

# Cytomorphological patterns of cervical Papanicolaou smear abnormalities based on 2014 Bethesda System in North Karnataka region

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## Abstract

**Introduction:** Cervical PAP smears are a cost effective, out-patient procedure to screen patients for cervical pathology. **Objectives:** To utilize cervical PAP smear examination in categorizing lesions according to the 2014 Bethesda System for cervical cytology, to analyse the spectrum of lesions and to evaluate its effectiveness as a screening procedure for detection of epithelial abnormalities in a teaching hospital in North Karnataka. **Methods:** A prospective one year study was carried on all conventional PAP smears received in the Department of Pathology, Navodaya Medical College, Raichur. Reporting was done in accordance with the 2014 Bethesda System for reporting cervical cytology. Correlation was done with the clinical findings. **Results:** A total of 578 cases were included in the study. The most common presenting complaint was abdominal pain (28.5%). The most frequent examination finding was white discharge per vaginum, WDPV (38.9%). 90.8% of PAP smears were categorized as 'Negative for intraepithelial Lesion or Malignancy'. Specific infections were seen in 8.1%, squamous metaplasia in 15.5% and atrophic changes in 3.2%. Epithelial cell abnormalities comprised 9.2% of cases, of which Atypical Squamous Cells of Undetermined Significance (ASC-US) was 3.2%, Low grade Squamous Intraepithelial Lesion (LSIL) was 3.6%, High grade Squamous Intraepithelial Lesion (HSIL) was 1.9%, Squamous Cell Carcinoma (SCC) was 0.2% and Atypical Glandular Cells– Not Otherwise Specified (AGC-NOS) was 0.9%. **Conclusion:** The overall prevalence of epithelial cell abnormalities concurred with studies done in other parts of India and constituted 9.2% of the total smears screened, LSIL being the most common lesion.

**Keywords:** PAP smear, Screening, Bethesda, Epithelial abnormalities

## Introduction

Cancer of the cervix is an increasing health problem and an important cause of mortality in women worldwide. According to the World Cancer statistics, >80% of all the cervical cancer cases are found in developing and low-resource countries, because of a lack of awareness and difficulty in running cytology-based screening programs. Almost nine in ten (87%) cervical cancer deaths occur in less developed regions [1].

Cervical cancer is the fourth most frequent cancer in women with an estimated 570,000 new cases in 2018 representing 6.6% of all female cancers. About 96,922 new cervical cancer cases are diagnosed annually in India and ranks as the 2nd leading cause of female

cancer in India [2]. In India, the peak age for cervical cancer incidence is 55–59 years [3]. Human Papilloma Virus (HPV) infection prevalence is 87.8%–96.67% among women with cervical cancer and 9.9% – 36.8% among women with no cancer or other gynaecological morbidities [3]. Fifty percent of women diagnosed with cervical cancer have never undergone cervical cytology testing and another 10% have not received screening in the five years preceding their diagnosis [4].

In India, unhealthy cervix is a common finding on per speculum examination in gynaecology Out Patient Department (OPD) and it is recommended to do the cervical cytology to detect any epithelial cell abnormality [4]. Early detection and appropriate treatment are possible if robust screening is implemented [5]. There has been a significant reduction

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in mortality from cervical cancer in developed countries, due to widespread screening programs. The pre-invasive stage of cervical cancer lasts for a long period, and only a small proportion of cervical intraepithelial neoplasia (CIN) progresses to an invasive lesion [6]. The appropriate management of CIN can prevent invasive cervical cancer [7]. The risk of developing cervical cancer has been associated with a number of socio-epidemiological factors such as age, parity, religion, socioeconomic status, educational level, and sexual behaviours [8].

Cervical cytology by Papanicolaousmears forms a simple and effective means of screening for pre-malignant lesions of the cervix and also to identify reactive conditions and infections. Due to easy availability, cost effectiveness and reliability, cervical smears became a valuable tool in screening and diagnosing various pathologies of the cervix even at peripheral level in rural places [9].

In this study, an attempt is made to analyse the various findings in conventional PAP smears, categorizing them in accordance with the 2014 Bethesda System for cervical cytology and correlating them with clinical details and biopsy findings, wherever available, for evaluating the efficacy of PAP smears as a screening procedure for neoplastic, pre-neoplastic and non-neoplastic lesions of cervix.

## Materials and Methods

**Setting and type of study:** A prospective study was carried out over a period of one year (2018) on all the

## Results

A total of 578 patients had samples of PAP smear taken due to various complaints in the calendar year 2018. The age ranged from 18 to 85 years (Mean = 38.9 years). Majority of the women belonged to age group between 31-40 years of age. (Figure 1) The mean age for non-neoplastic lesions was 38 years while that for suspected neoplastic lesions (ASCUS and beyond) was 49.8 years. The most common presenting complaint was abdominal pain (28.5%) followed by white discharge per vaginum (WDPV) and abnormal uterine bleeding (AUB). (Table 1) Eighteen patients were asymptomatic and came for routine screening PAP smears. Other rare symptoms included low back pain, itching in perineum, swelling in perineum etc.

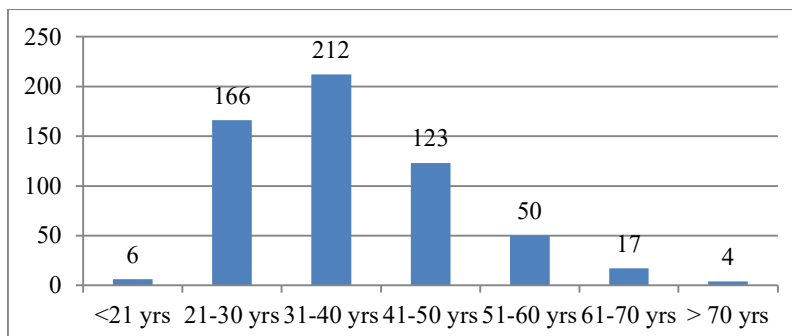


Figure-1: Bar graph representing the age distribution of patients (n=578)

PAP smears received in the Department of Pathology, NavodayaMedical College, Raichur. The patient details were obtained from the requisition forms, case sheets, out-patient slips and enquiry with the patient wherever necessary.

**Sampling Methods:** Sampling was done using cytobrush for endocervical component and Ayre's spatula and both samples were smeared onto two separate slides and fixed by using a spray fixative.

Each case had a minimum of two slides which were then stained by using modified PAP stain and examined by the pathologist.

**Inclusion Criteria:** All PAP smears which were satisfactory for evaluation based on the 2014 Bethesda System for cervical cytology were included in the study.

**Exclusion Criteria:** Patients who had undergone previous surgical procedures on the cervix, repeat PAP smears and those which were taken from the vaginal vault were excluded.

**Ethical Considerations and permissions:** Ethical clearance was obtained from the institutional ethical committee.

**Reporting format:** Reporting was done in accordance with The 2014 Bethesda System for reporting cervical cytology [10,11]. The findings were correlated with clinical details and histopathological findings, wherever available, in cases of suspected epithelial abnormalities.

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Greater proportion of the patients had examination findings of WDPV on per vaginal and per speculum examination comprising 39.8% either as the sole finding or along with others as elaborated in Table 2, Table 3 and Table 4. Cervical erosions were the most common finding associated with WDPV (7.6% of total cases). A normal gynaecological examination was observed in 22.1% of the patients. Other significant findings included UV prolapsed (10%), bulky uterus (8%), cervical hypertrophy (6.1%) and cervical erosions (5.5%). Bleeding on touch was a solitary finding in 10 patients and associated with WDPV in 9 patients. Other less common clinical findings included cervical pigmentation, cervical stenosis, cervical erythema etc. The clinical examination findings were not available in 8 cases.

Out of the 578 patients, 555 (96.03%) had PAP smears which were satisfactory for evaluation. The remaining 23 cases (3.97%) were unsatisfactory for evaluation due to various reasons as mentioned in Table 5. A minimum of approximately 8,000–12,000 well-preserved and well-visualized squamous epithelial cells were considered to categorize the conventional PAP smear as satisfactory for evaluation. Any specimen with abnormal cells [atypical squamous cells of undetermined significance (ASC-US), atypical glandular cells (AGC) etc.] was by definition satisfactory for evaluation [10].

**Table-1: Symptoms of women who had cervical PAP smears taken.**

Symptoms	Number (n=578)	Percentage
Pain Abdomen	165	28.5
White discharge per vaginum	134	23.2
Abnormal Uterine Bleeding	130	22.5
Mass per vaginum	75	13.0
Urinary complaints	24	4.2
Asymptomatic (screening)	18	3.1
Mass per abdomen	12	2.1
Others	12	2.1
Amenorrhoea	6	1.0
Post-coital bleeding	2	0.3
<b>Total</b>	<b>578</b>	<b>100</b>

**Table-2: Examination findings (per vaginum and per speculum) in patients from whom PAP smears were taken.**

Examination Findings	Number (n=578)	Percentage
WDPV	147	25.4
Normal	128	22.1
WDPV + Associated findings	83	14.3
Utero-Vaginal (UV) Prolapse	58	10.0
Bulky uterus	46	8.0
Cervical hypertrophy	35	6.1
Cervical erosion	32	5.5
Others	11	1.9
Bleeding on touch	10	1.7
Cervical polyp	8	1.4
Not available	8	1.4
Bleeding through os	6	1.1
Atrophic cervix	5	0.9
Cervical growth	1	0.2

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**Table-3: Distribution of cases with isolated finding of WDPV on examination.**

WDPV	Number (n=147)	Percentage
Serous	122	83
Curdy white	13	8.8
Foul smelling	8	5.4
Mucoid	2	1.4
Thick	2	1.4

**Table-4: Distribution of cases with other findings associated with WDPV on examination.**

Associated Complaints	Number (n=83)	Percentage
WDPV + Cervical erosions	44	53
WDPV + Bulky uterus	12	14.5
WDPV + Bleeding on touch	9	10.8
WDPV + UV Prolapse	7	8.5
WDPV + Cervical Hypertrophy	6	7.2
WDPV + Polyp	3	3.6
WDPV + Labial swelling	2	2.4

**Table-5: PAP smear sample adequacy.**

	Cases (n=578)	Percentage
Satisfactory for evaluation	555	96.03
Unsatisfactory	23	3.97
• Low cellularity	15 (23)	
• Obscuration by blood	06 (23)	
• Obscuration by fibrous strands	01 (23)	
• Broken slides	01 (23)	

Out of the 555 PAP smears which were found to be satisfactory, 504 were reported as “Negative for intra-epithelial lesion or malignancy” (ie. Non-neoplastic) and 51 were reported as having epithelial cell abnormality. (Table 6) PAP smears reported as inflammatory smears were 304 and this was the most common non-neoplastic finding. Among the non-neoplastic cellular variations, squamous metaplasia was the most often encountered finding comprising 15.5% of the PAP smears followed by reactive cellular atypia associated with inflammation and repair (7.9%) and atrophic changes (3.2%). Among the infectious conditions, 16 cases each of Bacterial Vaginosis and Candidiasis were reported (2.9% each). Trichomonas infection was seen in 13 cases (2.3%). No cases of actinomycosis or viral infection (Herpes Simplex Virus or Cytomegalovirus) were noted in our study. Incidental endometrial cells were seen in a single case of 40 year female presenting with abnormal uterine bleeding (AUB).

**Table-6: PAP smear findings – NILM vs Epithelial Cell Abnormality**

PAP smear report	Number (n=555)	Percentage
<b>NILM</b>	<b>504</b>	<b>90.8</b>
Inflammatory	307	55.3
Bacterial Vaginosis	16	2.9
Candidiasis	16	2.9
Trichomonas Vaginitis	13	2.3
Squamous metaplasia	86	15.5
Atrophy	18	3.2
Keratotic changes	2	0.4
Reactive atypia	44	7.9
Endometrial cells	1	0.2
No other findings	1	0.2
<b>Epithelial Cell Abnormality</b>	<b>51</b>	<b>9.2</b>

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Table-7: Distribution of cases with Epithelial Cell Abnormalities.

PAP Smear report	Number (n=51)	Percentage	Overall percentage (n=555)	Age range (years)	Mean age (years)
ASCUS	18	35.3	3.2	30-68	43.6
LSIL	20	39.2	3.6	31-77	51.5
HSIL	07	13.7	1.3	47-70	56.3
Squamous Cell Carcinoma	01	2.0	0.2	-	50
Atypical Glandular cells -NOS	05	9.8	0.9	38-55	47.6

Table-8: Comparison of PAP smear findings with previous studies.

Diagnosis	Elhakeem et al. <sup>19</sup> 2005	Altaf et al. <sup>20</sup> 2012	Bal et al. <sup>6</sup> 2012	Bukhari et al. <sup>22</sup> 2012	Kulkarni et al. <sup>16</sup> 2013	Verma et al. <sup>14</sup> 2014	Maleki et al. <sup>21</sup> 2015	Nandwani et al. <sup>18</sup> 2016	Sachan et al. <sup>13</sup> 2018	Present study 2019
Total cases (n)	2100	7297	300	1000	350	125	4274	5813	1544	578
NILM	-	-	91%	-	83.7%	68.8%	-	86.9%	48.8%	90.8%
Inflammatory	-	-	71.3%	-	72.0%	60.8%	89.4%	75.0%	42.7%	55.3%
Organisms	-	-	3%	-	1.7%	4.8%	2.4%	6.9%	-	8.1%
Squamous metaplasia	-	-	-	-	25.7%	-	4.0%	-	-	15.5%
Reactive cellular changes	-	-	-	-	-	-	0.2%	64.5%	-	7.9%
Atrophy	-	-	-	-	1.4%	3.2%	-	3.5%	-	3.2%
Epithelial Abnormality	7.9%	17.3%	4.7%	10.2%	12.3%	13.6%	4.0%	13.2%	8.5%	9.2%
ASCUS	2.7%	9.3%	0.3%	0.01%	0%	4.8%	1.9%	3.6%	2.9%	3.2%
ASC-H	0.2%	0.8%	0%	0%	0%	0%	0.9%	0%	0%	0%
LSIL	1.3%	2.7%	2.7%	4.7%	7.4%	5.6%	1%	2.6%	5.1%	3.6%
HSIL	0.7%	0.9%	0.7%	2.2%	4.3%	0.8%	0.1%	2.8%	0.5%	1.3%
SCC	0.3%	0.06%	1%	1.4%	0.6%	0.8%	0%	3.5%	0%	0.2%
AGC - NOS	2.6%	3.2%	0	0.4%	0.9%	0.8%	0.1%	0.4%	0%	0.9%
Adenocarcinoma	0%	0%	0.3%	0.6%	0%	0.8%	-	0.2%	0%	0%

Note: NILM– Negative for intraepithelial lesion or malignancy; ASCUS– Atypical Squamous Cells of Undetermined Significance; ASC-H– Atypical Squamous Cells cannot rule out HSIL; LSIL– Low grade Squamous Intraepithelial Lesion; HSIL- High grade Squamous Intraepithelial Lesion; SCC- Squamous Cell Carcinoma; AGC– Atypical Glandular Cells– NOS.

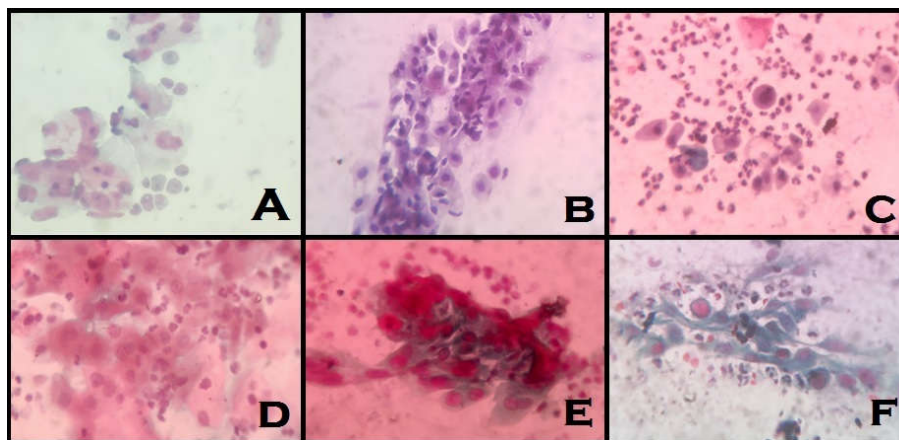


Figure-2: Microscopic images of various PAP smear findings: A – Trichomonas vaginalis trophozoites; B– Squamous metaplasia; C - ASCUS; D – LSIL; E – HSIL; F – SCC

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Among the cases with epithelial cell abnormalities, low-grade squamous intraepithelial lesion (LSIL) was the most common category comprising 20 of 51 cases followed closely by ASC-US comprising 18 of 51 cases. (Table 5) There were 7 cases of High grade Squamous Intraepithelial Lesion (HSIL) and one case of squamous cell carcinoma (SCC). Atypical glandular cells– Not Otherwise Specified (AGC-NOS) was the diagnosis furnished in 5 of 51 cases. No cases of endocervical adenocarcinoma in situ or frank adenocarcinoma were observed during the study period. The mean age of patients with PAP smears reported as ASCUS was 43.6 years being at least a decade lower than those reported as HSIL (Table 7).

**Discussion**

Cervical cancer is on the declining trend in India according to the population-based registries; yet, it continues to be a major public health problem for women in India [3]. Cervical cancer is the third largest cause of cancer mortality in India accounting for nearly 10% of all cancer-related deaths in the country [12] most cases of cervical cancer in India are diagnosed at later and more serious stages which will reduce the survival rate of women with cervical cancer. The prime reason for late stage diagnosis of disease is a lack of awareness about screening and preventive methods of cervical cancer. Screening would not only help to evaluate women without any obvious presenting symptoms but detect pre-malignant lesions of the cervix when are still amenable to curative therapy. PAP smears using standardized reporting criteria [10,11] (Bethesda System 2014) would greatly aid in identifying precursor lesions at an early stage and directing appropriate management.

The mean age of the women included in the study was 38.9 years. Majority of the women belonged to age group between 31-40 years of age. The mean age of women with epithelial abnormalities detected in their PAP smear was 49.8 years as opposed to those without epithelial abnormalities which was 38 years. This correlates with the studies of Verma et al. and Sachan et al [13,14].

As per the recommendations of American Cancer Society, cervical cancer screening should begin at age 21 years. For women 21–29 years of age, screening with cytology alone every 3 years is recommended. Women aged between 30–65 years should be screened with cytology and HPV testing (“cotesting”) every 5 years (preferred) or cytology alone every 3 years (acceptable) [15].

The most frequent presenting complaints in this study was abdominal pain (28.5%) followed by WDPV (23.2%) and AUB (22.5%). However, the most common clinical finding on per vaginal and per speculum examination was WDPV (43.08%) followed by normal findings in 22.1% of the cases. This correlates well with other studies conducted previously [4,13,14,16].

Among the smears obtained, 96.03% were found satisfactory for evaluation and 3.97% were unsatisfactory due to various reasons as mentioned in Table 3. This correlates with studies conducted by Bal et al [5]. A slightly higher percentage of unsatisfactory smears was observed in studies conducted by Vaghela et al. (4.8%), Sachan et al (6.42%) and Nandwani et al. (5.73%). [13,17,18]. The marginally lower rate of unsatisfactory smears could be due to better coordination between Obstetrics & Gynaecology and cytopathology departments in our institution and mandatory screening by the pathologist of all the PAP smears rather than by technologists.

The distribution of various PAP smear findings in comparison to other previous studies is displayed in Table 6.

Among the various findings, the category “Negative for intraepithelial lesion or malignancy(NILM)” constituted the most common with 90.8% cases.

This correlates with most of the previous studies except Sachan et al [13]. Who classified the cases separately as NILM and inflammatory whereas the other studies included inflammatory smears as a sub-category of NILM.

Micro-organisms encountered in PAP smears (Trichomonas, Candida, Gardenella etc.) comprised around 8% of the smears in the present study and this fact correlated well with the study conducted by Nandwaniet al [18]. The incidence of micro-organisms was less in other studies (Table 8) and this could be due to a more vigorous search for epithelial abnormality rather than presence of micro-organisms presence of which could easily be overlooked.

The occurrence of atrophic features in PAP smears comprising a predominance of parabasal cells was noted in 3.2% of the smears in the present study and this correlated well with other studies [14-18] Squamous metaplasia was seen as an additional finding in 15.5% of the cases. However, this finding was not adequately reported in many previous studies as it was an optional non-neoplastic finding [10].

The occurrence of epithelial cell abnormalities varied from 4% to 17.3% in various studies (Table 8). In the present study, the occurrence of epithelial cell abnormality was 9.2%. The differences may be due to different geographical areas and ethnicities as the studies were done in different parts of India and the world. LSIL was the most common lesion among the epithelial abnormalities in the present study contributing to 3.6% of the cases followed by ASCUS (3.2%). This fact correlated well with many of the Indian studies [5,13,14,16]. However, the studies conducted in other countries [19-21] and some Indian studies [18] showed greater occurrence of AS-CUS as compared to LSIL. ASC-H was not encountered in our study and probably contributes to a very negligible proportion (0.2-0.9%) of cases as observed in previous studies [19-21] with a larger sample size.

The mean age for non-neoplastic lesions was 38 years while that for epithelial cell abnormality (ASCUS and beyond) was 49.8 years. This correlates well with study conducted by Sachin et al [13] where the mean age for epithelial cell abnormality was 49 years. The mean age for HSIL category was approximately 5 years greater than the LSIL category in our study.

The LSIL to HSIL ratio was near equal to 2 or greater than 2 in most of the studies. Our study showed an LSIL to HSIL ratio of 2.77, asserting that LSIL is more than twice as commonly encountered as HSIL in PAP smears. This fact also correlates well with most other studies. (Table 8) Nandwani et al [18], however, reported an LSIL to HSIL ratio lesser than 1 in their study and the proportion of squamous cell carcinoma cases was more than the HSIL cases. The reason could be that this study was conducted in a tertiary care setup receiving greater proportion of referred cases from the periphery.

One case of squamous cell carcinoma (SCC) was reported in our study contributing to 0.2%, correlating with studies done by other authors [16,19,20]. A greater proportion of SCC in other studies [18, 22] may be due to a greater sample size and the study institute being a referral centre for malignancies. No case of adenocarcinoma was encountered in our study. This also correlates with other studies (Table 8) and may be due to a smaller sample size and a relatively lower occurrence of adenocarcinoma of cervix as compared to SCC.

Atypical glandular cells were seen to a lesser extent in our study comprising around 0.9%, which is consistent with other studies detailed in Table 6. In studies conducted by Elhakeem et al [19] and Altaf et al [20],

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the proportion of atypical glandular cells was higher. This could be attributed to the increased sample size and probably increased prevalence of endocervical lesions in these areas as both these studies were conducted in different provinces of Saudi Arabia.

We observed that the PAP smear is a cost effective, simple and reliable method for screening women presenting to OBG department with complaints related to the genitor-urinary tract. Though a majority of the cases (90.8%) were reported as Negative for Intraepithelial Lesion or Malignancy, the remaining cases were advised regular follow-up with repeat PAP smears or colposcopic biopsy to evaluate them further. Nearly 5% of the examined cases had a Squamous Intraepithelial Lesion (LSIL or HSIL) which mandated a biopsy.

The limitations of this study were a smaller sample size (n=578) and a study duration of 1 year. HPV testing which is an important adjunctive method for screening was not utilized in this study due to the cost constraints and availability issues. Histopathological correlation could not be done satisfactorily as many of the cases diagnosed with epithelial cell abnormalities were referred to specialist centres for follow up. Of the other cases, in which either a cervical biopsy or hysterectomy was done in our institute, two cases of HSIL had moderate and mild cervical dysplasia respectively and a case of SCC was confirmed on histopathological examination.

### Conclusion

Our study highlights conventional PAP smears with 2014 Bethesda System of reporting as an important screening tool for cervical lesions especially in the under-resourced countries where facilities for HPV testing and liquid based cytology techniques are of limited availability. Cost incurred by the patient also limits utility of more sophisticated screening techniques. In such situations, conventional PAP smears play an important role in filtering out a greater proportion of patients with a non-neoplastic cytological findings and identifying patients with epithelial abnormalities so that appropriate management can be instituted at the earliest. In addition, specific infections (trichomonas, candida etc.) can also be identified by optimal examination of PAP smears which help reduce patient morbidity due to these conditions.

### Contributions of Authors

- **Ashraf A. Zubair**– Played a major role is designing the study, collecting data, organising data and preparing the manuscript.

- **Shruti Kulkarni**– Contributed to organising the data, comparisons with other studies and preparing manuscript.
- **M. Preethi**– Contributed to data compilation and organisation.

#### What this study adds to the existing knowledge?

Systematic reporting of cervical PAP smears based on the 2014 Bethesda System aids in effective screening of patients for epithelial abnormalities, provides uniform terminology for clinicians and pathologists and caters to appropriate and better patient management to reduce mortality and morbidity.

**Findings:** Nil; **Conflict of Interest:** None initiated

**Permission from IRB:** Yes

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