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Original Article

Pathology Section

Cervical Cytological Abnormalities in Symptomatic Women: A Retrospective Cross-sectional Analysis of 945 Pap Smears at a Rural Tertiary Care Centre in Puducherry, India

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ABSTRACT

Introduction: Cancer of the uterine cervix remains a leading cause of mortality among rural Indian women, due to limited screening access and health illiteracy. Rural regions face a stable, high burden of disease, which is often complicated by a high prevalence of reproductive tract infections.

Aim: To delineate the cytological spectrum of conventional Papanicolaou (Pap) smears in a rural tertiary care centre in Southern India.

Materials and Methods: In this retrospective cross-sectional study, 945 conventional pap smears (between 01 April 2024-31 December 2025) performed in a rural tertiary care hospital at Pondicherry, India were analysed using the 2014 the Bethesda System for reporting cervical cytology. Demographic data, symptoms, clinical findings, adequacy of smears, Negative for Intraepithelial Lesion/Malignancy (NILM), Epithelial Cell Abnormality (ECA), and glandular cells were studied. Statistical Package for the Social Sciences (SPSS) version 23.0 for Windows was used for statistical analysis. Associations between cytological findings such as inflammation, NILM and infections were obtained using Chi-square and Fisher's exact tests.

Results: This cohort comprised 945 women with a mean age of 44.5±11.0 years (reproductive age group: 36.7±6.6 years; postmenopausal: 53.1±8.4 years). Specimen adequacy was 85.8%. About 907 (96%) were NILM, women in their reproductive years had a significantly higher infectious burden of Bacterial Vaginosis (BV) 78 (15%), *Candida* 47 (9.1%), and *Trichomonas vaginalis* 20 (3.9%). Postmenopausal women showed higher rates of reactive cellular changes and atrophic changes (p-value=0.03). The ECA rate was 4%, with an Atypical Squamous Cells of Undetermined Significance (ASC-US)/ Squamous Intraepithelial Lesion (SIL) ratio of 2.1:1. One invasive Squamous Cell Carcinoma (SCC) was identified in the postmenopausal group.

Conclusion: This study revealed a predominance of infectious lesions among NILM smears, in which inflammatory atypia may complicate the diagnosis. The persistence of high-grade lesions and SCC in postmenopausal women underlines the importance of continued screening beyond the reproductive years in rural settings.

Keywords: Bethesda System 2014, Cervical smear, Infections, Negative for intraepithelial malignancy, Papanicolaou test, Screening

INTRODUCTION

Cancer as a cause of premature death occupies either the 3rd or 4th position in 23 countries that includes the Indian subcontinent [1]. In developing countries like India, cervical cancer is the second most prevalent cancer among women [1]. Worldwide, the, aetiology of cervical cancer is due to Human Papilloma Virus (HPV) infection mainly with HPV types 16 and 18 [2]. About 90% of deaths due to carcinoma cervix occur in Low- and Middle-Income Countries (LMICs) [3]. This burden of cervical cancer is disproportionately borne by rural women who remain marginalised from both screening infrastructure and curative services available to their urban counterparts [4]. The urban incidence is reducing while the rural incidence remains stable [5]. Pap smear remains the most widely implemented, validated, and cost-effective primary cervical cancer screening tool [6]. In countries with established national programs, organised Pap smear screening has reduced cervical cancer incidence by 60-80% [5]. In resource-limited settings such as LMICs, the Pap smear remains the pragmatic first-line screening intervention.

The National Family Health Survey-5 (NFHS-5, 2019-21) reported that only 1.9% of people in India had undergone cervical cancer screening, with the highest rate reported in Tamil Nadu (9.8%) [7]. The social stigma surrounding pelvic examinations, lack of qualified or trained female health workers to collect smears, and limited health literacy among rural women regarding cervical cancer aetiology may partly contribute to the dismal percentages [8]. The Pap smear also provides a window into the microbiological environment of the lower female genital tract. Patterns identifiable on The Bethesda System 2014 (TBS, 2014) aid in the diagnosis of BV, *Candida* species, and *Trichomonas vaginalis* [9]. In India, the majority of Pap smear studies focus on the reporting of ECAs. Less attention has been paid to the categorisation of infective NILM subcategories [10]. Most studies on Pap smear cytopathology have been published from urban tertiary hospitals, medical colleges in metropolitan centres, and relatively, few studies from rural centres [11-13]. The TBS 2014 provides a structured, reproducible framework for Pap smear reporting. Specimen adequacy, general categorisation (NILM versus ECA), and subcategorisation of both negative and abnormal findings are assessed in the reporting system [9]. The present study aimed to describe the cytological spectrum of conventional Pap smears in women attending a rural tertiary care centre in Southern India, using the 2014 Bethesda System for reporting. The objective was to determine the differences in symptomatic postmenopausal and reproductive-age group women with regard to Pap smears.

MATERIALS AND METHODS

This was a retrospective cross-sectional descriptive study conducted in the Department of Pathology at a rural tertiary care medical college hospital in Pondicherry, Southern India (IEC/C-P/1/2026). Since the study was retrospective, individual patient consent was waived.

Inclusion criteria: The study included all symptomatic women ≥ 18 years attending Department of Gynaecology who had a conventional Pap smear between 01 April 2024 and 31 December 2025.

Exclusion criteria: Women with clinically evident cervical carcinoma prior to smear collection, and post-hysterectomy patients were excluded from the study.

Study Procedure

Conventional cervical smears were collected by trained gynaecologists using an Ayre's spatula and cytobrush. Smears were immediately fixed in 95% ethyl alcohol and stained with the standard Pap staining technique. The first author reviewed the archived slides. The demographic data (age, parity), symptoms {Abnormal Uterine Bleeding (AUB), Post-Coital Bleeding (PCB), pain, leucorrhoea}, and signs (bleeding on touch, cervical erosion, cervical ulcer and growth) were recorded from the case records. Cytopathological variables were based upon the TBS 2014 and were classified under four headings: (1) Specimen adequacy- each smear was assessed for adequacy and categorised as either satisfactory or unsatisfactory for evaluation; presence of obscuring factors such as haemorrhage, drying artefacts and presence of Transformation Zone (TZ) was noted; (2) Smears were then classified into NILM or ECA; (3) NILM was further subcategorised to identify inflammatory changes, infective organisms, and presence of endometrial cells; and (4) Smears suggestive of ECAs were further classified into ASC-US, Atypical Squamous Cells- cannot exclude High-Grade Squamous Intraepithelial Lesion (HSIL) (ASC-H), Low-grade Squamous Intraepithelial Lesion (LSIL), HSIL, SCC, Atypical Glandular Cells (AGC), and Adenocarcinoma [9]. The entire study group was divided into two (postmenopausal and reproductive age group, based on age onset of menopause) and the findings were compared between the two groups.

STATISTICAL ANALYSIS

The data was compiled and analysed using IBM SPSS Statistics Version 23.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were reported using frequencies and percentages. Relationships between categorical variables were assessed using Chi-square test (or Fisher's exact test as applicable). Comparisons were performed between postmenopausal women and women of reproductive age. Correlation was performed using Spearman rank correlation test. A p-value <0.05 was defined as statistically significant.

RESULTS

The study cohort comprised 945 women. Multiparity (95.2% vs 98.6%) was near-universal in both groups. Leucorrhoea (312/945; 33%) was the dominant presenting symptom overall but was significantly more prevalent in the reproductive age group (42.3% vs 21.4%). AUB showed a borderline significant difference between the two groups. PCB was recorded exclusively in the reproductive age group (p-value=0.04). On per-speculum examination, cervical erosion was significantly more frequent in reproductive-age women (p-value=0.005). Cervical ulceration, cervical growth, and bleeding on touch were not statistically significant between the two groups [Table/Fig-1].

Among the total, 811 smears (85.8%) were satisfactory [Table/Fig-2]. Unsatisfactory smears were attributable mainly due to drying artefacts and haemorrhage. The TZ component was significantly more prominent in reproductive-age smears. Active inflammatory changes (moderate and dense) were significantly present in the reproductive age group. Reactive cellular changes (35.6% vs 26.6%) and atrophic changes (13.2% vs 6.4%) were significantly more common in the postmenopausal group [Table/Fig-2]. All three identified infective organisms were significantly more prevalent in the reproductive age group [Table/Fig-2,3a,b]. ECAs were identified in 14 postmenopausal women including one SCC. Among the ECAs, ASC-US was the most frequent finding. HSIL and LSIL were seen only in three cases each [Table/Fig-3c,d]. Among glandular abnormalities, AGC were identified in nine cases, giving a total of 38 ECAs (ASC-US, ASC-H, HSIL, LSIL, SCC and AGC).

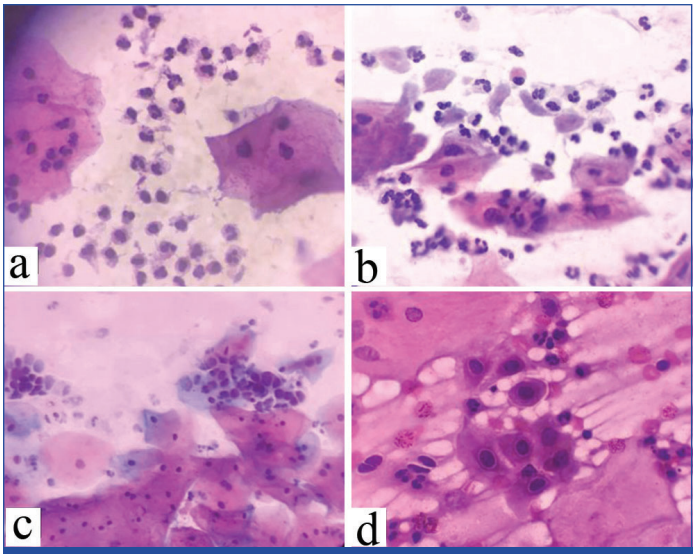
The BV was highest in the 20-29 years age group and showed a linear decline over the next decades [Table/Fig-4]. The linear association between BV infection and age yielded a weak negative correlation (Spearman correlation coefficient of -0.144; p-value <0.001). NILM showed no significant difference in rate between the two groups (p-value=0.81).

Variables	Postmenopausal age group n (%) n=425	Reproductive age group n (%) n=520	p-values
Age, mean (years) (M±SD)	53.1±8.4	36.7±6.6	**0.003**
Multiparous (n)	419 (98.6)	495 (95.2)	**0.005**
Leucorrhoea (n)	91 (21.4)	220 (42.3)	**<0.001**
Abnormal uterine bleed (n)	77 (18.1)	70 (13.5)	**0.05**
Post-Coital Bleeding (PCB) (n)	0	5 (1)	**0.04**
Lower abdominal pain (n)	70 (16.5)	123 (23.7)	**0.06**
Cervical erosion (n)	30 (7.1)	66 (12.7)	**0.005**
Cervix ulcer (n)	5 (1.2)	5 (1)	0.75
Cervix growth (n)	4 (0.9)	6 (1.2)	0.74
Bleeding on touch (n)	10 (2.4)	14 (2.7)	0.73

[Table/Fig-1]: Symptoms and signs in the patient cohort.
*Comparison was made using Chi-square test

Variables	Postmeno-pausal n (%)	Reproductive n (%)	p-values
Specimen adequacy			
Satisfactory (n)	359 (84.5)	452 (87)	0.25
Drying artifact (n)	21 (5)	23 (4.4)	0.71
Haemorrhage (n)	14 (3.3)	14 (2.7)	0.59
Transformation component (n)	174 (40.9)	260 (50)	**0.005**
Inflammation			
Mild (n)	88 (20.7)	109 (21)	**0.02**
Moderate (n)	25 (5.9)	55 (10.6)	
Dense (n)	33 (7.8)	52 (10)	
Abnormality in glandular cells NOS (n)	3 (0.7)	6 (1.1)	0.06
NILM (n)	408 (96)	499 (96)	0.81
ASC-US (n)	6 (1.4)	7 (1.2)	
ASC-H (n)	4 (0.9)	5 (1)	
SCC (n)	1 (0.2)	0	
LSIL (n)	2 (0.5)	1 (0.2)	
HSIL (n)	1 (0.2)	2 (0.4)	
Reactive cellular changes (n)	151 (35.6)	138 (26.6)	**0.03**
Atrophy (n)	56 (13.2)	33 (6.4)	**0.03**
Endometrial cells (n)	10 (2.4)	4 (0.8)	**0.04**
Abnormality in squamous cells (n)	17 (4)	13 (2.5)	0.19
Infections			
Candida (n)	21 (4.9)	47 (9.1)	**0.01**
Bacterial Vaginosis (BV) (n)	35 (8.2)	78 (15)	**0.001**
Trichomonas vaginalis (n)	7 (1.6)	20 (3.9)	**0.04**

[Table/Fig-2]: Comparison of cytopathological variables between the two groups.
*Comparison was made using Chi-square test; NILM: Negative for intraepithelial lesion or malignancy; ASC-US: Atypical squamous cells of undetermined significance; ASC-H: Atypical squamous cells-cannot exclude HSIL: LSIL: Low-grade Squamous intraepithelial lesion; HSIL: High-grade squamous intraepithelial lesion; SCC: Squamous cell carcinoma



[Table/Fig-3]: a) Shows superficial cells, acute inflammatory cells and eosinophilic budding fungal organisms, morphologically consistent with *Candida* species (Pap, 40x); b) Shows superficial squamous cells, acute inflammatory cells, and pear shaped cyanophilic *Trichomonas vaginalis* organisms (Pap, 40x); c) Shows high-grade squamous intraepithelial lesion: cluster of pleomorphic squamous cells having high nuclear cytoplasmic ratio, with hyperchromatic nucleus, irregular nuclear membrane, and fine chromatin (Pap, 40x); d) Shows low grade squamous intra epithelial lesion: superficial squamous cells with enlarged nucleus, mild increase in nuclear cytoplasmic ratio, slightly irregular nuclear membrane, coarse chromatin with koilocytosis (Pap, 40x).

Age groups (years)	Total (n)	*Candida* species (n)	Bacterial Vaginosis (BV) (n)	*Trichomonas* (n)
20-29	84	7 (8.3%)	18 (21.4%)	5 (6.0%)
30-39	238	23 (9.7%)	38 (16.0%)	10 (4.2%)
40-49	359	20 (5.6%)	41 (11.4%)	9 (2.5%)
50-59	176	14 (8.0%)	15 (8.5%)	1 (0.6%)
60-69	59	2 (3.4%)	0	2 (3.4%)
70-79	27	2 (7.4%)	1 (3.7%)	0
80-89	2	0	0	0
Total	945	68 (7.2%)	113 (12%)	27 (2.9%)

[Table/Fig-4]: Age-stratified prevalence of infection.

could reflect the heightened state of cervical and vaginal mucosal response in symptomatic women. The increased responsiveness may be due to an ongoing infection hormonal fluctuations or microtrauma all of which affects the cytological characteristics in the Pap smear. The current study was remarkable with a lower inflammatory and ECA burden (4%) with a higher percentage of infectious organisms. Infections were not focussed upon in some studies [15-19] [Table/Fig-5].

The postmenopausal group in this study was dominated cytologically by atrophic changes (9.8% of total NILM). The nuclear enlargement and irregular chromatin distribution of parabasal cells in smears with an atrophic background can mimic HSIL or even SCC [9]. The overall ECA rate of 4% was similar to a study from Himachal Pradesh but much lower than what has been reported in other series [10,11]. The ASC-US/SIL ratio was 2.1:1 (13:6) in the current study which was within the benchmark of ≤3:1, confirming appropriate diagnostic calibration [20]. The higher occurrence of ASC-US (n=13, 1.4%) compared to definitive SIL categories indicates the reactive-inflammatory burden of the study cohort, where inflammatory atypia may cross the diagnostic threshold into atypical categories. This finding underlines the importance of reflex HPV testing in these individuals or repeat cytology testing, both of which could not be addressed in this retrospective study [21]. The detection of three cases each of LSIL and HSIL, respectively, along with one case of invasive SCC in the postmenopausal group, emphasises the continued risk of cervical neoplasia in older women; thus, the need for regular screening in postmenopausal women, especially in rural settings where the screening coverage maybe less than optimal. Worldwide, cervical cancer incidence peaks in the sixth decade, and a considerable proportion of cases occur in women who have never been screened or were screened inadequately during their reproductive years [5]. There were nine cases of AGC in the current study. This warrants particular attention, as glandular abnormalities may carry a higher risk of significant underlying pathology [9].

The significantly higher prevalence of the TZ component in the reproductive-age group when compared to postmenopausal women (50% vs 40.9%, p-value=0.005) reflects the physiological regression of

Authors, year	Centre	Study population; sample size	Adequacy	NILM/ ECA	Inflammation/infection
Chakma R et al., 2023 [15]	Karnataka	Rural; Prospective, 42 Housekeeping women (21-65y)	Bethesda 2014; adequacy-no data	ASCUS-1(2.27%)	Inflammatory 21 (47.7%) Infection 11 (25%)
Bansal M et al., 2021 [16]	Uttar Pradesh	Urban; Retrospective, 450 women	Bethesda 2014; Unsatisfactory 8(1.7%)	NILM 411 (91.3%) ECA 31 (6.9%) SCC 1 (0.2%)	Inflammatory 357 (79.3) Infection 53 (11.7%)
Agale SV et al., 2025 [17]	Maharashtra	Rural tertiary care; Retrospective, 538 women	Bethesda 2014; Unsatisfactory 11 (2.04%)	ECA 34 (6.32%) NILM 493 (91.6%)	Inflammatory 287 (53.5%) Infections 106 (19.7)
Bhutia TW et al., 2021 [18]	Sikkim	Urban multispecialty hospital; Retrospective, 1256 women	Bethesda III 2001; Unsatisfactory 70 (5.57%)	NILM 619 (49.3%) ECA 567 (45.1%)	Inflammatory 1104 (87.8%)
Chinmayee D et al., 2024 [19]	Karnataka	Rural, retrospective; 605 women	Bethesda 2014	NILM 94.8% ECA 33 (6.05%)	-
Vijayakumar S and Randive R, 2026	Pondicherry	Rural, retrospective; 945 women	Bethesda 2014; Unsatisfactory 14.2%	NILM 907 (96%) ECA 38 (4%)	Inflammatory 362 (38.3%) Infections 208 (22%)

[Table/Fig-5]: Comparison with recent studies from India [15-19].

DISCUSSION

This study of 945 Pap smears from a rural tertiary care centre in Southern India confirmed a pattern of infection-dominant NILM cytology (n=907, 96%). Smears were satisfactory in 85.8%, which was lesser when compared to reported rates of 90-98% in Indian cytology literature [14]. Symptomatic women as in this study can present with leucorrhoea, contact bleeding, or AUB, all of which may compromise smear quality through the presence of excess inflammatory exudate or erythrocytes. Non-infective NILM encompasses reactive changes and atrophic changes. In the present study, the purely non-infective reactive-inflammatory NILM subcategory constituted 71.9% of NILM. These observed findings the squamocolumnar junction with advancing age. The TZ component is critical for adequate cervical cancer screening, since most of the precancerous lesions arise at the squamocolumnar junction [20].

The infection burden documented in this study, particularly that of BV (12%) and *Candida* (7.2%), may suggest the limited access to treatment of sexually transmitted infections, inadequate partner notification, and possibly, a weak integration of cervical cancer screening with reproductive health services. The infective subcategories of NILM comprised BV, *Candida*, and *Trichomonas*, and these frequently co-existed with the non infective reactive patterns of the smears. Non infective reactive changes possess the potential to obscure the underlying pathology in two ways: cellular atypia

may mimic low grade abnormalities and yield false-positive results. Alternatively, underlying dysplastic or premalignant conditions may be masked leading to false-negative interpretation. For example, the heavy inflammatory exudate seen in cases of BV and Trichomonas vaginalis infection can obscure nuclear detail making it challenging to interpret ASC-US [9]. In such cases, appropriate treatment for the underlying infection followed by a repeat Pap smear is needed but may present with practical difficulties in rural settings.

This high infection burden reported in this study also supports the rationale for HPV vaccination programs which targets adolescent girls before age 15 years. This primary prevention strategy could dramatically reduce the future incidence of cervical cancer [21]. The Government of India had introduced the HPV vaccination as part of the Universal Immunisation Program in 2023 and targeted girls aged 9-14 years, which is a critical step forward in the prevention of cervical cancer [22]. BV and *Candida* infections in younger women may reflect the intersection of peak sexual activity, multiparity, hormonal changes, and glucose intolerance. Biochemical investigations were not available for these patients to correlate with the infections. The rate of BV in this study is slightly higher than the findings of Madhivanan P et al., wherein 21% (20-29 years, current study) had BV compared to 19% of reproductive age women aged 15-30 years [23]. The progressive decline in infection prevalence with advancing age is probably due to the diminishing oestrogen milieu and reduced sexual activity in the peri- and postmenopausal period [2].

Limitation(s)

It is a single centre study, and the findings may not apply to other institutions or communities. The retrospective design did not allow us to correlate the cytological findings with histopathological outcomes, or clinical follow-up data. The study population comprised only symptomatic women, thereby introducing a selection bias and thus limiting generalisability to the community. Opportunistic screening in symptomatic women as in this study may overestimate the infection prevalence in the community. The lack of HPV genotyping data in our patients since this facility was not available at our institute prevents assessment of high-risk HPV prevalence and co-infection patterns with bacterial and fungal pathogens.

CONCLUSION(S)

This comprehensive analysis of 945 Pap smears from a rural tertiary care centre in Southern India revealed a predominantly infection-driven NILM pattern. The ASC-US/SIL ratio of 2.1:1 meets established cytological diagnostic standards in this centre. Premenopausal women showed a significantly higher infection burden and TZ representation. In contrast, postmenopausal women exhibited greater atrophic changes and had the sole case of SCC. The findings in this study collectively reflect the compounded vulnerability of rural Indian women to high infection prevalence. This study also reinforces the continued relevance of conventional Pap smear cytology as a cost-effective primary screening tool in resource-limited settings. More prospective studies coupled with HPV testing, appropriate follow-up, and integration with HPV vaccination programs will help in sustainable cervical cancer prevention.

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