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Cytology Triage for HPV-Positive Postmenopausal Women in a Setting of Cervical Cancer Screening

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ABSTRACT

Background: Globally, cervical cytology continues to serve as the cornerstone of cervical cancer screening programs, but WHO 2021 guidelines advocate HPV DNA testing as the primary screening modality due to its heightened sensitivity. This method necessitates additional triage with cytology or colposcopy to detect precancerous lesions. Hormonal shifts and anatomical alterations in postmenopausal women may impact diagnostic outcomes in either modality.

Aim: To describe the spectrum of cytological lesions in HPV-positive postmenopausal women to detect precancerous lesions as part of cervical cancer screening.

Methods: Conventional cytology smears of high-risk HPV-positive postmenopausal women were reported according to The Bethesda System 2014. Results of follow-up biopsies of the positive smears were recorded, and cyto-histological correlation was performed.

Results: The retrospective study included conventional cytology smears of 124 postmenopausal women positive for high-risk HPV DNA with a mean age of 52 years. Of the 124 cases, 68 were positive for HPV 16 and/or 18, and 56 were positive for other high-risk HPV. On cytology, 78% were negative for intraepithelial lesions. HSIL+ lesions were noted in 12%, and low-grade lesions were noted in 10% of smears with HPV16/18 predominance. Follow-up biopsies of 17 smears revealed SCC in two cases, HSIL+ in 11 cases, LSIL in 2, and 2 were negative/benign lesions. Of the cyto-histological discordant cases, four were upgraded on review.

Conclusion: Cytology is a useful triage tool in detecting preinvasive and early invasive tumors in HPV DNA-positive postmenopausal women.

1 | Introduction

Cervical cancer is a preventable malignancy, caused by human papilloma virus (HPV) infection, and a substantial decrease in incidence and mortality has been achieved by organised screening methods. HPV testing has higher sensitivity but lower specificity as compared to cytology-based screening, resulting in extended periods of screening intervals. Hence, HPV testing has been accepted as the primary screening modality for cervical cancer [1].

Postmenopausal women are particularly vulnerable to cervical cancer due to a combination of factors including hormonal changes, immunosenescence, and a potentially undetected or latent HPV infection, which may reactivate after menopause [2]. Cervical cancer is most common in women under 50, but a

significant proportion of cases still occur in older women, aged 65 or older, which is projected to increase as life expectancy rises globally [3]. The incidence of cervical cancer in postmenopausal women may be underestimated due to inadequate or infrequent screening, resulting in delayed diagnosis and worse outcomes [2].

There remains a critical gap in cervical cancer screening practices for postmenopausal women. Most of the developed countries with organised screening programmes advocate the age of 65 as a cutoff for cessation of screening; studies indicate that a significant number of cases are diagnosed after 69 years of age [3, 4]. In India, The Federation of Obstetric and Gynaecological Societies of India's (FOGSI) Good Clinical Practice Recommendations (FOGSI GCPR) advocate cessation of screening at the age of 65 years [5].

Potential primary screening modalities under investigation include HPV testing, co-testing (Pap smears combined with HPV testing), liquid-based cytology, and even biomarker identification [4]. The sensitivity of cytology in detecting epithelial cell abnormalities is low in postmenopausal women due to the inability to obtain a satisfactory sample caused by vaginal atrophy or anatomical difficulties in reaching the retracted transformation zone. A negative HPV result is a better indicator than a corresponding negative cytology, and HPV testing with a triage of cytology increases the sensitivity of finding high-grade precancerous lesions in postmenopausal women [3]. The present study aims to describe the spectrum of the cytological lesions with their histological correlation in HPV-positive postmenopausal women in an unscreened population.

2 | Methods

This single institutional study was conducted after obtaining the approval of the Institutional Ethics Review (Ref. Study No. 135/2024). The waiver of consent was obtained because of the retrospective nature of the study. A total of 9650 women underwent HPV DNA testing as a part of the community screening programme between April 2023 and April 2024. The study population was unscreened for intraepithelial malignancy. The Federation of Obstetric and Gynaecological Societies of India's (FOGSI) Good Clinical Practice Recommendations (FOGSI GCPR) advocates cervical cancer screening in the age group of 30–65 years in limited resource settings with VIA as the screening methodology, whereas cytology or HPV DNA testing is recommended in good resource setting [5]. However, with no organised screening programme, screening is opportunistic, and hence most of the women are unscreened.

HPV real-time PCR assay was done by Cobas 6800 system (Roche Diagnostics). High-risk HPV DNA was detected in 9.6% of the women. Of these, 124 (1.2%) postmenopausal women who tested positive for high-risk HPV DNA underwent cytology triage for the detection of cytological abnormalities. The conventional smears fixed in 95% ethanol, thus received, were reported according to the Bethesda system 2014. The demographic details and the per vaginal examination findings were collected from the clinical records. The histological findings of the follow-up cervical biopsies and cone resections were obtained from the pathology database. The cytological diagnoses were compared with the histological diagnosis when available, and the discordant cases were reviewed. The data was entered into an Excel sheet and basic statistics were performed.

3 | Results

3.1 | Study Population and HPV Status

The study included 124 conventional cytology smears from postmenopausal women who were unscreened for cervical intraepithelial malignancy and who tested positive for high-risk HPV DNA. The mean age was 52 years (range 40–80 years), and 91% were under 60 years. Among these, 68 (54%) were positive for HPV 16 and/or 18, and 56 tested positive for other high-risk HPV types, reflecting a varied distribution of high-risk HPV infections in this age group.

On per-vaginal examination of the cases evaluated, 76% had normal findings, 20% of the patients showed atrophic changes, and 4% exhibited abnormal changes including acetowhite areas, bleeding on touch, and warts, of which 80% were noted in HPV 16/18 positive women.

3.2 | Cytological Findings

Among the 124 cases examined in this study, 27 (22%) tested positive for cytological abnormalities, while the remaining 97 cases were negative for intraepithelial lesions or malignancy. 62% of the lesions were noted in association with the HPV 16/18 group. The distribution of positive cases based on HPV typing is depicted in Figure 1.

Among the positive cases, 46% had low-grade lesions with eight cases of atypical squamous cells of undetermined significance (ASCUS) and four cases of low-grade squamous intraepithelial lesions (LSIL). High-grade lesions were noted in 54%, of which 37% were positive for HPV 16/18. There were nine cases of high-grade squamous intraepithelial lesion (HSIL), two cases of atypical squamous cells—cannot exclude high-grade (ASC-H), and four patients had carcinoma. Of the cases with > HSIL, the ages ranged from 47 to 59 for invasive carcinoma and 46–54 years for HSIL.

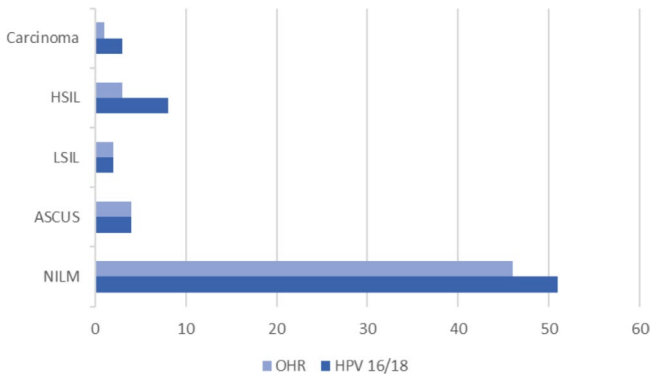

FIGURE 1 | Distribution of cytological diagnosis with respect to HPV genotypes. [Color figure can be viewed at wileyonlinelibrary.com]

3.3 | Cyto-Histological Correlation

Follow-up biopsies were available for 17 of the 27 cytologically positive cases, enabling cytology-histology correlation, which was performed as per the ASC guidelines [6] (Table 1). Histology of all the cytological high-grade lesions, HSIL, ASC-H, and carcinoma were concordant. Of the six discordant cases identified, four were upgraded and two were downgraded on histology. Discordance was frequent in the ASCUS category with five smears; two were upgraded to HSIL, one to LSIL, and two were downgraded as negative on biopsy. One of the cases identified as LSIL on cytology had focal HSIL on biopsy. On reviewing the slides, the interpretive errors were mainly due to atrophic changes noted and due to the paucity of cells exhibiting the high-grade changes (Figure 2).

3.4 | Accuracy and PPV

In this study, cytology demonstrated a high level of diagnostic accuracy in detecting cervical neoplasia, with an overall accuracy of 88.2% and a PPV of 100%. This indicates that cytological triage adequately identifies abnormalities in hrHPV-positive postmenopausal women.

4 | Discussion

The prevalence of HR HPV in unscreened postmenopausal women in a community-based screening programme was 1.2% in the present study. Twenty-two percent of these women had abnormalities on subsequent triage with cytology, of which 12% were confirmed on histology, with 10.4% presenting high-grade lesions.

Epidemiological data is scant regarding the exact prevalence of HPV infection in women 40–60 years, which is highly variable and is estimated to be 1%–22% [7]. Contrary to the long-standing belief that HPV infection rates decrease with increasing age, there is a secondary peak in the prevalence of cervical cancer in the older population [8]. Persistence of infection, particularly with certain types of high-risk HPV in postmenopausal women, leads to a higher risk of progression to neoplasia, thereby leading to a higher incidence of neoplastic lesions. Additionally, many women in this age group lack regular gynecological care after their reproductive years [7, 9]. There is inconclusive evidence regarding an upper age limit for cessation of screening, and guidelines for screening vary [10]. Though the age range in the present study spanned four decades, the high-grade lesions were noted predominantly in women less than 60 years, with high-grade intraepithelial lesions occurring slightly earlier (46–54 years) than the invasive carcinoma (47–50 years) [1].

The genotypic profile of HPV infection noted in PM women in exit screening studies across the world is variable. HPV 16 is most prevalent in central and South America and Europe, whereas other high-risk types are prevalent in Asian and African women. These differences might not necessarily reflect true HPV-type distribution but could be influenced by HPV testing methods and correlate with the cervical cancer burden [11]. The present study reports an almost equitable distribution of HPV genotypes with a relative increased prevalence of other high-risk types as compared to premenopausal women where HPV 16/18 are dominant, similar to other Indian study [12].

The effective mode of screening for cervical neoplasia in postmenopausal women is debatable. The sensitivity of cytology as a screening tool is low, less than 60% [13], and further lower in postmenopausal women, due to difficulty in obtaining quality samples from an atrophic and retracted transformation zone leading to unsatisfactory smears [14]. Hence, primary HPV DNA screening is the choice and a triage of cytology in HPV-positive postmenopausal women in detecting precancerous lesions increases the sensitivity by three fold [2] and this approach eliminates the ASCUS diagnosis of atrophy. This bimodal screening strategy in the present study led to the detection of pre-cancerous and early neoplastic lesions in 22% of the cases, which is much higher compared to previous studies reporting 13.9% detection [4] which employed HPV DNA testing with a triage of colposcopy where atrophic changes compromise the visualisation of the TZ. Further, prior knowledge of HPV DNA status increases the specificity of cytology as compared to cytology results when blinded to HPV status [15]. On the contrary, the higher rates observed in the present study may be due to unscreened women included in the study. Primary HPV screening with cytology triage will effectively reduce colposcopy and biopsy referrals which are technically demanding and require expertise.

The frequency of cytological diagnosis of ≥ASCUS varies in the literature. The present study reports 22% of >ASCUS lesions and 12% with high-grade lesions as opposed to 10% with cytology as the primary screening modality, as reported in the literature [16]. The prevalence rate of>ASCUS in the literature is 3.8%–6.2%, with high-grade lesions ranging from 8.7% to 17.2% [4]. The frequency of high-grade lesions in post-menopausal women with cytology as the primary screening modality with reflex HPV testing in the ASCUS category ranges from 10.7% to 27.5%, as reported in literature [7, 10]. The ASCUS category in the present

TABLE 1 | Discrepancy assessment grid.

Cyto\Histo	Negative	LSIL	HSIL	Carcinoma	Total
ASCUS/AGC	**2**	**1**	**2**	—	5
LSIL	—	1	**1**	—	2
ASC-H & HSIL	—	—	8	—	8
Carcinoma	—	—	—	2	2
Total	2	2	11	2	17

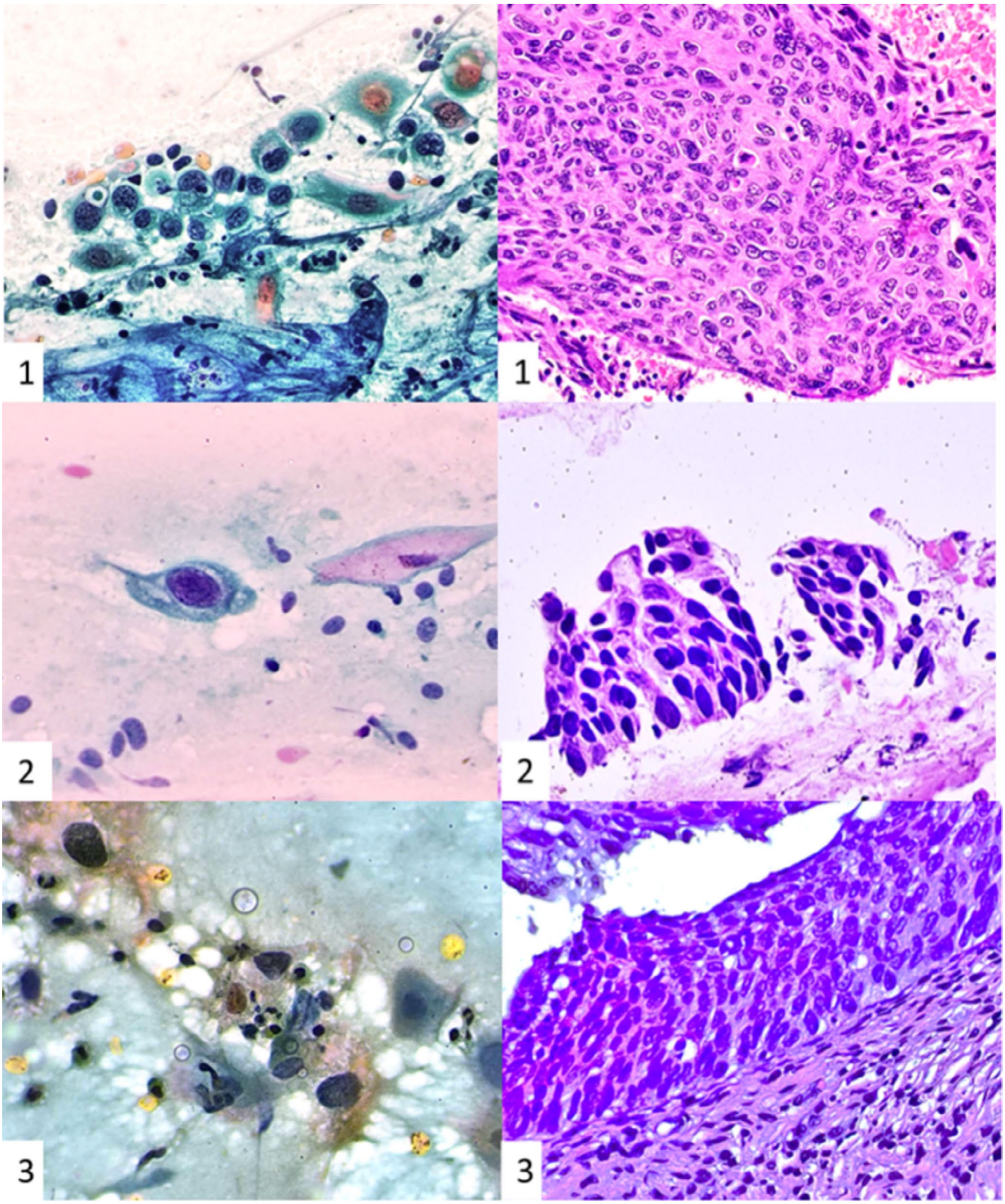

FIGURE 2 | Cytohistology discordant cases of ASCUS category. Smears with low cellularity and occasional atypical parabasal cells (X400; Pap) on histology showed high-grade squamous intraepithelial lesion (X200; H&E). [Color figure can be viewed at wileyonlinelibrary.com]

study harbored a high rate of SIL (60%) and HSIL+ (40%) lesions. Hence, primary screening with HPV testing eliminates the reactive category of ASCUS, thereby reducing the number of colposcopic referrals or reflex cytology testing, thereby channeling the resources towards deserving women.

Interpretation of cytology in postmenopausal women is often challenging due to decreased cell yield and atrophic changes, with ASCUS being a common diagnosis. The parabasal cells obscuring the high-grade atypical cells or the parabasal cells mimicking the high-grade atypical cells led to discordant

diagnoses in the present study. Interestingly, the proportion of high-grade lesions (40%) associated with ASCUS in our study was higher than that reported in existing literature, likely due to the use of HPV testing as the primary screening method [9]. Therefore, HPV screening with cytology triage can help differentiate reactive ASCUS due to atrophy from true neoplastic lesions, improving the detection of underlying malignancies in postmenopausal women.

The study results are based on a small sample size and do not include colposcopy findings. Further, conventional cytology was used as a triage method rather than performing liquid-based cytology (reflex cytology) on the original sample.

The continued risk of persistent HPV infection and cancer development even after menopause necessitates developing the most appropriate screening modalities and evidence-based guidelines for early detection and to improve outcomes. The present study highlights the advantages of HPV testing as a primary screening modality, with cytology as a triage method in postmenopausal women, which needs to be validated in large cohorts.

Ethics Statement

The study was approved by the Institutional Ethics Committee of St. John's National Academy of Health Sciences.

Consent

Waiver of consent obtained due to the retrospective nature of thestudy.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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