Original Article

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Pathology Section

Cytomorphological Variations in Pap Smears amongst Postmenopausal Women Reporting at a Tertiary Care Centre, Tamil Nadu, India

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ABSTRACT

Introduction: In developing countries, cervical cancer is the leading cause of death amongst women. Since women spend one third of their lives in the postmenopausal period, which is most neglected phase, needs a technically simple, non invasive and, cost-effective screening tool for cervical cancer detection and the exact answer is the Pap smear screening test. This simple screening test when performed at regular intervals in post menopausal population, can detect precursor lesions there by reducing cervical cancer morbidity and mortality.

Aim: This study was undertaken to evaluate the cytomorphological variations in Pap smears amongst postmenopausal women.

Materials and Methods: In this prospective study, a total of 138 satisfactory smears were considered for study amongst 146 postmenopausal women from August 2020 to July 2022 at Bhaarath Medical College and Hospital and then evaluated and categorised based on Bethesda system for reporting cervical cytology 2014. Reporting elements include specimen type, specimen adequacy, general categorisation, interpretation/ result includes general categories of negative for Intraepithelial Lesion or Malignancy (NILM), epithelial cell abnormalities and other malignancies.

Results: Out of 138 satisfactory cases, 78.26% (108 cases) 14.5% (20 cases) and 7.24% (10 cases) were diagnosed as of inflammatory/benign conditions, epithelial abnormalities and normal cytology respectively. The mean age for inflammatory/ benign lesions was 57.5 years and majority presenting with postmenopausal bleeding and white discharge. Amongst epithelial abnormalities Atypical Squamous Cells of Undetermined Significance (ASCUS) and Low-grade Squamous Intraepithelial Lesions (LSIL) were commonest. The incidence of ASCUS 7.24% (10 cases), and incidence of LSIL 2.89% (4 cases). The mean age was 57.5 years and majority of patients presented with postmenopausal bleeding and mass per vaginum. Incidence of High grade Squamous Intraepithelial Lesion (HSIL) was 2.17% (three cases), the mean age was 65.5 years and presented with white discharge and postmenopausal bleeding. Incidence of SCC was 2.17% (three cases) and the mean age was 70.5 years, presented with, postmenopausal bleeding and white discharge.

Conclusion: Pap smear is a simple, cheap, safe and gold standard diagnostic tool for screening postmenopausal women for all cervical abnormalities in particular pre-invasive and invasive lesions.

Keywords: Benign conditions, Epithelial abnormalities, Postmenopausal bleeding, Satisfactory cases

INTRODUCTION

A woman's life after puberty is broadly divided into reproductive and menopausal phases. The reproductive cycle is normal until the age of 40, with menstruation occurring on a regular basis. Premenopause begins between the ages of 40 and 45, when the ovaries gradually produce less oestrogen, and postmenopause (≥45) occurs when the oestrogen level [1] is sustained and the progesterone level is higher, resulting in atrophic changes in the lower female genital tract. Cervical cancer incidence peaks between the ages of 54 and 59 years, with older women being more likely to develop cancer [2]. It occurs in about one in 53 Indian women compared to one in 100 women in more developed regions of the world during their lifetime [3]. As majority of cases present in late and advanced stages, it is critical to detect early changes in cervical cytology using the most simple cervical cancer screening tool, the Papanicolaou (Pap) smear. It is a preventable disease, if detected early (precancerous) and treated with ablation [4]. Cervical cancer rates have decreased in most developed countries since the introduction of the Pap smear screening test. Death rates are higher in countries where screening and appropriate treatment are unavailable [5-7].

The Human Papillomavirus (HPV) causes the development of abnormal cells in the cervix [8]. Because HPV can remain dormant for many years, even if a woman is not currently sexually active, she may be infected as a result of previous sexual activity. A regular Pap smear is the only way for women to ensure that any abnormal cells they develop are dealt with appropriately, so it is critical to raise awareness about the importance of regular screening, including the postmenopausal group [9,10]. The Pap smear screening test not only aids in the detection of cervical cancer and its precursor lesions, but it also aids in the diagnosis of other conditions such as infectious and inflammatory diseases [11].

Study objective:

- To study the spectrum of epithelial abnormalities in Pap smears amongst postmenopausal women.
- To estimate the frequency of abnormal Pap smears and its relation to clinical symptoms.

MATERIALS AND METHODS

This prospective observational study was conducted from August 2020 to July 2022 in the Department of Pathology at Bhaarath Medical College and Hospital with approval from the Institutional Ethical Committee (BIEC-048-22). Each patient provided informed written consent.

Inclusion criteria: The study included the satisfactory Pap smears of all postmenopausal women, who came for routine screening as well as those who presented with excessive vaginal discharge, blood stained discharge, postcoital bleeding and postmenopausal bleeding, mass per vaginum, lower abdominal pain, fibroids, and chronic pelvic inflammatory disease.

Exclusion criteria: The study excluded all the unsatisfactory Pap smears from postmenopausal group, women aged less than 45 years and also older with a history of hysterectomy/invasive carcinoma at

the time of clinical evaluation and previously treated for cervical or any other genital malignancy are excluded from the study.

Study Procedure

Conventional method of Pap smear screening test was applied in collecting smears.

A valid requisition must include the following information:

- Identification details- name of the patient, age, clinical details and lab number.
- Date and time of specimen collection and signature of collector.

Sample collection:

- The use of gloves is required when handling all biological specimens.
- The frosted end of the slide has label with patient's full name and lab number, using a lead pencil.
- The speculum is inserted (lukewarm water may be used to lubricate speculum).
- Cervix is visualised. Ayre's spatula is used to obtain two cervical scrape smears (for each patient) one from ectocervix and other endocervix, and the smears are fixed in 95% isopropyl alcohol for 20-30 minutes.
- Smears were then stained using the Pap staining method.

The smears were finally evaluated based on Bethesda system for reporting cervical cytology 2014 [12].

STATISTICAL ANALYSIS

Descriptive analysis was done by calculating Frequency and Percentage only, No stats test was applied.

RESULTS

A detailed clinical history as well as demographic characteristics such as age, parity, smoking history and age at menopause is documented in [Table/Fig-1]. The mean age of the study participants was 57.5 years.

Age group (years)	No. of cases
45-50	68
51-55	27
56-60	22
61-66	11
66-70	07
71-75	02
76-80	01
Total	138
Parity	**No. of cases**
0	3
1	7
2	117
3	6
4	4
5	1
Total	138
Smoking history	**No. of cases**
Smokers	4
Non smokers	134
Menopausal timing (age group)	**No. of cases**
44-46	52
47-48	43
49-50	34
>50	09

[Table/Fig-1]: Distribution cases as per age group, parity, smoking status and age at menopause.

A total of 386 cases (one case=two Pap smears, one from ectocervix and one from endocervix) were reported from August 2020 to July 2022. Amongst these, 146 cases were from postmenopausal women and their smears were considered for the study. Amongst these 146 cases, eight cases were unsatisfactory for evaluation due to low squamous cellularity and they were excluded from the study. Distribution of remaining satisfactory cases (138) based on transformation zone shown in [Table/Fig-2].

Adequacy	No. of cases (%)
Satisfactory with transformation zone	88 (63.76)
Satisfactory without transformation zone	50 (36.24)
Total	138 (100%)

[Table/Fig-2]: Distribution of satisfatory cases based on transformation zone.

Out of 138 satisfactory cases, 108 (78.26%) cases, 10 (7.24%) cases and 20 (14.5%) cases were diagnosed as inflammatory/benign conditions, normal cytology and epithelial abnormalities respectively [Table/Fig-3]. Among 108 cytologically diagnosed cases of inflammatory (benign lesions the most common was inflammatory smears including reactive cellular changes with 55 (39.8%) cases, atrophic smears in 41 (29.7%) cases lastly are the other benign conditions 12 cases (shift in flora and candidiasis) [Table/Fig-4,5]. Most common presenting symptoms associated was postmenopausal bleeding in which incidence of epithelial abnormalities were 25.8% and white discharge in which incidence of epithelial abnormalities were 28% [Table/Fig-6].

Cytology report	No of cases (%)
Normal cytology	10 (7.24)
Inflammatory/Benign conditions	108 (78.26)
Epithelial abnormalities	20 (14.5)
Total	138 (100%)

[Table/Fig-3]: Distribution of satisfactory cases based on cytological examination.

S. No.	Diagnosis	No. of cases	Incidence %
A.	**Normal**	10	7.24%
B.	**Inflammatory and other benign conditions**	108	78.26%
1	Shift in flora	8	5.79%
2.	Candidiasis	4	2.89%
3.	Inflammatory smear	38	27.53%
	Reactive cellular changes with inflammation	17	12.31%
4.	Atrophic smear	25	18.15%
	Atrophic smear with reactive cellular changes	5	3.62%
	Atrophic smear with inflammation	11	7.97%
C.	**Epithelial abnormalities**	20	14.5%
1.	ASC-US	10	7.27%
2.	LSIL	4	2.89%
3.	HSIL	3	2.17%
4.	SCC	3	2.17%
	Total	138	100%

[Table/Fig-4]: Distribution of cases based on Pap smear diagnosis.

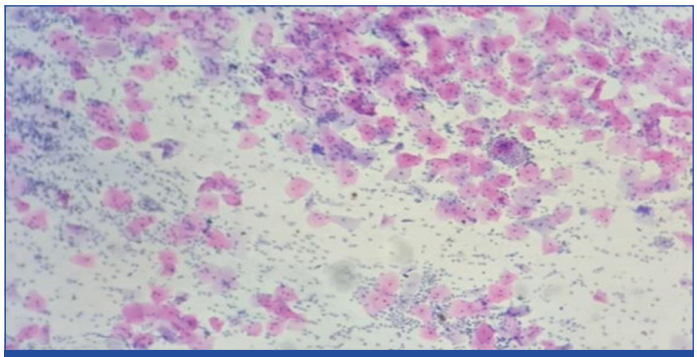
The mean age for inflammatory [Table/Fig-7]/benign lesions was 57.5 years and 41 (29.71%) cases presented with postmenopausal bleeding and white discharge. Epithelial abnormalities accounted for 20 cases with overall incidence of 14.5%. ASCUS accounted for 10 (7.27%) cases with a mean age of incidence being 57.5 years and majority of patients presented with postmenopausal bleeding or mass per vaginum [Table/Fig-8]. LSIL accounted for 4 (2.89%) cases with a mean age of incidence being 55.5 years and presented with white discharge and postmenopausal bleeding [Table/Fig-9]. HSIL accounted for 3 (2.17%) cases with a mean age

S. No.	Age (years)	No. of cases	Shift in flora and Candidiasis	Inflammatory smear	Atrophic smears	ASC-US	LSIL	HSIL	SCC	Normal
1.	45-50	68	9	37	10	4	1	0	0	7
2.	51-55	27	2	9	10	3	1	1	0	1
3.	56-60	22	1	5	12	1	1	0	0	2
4.	61-65	11	0	3	7	1	0	0	0	0
4.	66-70	7	0	0	2	1	1	1	2	0
5.	71-75	2	0	1	0	0	0	0	1	0
6.	76-80	1	0	0	0	0	0	1	0	0
Total		138	12	55	41	10	4	3	3	10

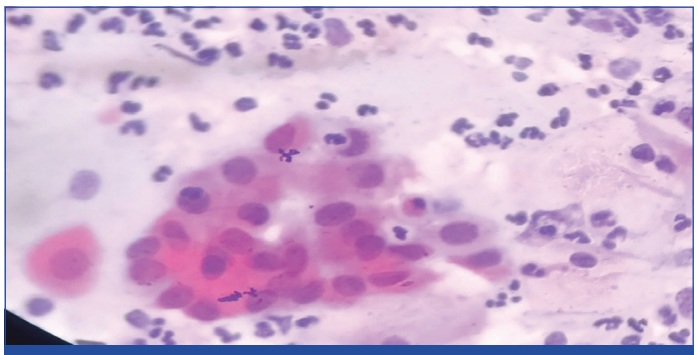
[Table/Fig-5]: Relation between abnormal Pap smears and age.

S. No.	Symptoms	No. of cases	Benign	ASC-US	LSIL	HSIL	SCC
1.	Postmenopausal bleeding	31	23	4	1	1	2
2.	White discharge	26	18	2	3	2	1
3.	Lower abdomen pain	03	02	1	0	0	0
4.	Mass per vaginum-UV Prolapse	05	4	1	0	0	0
5.	Cervical polyp	03	1	2	0	0	0
6.	On USG -Fibroid	01	1	0	0	0	0
7.	On USG- PID	01	1	0	0	0	0
8.	On USG-Thickened endometrium	01	1	0	0	0	0
9.	Routine screening	67	0	0			
	Total	138					

[Table/Fig-6]: Relation between abnormal Pap smears and symptoms in satisfactory cases.

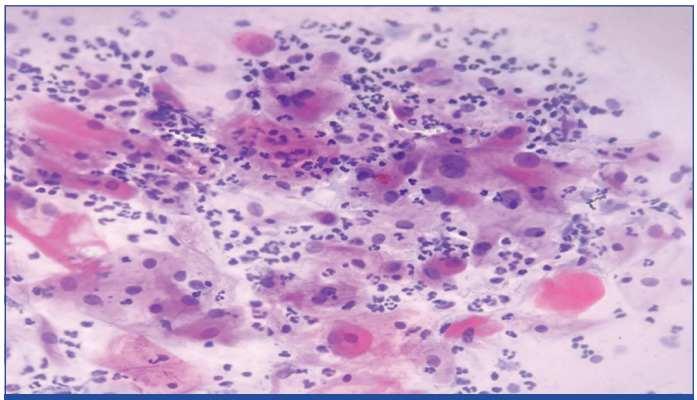


[Table/Fig-7]: Papanicolaou stained smear shows predominantly superficial squames with dense polymorphs in the background (H&E, 10X) (Inflammatory Smear).

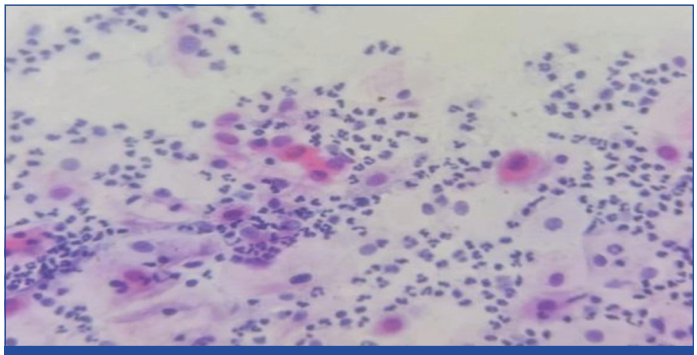


[Table/Fig-8]: Papanicolaou stained smear shows squamous epithelial cells with high nucleus/cytoplasm (n/c) ratio but no nuclear membrane irregularities (H&E, 40X) (ASC-US).

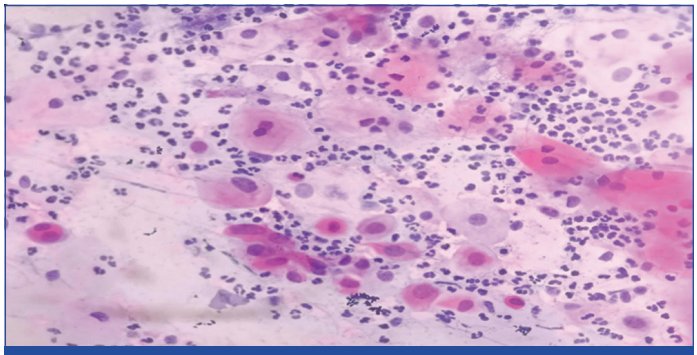
of incidence being 65.5 years and presented with white discharge and postmenopausal bleeding [Table/Fig-10,11]. Squamous Cell Carcinoma (SCC) accounted for 3 (2.17%) cases with a mean age of incidence being 70.5 years, presented with postmenopausal bleed and white discharge [Table/Fig-12,13].



[Table/Fig-9]: Papanicolaou stained smear shows predominantly squamous epithelial cells with high nucleus/cytoplasm (n/c) ratio, bi or multinucleation, perinuclear halo and polymorphs in the background (H&E, 40X) (LSIL).

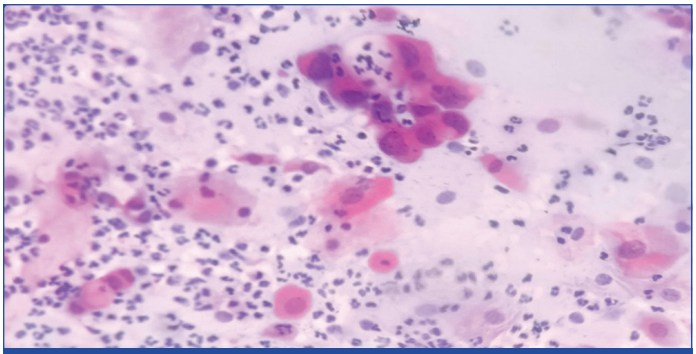


[Table/Fig-10]: Papanicolaou stained smear shows predominantly parabasal and basal cells with high nucleus/cytoplasm (n/c) ratio with dense polymorphs and its debris in the background (H&E, 40X) (HSIL).

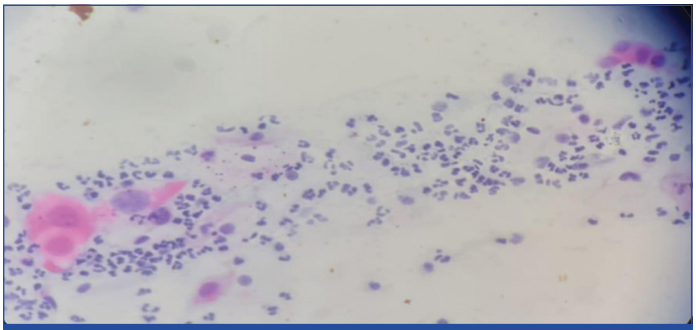


[Table/Fig-11]: Papanicolaou stained smear shows predominantly parabasal and basal cells with high nucleus/cytoplasm (n/c) ratio and dense polymorphs with its debris in the background (H&E, 40X) (HSIL).

Among 138 cases, 71 (51.44%) cases of the patients were symptomatic and 67 (48.56%) cases were asymptomatic. The most common presenting complain was postmenopausal bleeding in 31 (43.67%) cases, white discharge per vaginum in 26 (36.61%) cases followed by mass per vaginum (UV prolapse and cervical polyp) in 8 (11.26%) cases, lower abdomen pain three cases, with ultrasound showing changes of thickened endometrium in one cases, fibroid one case and chronic pelvic inflammatory disease in one case.



[Table/Fig-12]: Papanicolaou stained smear show squamous cells with high nucleus/cytoplasm (n/c) ratio with nuclear pleomorphism admixed with tadpole cells, also showing neutrophilic debris in the background (H&E, 40X) (SCC).



[Table/Fig-13]: Papanicolaou stained smear show squamous cells with high nucleus/cytoplasm (n/c) ratio and nuclear pleomorphism admixed with tadpole cells, also showing neutrophilic debris in the background (H&E, 40X) (high power 40X) (SCC).

DISCUSSION

Amongst 146 cases of postmenopausal women, only 138 (94.53%) cases were satisfactory for evaluation and were included and 8 (5.47%) cases were excluded from study population as they were unsatisfactory which was close to study by Chate MT et al., (4.95%) [13]. High incidence of unsatisfactory smears was reported by Mahadik J et al., (14.85%) and Malik NP et al., (18.94%) [14,15]. This variation may be due to difference in number of study population. The age of patients ranged from 45-80 years with a mean age of 54.6 years in this study which is comparable to Cakmak B and Köseoglu DR study and Kaiho N et al., study which include age group 45-80 years and 44-79 years, respectively [16,17]. Maximum number of cases who underwent Pap procedure, were in the age group of 45-50 years followed by 50-55 years which was similar to studies by Mahadik J et al., and Kaiho N et al., [14,17].

On cytological examination 108 (78.26%) cases were reported as inflammatory including reactive cellular changes associated with it and other benign condition (Bacterial vaginosis and Candidiasis), 10 (7.24%) cases had normal cytology and 20 (14.5%) cases were diagnosed as having epithelial abnormalities, which were comparable with the study done by Bansal S et al., and Bal MS et al., [2,18]. Incidence of Bacterial vaginosis in present study was 5.79% with 8 cases and candidiasis was 2.89% was comparable to 0.83% and 0.63%, respectively from Mahadik J et al., [Table/Fig-14] [2,10,13,14,18,19].

In the present study, overall Incidence of ASCUS was 7.27% which is similar to Bansal S et al., lower than Cakmak B and Köseoglu DR study, and higher than Bal MS et al., study and Shashidhar MR et al., [2,16,18,19]. Incidence of LSIL was 2.89% which was and lower than Bansal S et al., and similar to Bal MS et al., while higher than Shashidhar MR et al., [2,18,19]. Chate MT et al., reported highest incidence of LSIL (13.28%) overall [13]. HSIL was reported in 2.17% cases which was similar to the study by Cakmak B and Köseoglu DR (4%) and Kaiho N et al., (3.9%) [16,17]. SCC was 2.17% in present study which was also almost similarly seen in study by Chate MT et al., (2.4%), Kaiho N et al., (2.9%) and Bal MS et al., (1.3%) [13,17,18]. ASCUS and LSIL were the most common epithelial abnormalities reported [Table/Fig-15] [2,10,13,16-19].

In the present study, 50% of epithelial abnormality cases had ASCUS, which was higher than the study done by Bansal SM et al., (31.5%) [2]. The incidence of LSIL in this study was 20%, which was consistent with the findings of Shaki O et al., (32.3%) [10]. HSIL was 15% in the present study and was similar to 16.3% of Shaki O et al., study [10]. The prevalence of SCC was 15% in the current study and 13.7 % in the Bukhari MH et al., study [9]. Difference in incidences is due to different age and geographical area at time of presentation. In the current study, ASCUS was more prevalent in the 45-55 age group, with a mean age of 50.5 years, whereas it was 37.6 years in the Jetley S et al., study [20]. LSIL was more prevalent in the 45-70 year age group, with a mean age of 56.6 years, compared to Jetley S et al., study, which had a mean age of 33.4 years and 32.4 years in Bal MS et al., study [8,20]. HSIL was more prevalent in people aged 51-80 years, with a mean age of 61.78 years, compared to 44 years in the Jetley S et al., study and 40.5 years in the Bal MS et al., study [18,20].

S. No.	Study	Normal %	Inflammatory and other benign %	Epithelial abnormalities %
1.	Bal M S et al., [18] (2012)	16.7	74.3	5
2.	Mahadik J et al., [14] (2013)	30	66.3	3.23
3.	Chate MT et al., [13] (2017)	25.23	45.49	24.3
5	Shashidhar MR et al., [19] (2017)	51.30	42.17	2.9
6.	Shaki O et al., [10] (2018)	52.8	23.8	19.1
7.	Bansal S et al., [2] (2021)	26.2	55.9	17.9
	Present study	7.24	78.26	14.5

[Table/Fig-14]: Comparison of cytological findings between present study and other studies amongst satisfactory cases [2,10,13,14,18,19].

S. No.	Study	ASCUS %	LSIL %	HSIL %	SCC %
1	Bal MS et al., [18] (2012)	0.3	2.7	0.7	1.3
2	Cakmak B and Köseoglu DR [16] (2014)	15.7	3	4	0.5
3	Kaiho N et al., [17] (2016)	1.9	4.9	3.9	2.9
4	Shashidhar MR et al., [19] (2017)	1.62	0.6	0	0.6
5	Chate MT et al., [13] (2017)	2.03	13.28	4.94	2.4
6	Shaki O et al., [10] (2018)	0	6.8	6	0
7	Bansal S et al., [2] (2021)	5.6	6.7	4.8	0.8
	Present study	7.27	2.89	2.17	2.17

[Table/Fig-15]: Comparison of overall incidence of each epithelial abnormality with other studies [2,10,13,16-19].

SCC was discovered in the 66-75-year-old age group, with a mean of 63.7 years, which was comparable to 59 years in the Jetley S et al., study and 57 years in Bal MS et al., [18,20]. One possible explanation for the difference in mean age is that the age groups in both studies are different. In the current study, the incidence of epithelial abnormalities in women with postmenopausal bleed, discharge per vaginum, mass per vaginum, and lower abdominal pain was 40%, 40%, 15%, and 5%, respectively, which was similar to previous studies by Misra JS et al., Bansal S et al., and Kaiho N et al., [Table/Fig-16] [1,2,17]. Variations in findings when compared to other studies could be attributed to differences in sample size and study duration.

S. No.	Study	Postmenopausal bleeding %	White discharge %	Mass per vaginum %	Abdomen pain %
1	Bal MS et al., [18] (2012)	1	59	1	19.3
2	Shaki O et al., [10] (2018)	5	36.5	1	7.4
3	Misra JS et al., [1] (2018)	1.3	12.6	2	19.2
4	Bansal S et al., [2] (2021)	1	43.4	2	36.4
	Present study	22.46	18.84	5.79	2.17

[Table/Fig-16]: Comparison of presenting complains with other study [1,2,10,18].

Limitation(s)

In the present study, sample collection was the most common issue in postmenopausal women due to lack of motivation and their fear factor regarding the procedure itself and also their hormone status made the cervix relatively difficult for collection. Inadequacies in sample were the next potential drawback, for false negative results brought on by background inflammatory cells, blood, mucus, and mucus-producing mucus that could obscure aberrant cells.

CONCLUSION(S)

The current study gave an overall insight on the common cervical cytomorphological changes in postmenopausal women and their presenting symptoms. The epithelial abnormalities ASCUS and LSIL accounted for the major portion and the predominant complaints were postmenopausal bleeding and white discharge. Most of the cervical epithelial abnormalities were found in symptomatic cases. Symptomatic cases took screening test promptly and also most of them had a follow-up. Hence, Pap smear screening test is an important step in knowing early changes in cervical epithelium and also acts as a clue for early treatment in postmenopausal women.

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