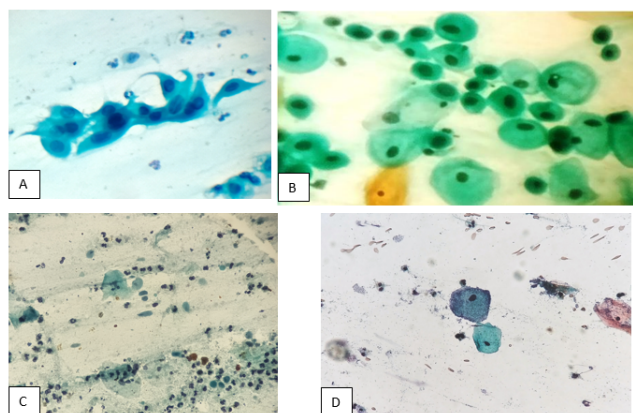
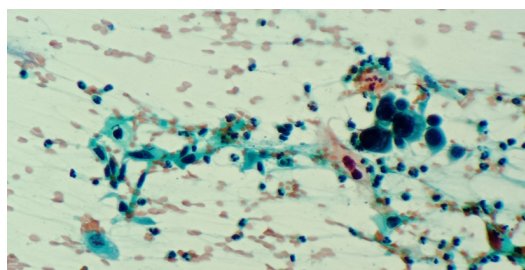
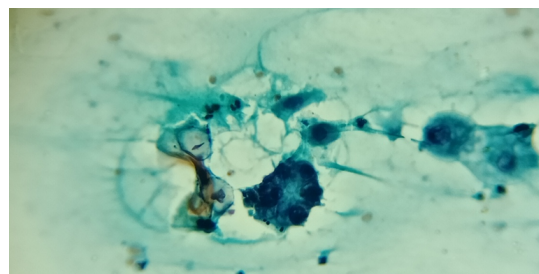
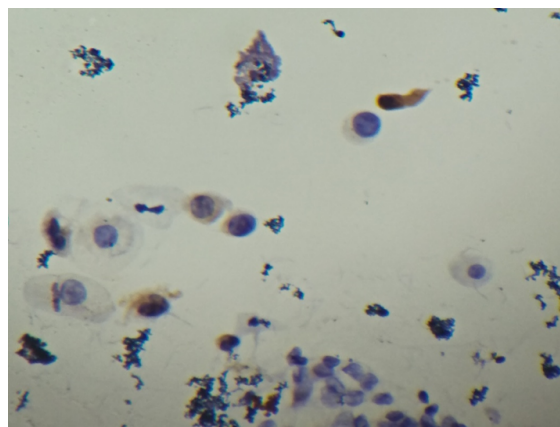
Interpretation	No. of cases	Percentage (%)
Unsatisfactory	07	3.5
NILM	175	87.5
ASC-US	02	01
LSIL	02	01
ASC-H	01	0.5
HSIL	04	02
SCC	07	3.5
AGC-NOS	02	01
Total	200	100

**Table 5:** Distribution of cases interpreted as negative for intraepithelial lesion or malignancy

Interpretation/ Results	No. of cases
Inflammatory smear	139
Atrophy	09
Pregnancy associated changes	03
Lymphocytic cervicitis	02
Trichomonas vaginalis	09
Bacterial vaginosis	11
Leptothrix	02
No pathology	30

**Table 6:** Correlation of cytomorphological features of pap smears with p16<sup>INK4a</sup> results

Cytomorphological features	p16 <sup>INK4a</sup> results		Number of cases
	Positive	Negative	
Squamous metaplasia/HSIL	01	02	03
Atrophy/HSIL	01	02	03
Total	02	04	06

**Fig. 1:** Photomicrograph showing a): Squamous metaplastic cells, b): Atrophic smear; c): Trichomonas vaginalis; d): Clue cell in Bacterial vaginosis (Pap, x400)**Fig. 2:** Photomicrograph showing features of SCC (Pap, x400)**Fig. 3:** Photomicrograph showing features of AGC-NOS (Pap, x400)**Fig. 4:** Photomicrograph showing nucleocytoplasmic positivity of p16<sup>INK4a</sup> in tumor cells (p16<sup>INK4a</sup>, x400)

Bethesda system 2014. In the current study a total of 200 participants who met all the inclusion criteria were studied and p16<sup>INK4a</sup> immunocytochemistry was done on a total of six cases. The median age of 200 participants was 41.4 years with a range of 20 to 75 years. Out of 200 cases majority (30%) were in the age group of 30-39 years. The Pap smear screening should start in the age group of 20-30 years, depending on burden of the disease and the younger age group should be screened for premalignant conditions.<sup>7</sup> In our study only 19% of cases were observed in 20-29 years age group. The cases were not uniformly distributed (P value <0.001). It is important to screen for premalignant conditions in this age group as low grade dysplasia are

mostly asymptomatic and studies have shown that 10% of LSIL cases go for high grade lesion.<sup>2</sup> Hence regular follow up of cases with low grade dysplasia is required. Similar studies were done by Umarani et al,<sup>1</sup> Vaghela et al,<sup>8</sup> Nikumbh et al,<sup>9</sup> Das et al,<sup>10</sup> Patel et al,<sup>11</sup> Suryawanshi et al,<sup>12</sup> Shekhar et al<sup>13</sup> and Rana et al<sup>14</sup> in which predominant cases screened were in 31–40 years age group.

In the present study white discharge per vagina was the commonest presenting complaint most commonly in 30-39 years age group. This may be due to high rates of infectious diseases in reproductive age group. The smears interpreted as epithelial cell abnormalities, with most of the cases as SCC presented with postmenopausal bleed. The cases were not uniformly distributed (P value <0.001). These findings were correlated with other studies done by Nikumbh et al,<sup>9</sup> Das et al,<sup>10</sup> Patel et al,<sup>11</sup> Rajput et al<sup>15</sup> and Verma et al.<sup>16</sup>

It was observed that in our study majority of cases (52%) had no gross pathology on per speculum examination. Visible cervical growth was observed in 2.5% of cases and smears were interpreted as SCC. The cases were not uniformly distributed (P value <0.001). Similar observation was made by Nikumbh et al<sup>9</sup> with 1.3% cases presenting with cervical growth.

In the present study out of 200 participants 3.5% cases were unsatisfactory for evaluation due to the low cellularity from improper technique. Similar observations were made by Umarani et al,<sup>1</sup> Vaghela et al,<sup>8</sup> Rajput et al<sup>15</sup> and Bamanikar et al.<sup>17</sup>

In the present study 87.5% of NILM cases were observed. Smears with non specific inflammatory changes (69.5%) were the most common and seen mainly in women in the reproductive age group and specific causes like trichomonas vaginalis and Gardnerella vaginalis was observed to be affecting 4.5% and 5.5% of cases respectively. The incidence of trichomonas vaginalis was found to be decreased. It may be due to the treatment of patients presenting with white discharge per vagina at the peripheral health centers with metronidazole, which is considered to be a effective treatment against TV.<sup>18</sup> Non-specific inflammatory smears formed the majority of cases in the studies of Umarani et al(97%),<sup>1</sup> Vaghela et al(53%)<sup>8</sup> and Nikumbh et al(91.5%).<sup>9</sup>

We noted epithelial cell abnormalities in 9% cases. Study done by Mahadik et al<sup>3</sup> showed 5.36% cases with epithelial cell abnormality, which is comparable to study done by Nikumbh et al (5.8%).<sup>9</sup> The incidence of epithelial abnormalities in India varies from 1.87 to 5.9%, as stated in a study by Mulay et al.<sup>19</sup> This study showed 88% of all abnormal epithelial lesions in women above 40 years of age, whereas in a study done by Hirachand et al<sup>20</sup> and Ranabhat et al<sup>21</sup> it was found to be 60% and 80% respectively. Another study by Mishra et al<sup>22</sup> has found that 51.5% of squamous intraepithelial lesion cases and 75.3% of carcinoma cases were detected in women above 40 years

of age. Although in many studies abnormal epithelial lesions were found in the age group above 40 years, the screening programme should start in the age group of 20-30 years.<sup>7</sup> So if premalignant lesions are detected at early age it can help in preventing further development of cancer.

Malignancy rate in our study was 3.5%, whereas studies done by Bamanikar et al,<sup>17</sup> Banik et al<sup>23</sup> and Edelman et al<sup>24</sup> the malignancy rate was found to be 0.71%, 0.35%, and 0.2% respectively. The high percentage of SCC was observed in our study. It may be due to the patients presenting late with the disease which could be because of ignorance and lack of knowledge regarding diagnosis and treatment of cervical cancer precursor lesions. The majority of cases with malignancies in the present study were noted in 60-69 years age group. Mahadik et al<sup>3</sup> and Reddy et al<sup>25</sup> found the highest incidence of malignancies in the age group of 51-60 years as 1.93% and 4.6% respectively. In India the peak age for cervical cancer incidence is 55–59 years.<sup>2,26</sup>

Out of 2 cases interpreted as AGC-NOS, one case was diagnosed to be endometrial adenocarcinoma on biopsy and other case was lost for followup. Early endometrial carcinoma detection is difficult as it is not easy to get the representative sample in routine Pap smears. It was comparable with other studies done by Mahadik et al(0.16%),<sup>3</sup> Nikumbh et al(0.40%)<sup>9</sup> and Mulay et al(0.31%).<sup>19</sup> Chhieng et al conducted a 5 year study on the clinical implications of atypical glandular cells of undetermined significance. The AGUS rate was found to be 0.56% among general population in this study, whereas the reported rate of AGUS generally range from 0.17% to 1.83%. In this study majority of postmenopausal patients turned out to have either endometrial adenocarcinoma, endocervical adenocarcinoma or endometrial hyperplasia with atypia and few with benign/reactive process.<sup>27</sup> Hence postmenopausal women should be evaluated further for premalignant/malignant lesions of endometrial/ endocervical origin in case of AGUS.

p16<sup>INK4a</sup> ICC was done on 6 doubtful cases of HSIL out of which 2 cases showed score more than 2 which was considered to be positive based on scoring method used by Wentzensen et al.<sup>28–30</sup>

Cervical cancer has a long latent period which begins as dysplasia also known as cervical intraepithelial neoplasia (CIN), starts at the transformation zone in relation to the squamous metaplasia and reserve cell hyperplasia.<sup>31</sup> The detection of CIN at an early stage is curable. Cervical screening is the most effective approach to prevent the development of cervical cancer. The long latent period and the ability to detect epithelial abnormalities in cervical cytology helps to control cervical cancer by population screening.

## 5. Conclusion

The Pap smear is a simple, safe and effective test which contributes to diagnosis by providing an interpretation along with patient history and examination findings. The Bethesda System 2014 reporting of cervical Pap smear provides uniformity in reporting and conveys relevant information to the clinician. Cervical Pap smear is useful technique to detect and treat the cause for inflammatory conditions. The 9% incidence of epithelial cell abnormalities observed in our study indicates the need to cover the early age groups to detect premalignant lesions. p16<sup>INK4a</sup> helps in differentiating HSIL from benign mimics such as immature squamous metaplastic cells, atrophy and reparative changes. The accurate diagnosis of which helps the clinician to plan further management. Cervical cancer if detected at an early stage can decrease the morbidity and mortality.

## 6. Source of Support

Nil.


## 7. Conflict of Interest

Nil.

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