Research Article

Pattern of cervical biopsy results in cases with cervical cytology interpreted as higher than low grade in the background with atrophic cellular changes

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ABSTRACT

Objective: The cytomorphological changes associated with atrophic cellular pattern (ACP) in cervical cytology smears may mimic high-grade squamous intraepithelial lesion (HSIL). Due to this, there may be higher chances of cytomorphological overinterpretation in cases with ACP. Estrogen therapy (ET) (topical or systemic) would reverse the changes related to atrophy and repeat Pap smear after ET should correct the false positives. This approach would minimize the unindicated invasive interventions. However, performing immediate biopsies following “higher than low-grade squamous intraepithelial lesion (LSIL) (atypical squamous cells—cannot exclude HSIL, low-grade squamous intraepithelial lesions—cannot exclude HSIL, and HSIL) interpretations” in such cases, is a general trend. Pap smears with “higher than LSIL interpretations” in association with ACP over a period of 10 years were selected.

Materials and Methods: A total of 657,871 cases over 10 years were reviewed, of which 188 Pap smears interpreted as higher than LSIL interpretations with ACP were selected randomly for this study.

Result: Of these 188 cases, 67 underwent biopsies which were reviewed and compared with 67 biopsies performed for “higher than LSIL interpretation” cases without ACP. The follow-up biopsy material was reviewed including elective p16 immunohistochemistry with other clinical details including high-risk HPV test results as indicated.

Conclusion: The findings demonstrated that Pap smears with ACP have higher false positives due to tendency for cytomorphologic overinterpretation as compared to non-ACP group.

Keywords: Hyperchromatic crowded groups, Atrophic cellular pattern, High-risk HPV test

INTRODUCTION

Cervical carcinoma is the fourth most frequent cancer in women and represents 6.6% of all female cancers worldwide.[1] There has been a tremendous decline in the morbidity and mortality of cervical carcinoma due to the successful screening with cervical cytology smear (Pap smear) with early treatment of precancerous lesions.[2] Bethesda terminology and other advanced ancillary tests such as high-risk HPV test (hrHPVT), p16 immunohistochemistry (IHC), and liquid-based cytology preparation have participated in continued improvement in interpretations.[3,4]

Pap smear is still the standard screening test for cervical dysplasia worldwide; however, similar to other screening methods, it has false-positive and false-negative components.
Atrophic vaginitis is commonly seen in women with low estrogen level such as during postmenopause. The vaginal wall would be thin and dry together with inflammation. Pap smears with atrophic cellular pattern (ACP) present usually as scant cellularity with many hyperchromatic crowded groups (HCGs) of parabasal/basal cells[5,6] with cytomorphological variables, ranging from singly scattered naked nuclei to syncytial-like sheets of HCG of atypical cells with high nucleus-to-cytoplasm (N/C) ratio in some cells. Due to degeneration, the chromatin may be smudgy with hyperchromasia. Severe atrophy may be associated with significant inflammation and debris. All these features may lead to false-positive interpretations including high-grade squamous intraepithelial lesion (HSIL), atypical squamous cells-cannot exclude HSIL (ASC-H), low-grade squamous intraepithelial lesions-cannot exclude HSIL (LSIL-H), and even invasive carcinoma. For the current study, all these categories are grouped as "higher than LSIL interpretations" (HGI).[6,8]

Estrogen therapy (topical or systemic) (ET) would reverse the changes related to ACP and repeat Pap smear after ET would correct the false positives.[9,10] Short-term local estrogen cream is preferred, and it will restore normal vaginal pH levels and revitalize the epithelium. This approach of repeating Pap smears in cases with HGI after ET should correct false-positive interpretations and minimize the unindicated invasive interventions.

PAP smear testing with liquid-based cytology has provided the opportunity for testing the same cytology sample for hrHPVT.[5,11-13] In this study, we analyzed cases with ACP cytomorphology interpreted as HGI with follow-up biopsy over the past 10 years with hrHPVT results in some cases and elective IHC for p16 as indicated in biopsies with indeterminate histomorphology. The result patterns between cases with ACP and without ACP (non-ACP) were compared.

RESULTS

Of 188 cases with ACP and interpreted as HGI (159 – ASC-H, 14 – LSIL-H, and 15 – HSIL), 67 (57 ASC-H, 5 LSIL-H, and 5 HSIL) had follow-up biopsies. Thirty-six biopsies showed “higher than cervical intraepithelial neoplasm (CIN2) dysplasia” (HGD) with relatively lower positive predictive value (PPV) of 54% (36/67).

Specific interpretation under HGI general category in ACP group was mostly in ASC-H (57/67). In non-ACP group, ASC-H (28/67) and LSIL-H (27/67) interpretations were more frequent. The number of definitive interpretations as HSIL was more frequent in non-ACP group (12/67) than ACP group (5/67) [Table 1].

Thirty-one biopsies were negative for HGD, of which 19 biopsies with adequate squamous component for IHC were reviewed further. Eleven cases showed indeterminate histomorphology, so they were evaluated further with p16 IHC. All 11 (100%) biopsies demonstrated a lack of p16 nuclear immunoreactivity in abnormal indeterminate squamous epithelium, consistent with lack of HPV-related dysplasia.

hrHPVT results were available in 48 ACP cases and in 59 non-ACP cases. PPV of hrHPVT was relatively low (67%) in cases with ACP as compared to non-ACP group (89%). On the other hand, negative predictive value (NPV) showed the reciprocally opposite pattern. The sensitivity of hrHPVT was also low (67%) but with better specificity (74%), as compared to reverse pattern in non-ACP group [Table 2]. This highlights the application of considering gray-zone cytopathologic interpretations in cases with ACP to be downgraded to negative if hrHPVT result is negative.

| Group    | HGI with specific cytopathologic interpretation |
|----------|-----------------------------------------------|
|          | ASC-H | LSIL-H | HSIL |
| ACP      | 57    | 5      | 5    |
| Biopsy result: | 29+, 28− | 4+, 1− | 3+, 2− |
| Non-ACP  | 28    | 27     | 12   |
| Biopsy result: | 19+, 9− | 22+, 5− | 11+, 1− |

+: Positive for HGI, −: Negative for HGI, HSIL: High-grade squamous intraepithelial lesion, LSIL: Low-grade squamous intraepithelial lesion, HGI: Higher than LSIL interpretation, ACP: Atrophic cellular pattern, Non-ACP: Without ACP, ASC-H: Atypical squamous cell-cannot exclude HSIL, LSIL-H: LSIL-cannot exclude HSIL.
The findings noted in cases with ACP were compared with the pattern noted in 67 non-ACP cases from the same population but interpreted as HGI. Of these 67 ACP cases, only 36 cases were positive for HGD in biopsy with relatively lower PPV of 54% (36/67) as compared to non-ACP group [Table 3]. The difference of PPV of HGI between cases with ACP (54%) and non-ACP (78%) was statistically significant ($P = 0.006$). These findings confirmed our hypothesis that cases with ACP have higher chances of false-positive HGI.

Various cytomorphological pitfalls lead to HGI in the background of ACP. Many of these were related to HCG of parabasal cells. This was enhanced further due to crushing artifact and degenerative changes/poor preservation associated with Pap smear with ACP. Singly scattered parabasal/basal cells with degenerative changes overinterpreted as HGI were noted in some cases [Figure 1]. Enlargement of some nuclei (unrelated to dysplasia such as folate deficiency and age-related phenomenon) in some cases may be interpreted as LSIL, and if this is associated with some cells suspicious for HSIL, such cases were upgraded to LSIL-H.

**DISCUSSION**

Pap smear is still the standard screening method for cervical cancer, the most common HPV-associated cancer.$^{[14]}$ We

### Table 2: Correlation of high-risk human papillomavirus test results with biopsies in atrophic cellular pattern and without atrophic cellular pattern group.

| hrHPVT result | Biopsy positive for HGD | Biopsy negative for HGD | PPV (%) | NPV (%) |
|---------------|-------------------------|-------------------------|---------|---------|
| **ACP group (total 48*)** | | | | |
| HPV+ (T: 21) | 14 | 7 | 67 |
| HPV− (T: 27) | 7 | 20 | 74 |
| Sensitivity (%) | 67 | | |
| Specificity (%) | | 74 | |
| **Non-ACP group (total 59**) | | | | |
| HPV+ (T: 48) | 41 | 7 | 85 |
| HPV− (T: 11) | 5 | 6 | 55 |
| Sensitivity (%) | 89 | | |
| Specificity (%) | | 46 | |

*Of 67 cases in ACP group, hrHPVT results were available in 48 cases, **Of 67 cases in non-ACP group, hrHPVT results were available in 59 cases. +: Positive, -: Negative, ACP: Atrophic cellular pattern, Non-ACP: Without ACP, HPV: Human papillomavirus, hrHPVT: High-risk HPV test, CIN2: Cervical intraepithelial neoplasm 2, HGD: Higher than CIN2 dysplasia, PPV: Positive predictive value, NPV: Negative predictive value

### Table 3: Comparison of results on cervical biopsies in cases interpreted as higher than low-grade squamous intraepithelial lesion in Papanicolaou smear in atrophic cellular pattern and without atrophic cellular pattern groups.

| PAPs interpreted as HGI | Total | Biopsy positive for HGD | Biopsy negative for HGD | PPV (%) |
|-------------------------|-------|-------------------------|-------------------------|---------|
| ACP                     | 67    | 36                      | 31*                     | 54      |
| Non-ACP                 | 67    | 52                      | 15*                     | 78      |
| Total                   | 134   | 88                      | 46                      |         |

*Objectively confirmed with elective IHC for p16 in indeterminate cases. Fisher's exact test. The two-tailed $P=0.0060$. The difference between rows (groups) and columns (outcomes) is considered to be very statistically significant. (GraphPad. https://www.graphpad.com/scientific-software/prism/). CIN2: Cervical intraepithelial neoplasm 2, IHC: Immunohistochemistry, ACP: Atrophic cellular pattern, Non-ACP: Without ACP, LSIL: Low-grade squamous intraepithelial lesion, HGI: Higher than LSIL interpretation, HGD: Higher than CIN2 dysplasia, PAPs: Papanicolaou smear, PPV: Positive predictive value

**Figure 1**: (a) Cervical smear, Pap stained ThinPrep (a 64-year-old female). Small group of cells with checkerboard pattern. The cells have a high nucleus-to-cytoplasm ratio with smudgy nuclei. The smear was hypocellular with atrophic cellular pattern. (b) Cervical biopsy, HE stain (follow-up biopsy). The biopsy shows fragments of superficial portion of atrophic squamous epithelium in this case with postmenopausal changes. The findings focally may resemble atypical squamous metaplasia which may be overinterpreted as high-grade squamous intraepithelial lesion. (c) Cervical biopsy, p16 immunohistochemistry (follow-up biopsy). The biopsy shows scant focal nuclear (and cytoplasmic) immunoreactivity for p16. This is consistent with HPV-related dysplasia; however, the immunostaining pattern is not strong and diffuse usually associated with high-grade squamous intraepithelial lesions. Low-grade dysplasia was further confirmed with low Ki67 proliferation index. (d) Cervical biopsy, Ki67 immunohistochemistry (follow-up biopsy). The biopsy shows very few squamous epithelial cells with nuclear immunoreactivity due to very low Ki67 proliferation index.
observed during our routine quality assurance analysis with cytology–histology correlation that false positives are more frequent with ACP. This may be due to the overlapping cytomorphological look-alikes associated with ACP. ACP typically demonstrates single or syncytial groups of parabasal cells with degeneration-related enhanced nuclear hyperchromasia. The HCG may mimic HGI. This may initiate unindicated interventions with potential morbidity and higher cost.

Some studies have demonstrated a higher risk of false positives due to atrophy-related epithelial changes. Ancillary tests such as hrHPVT on residual cytology specimen and p16 with or without Ki67 on surgical specimen are helpful. However, there is a relative paucity of data correlating results of hrHPVT, p16, and Ki67 with cytomorphology interpreted as HGI in cases with ACP.

This study compares ACP and non-ACP cases followed by the surgical biopsy or loop electrosurgical excision procedure. Ki67 IHC for proliferation index and p16 IHC as a surrogate marker for HPV-related dysplasia are helpful to discriminate between atrophy and dysplasia. Lack of nuclear immunoreactivity for p16 with low Ki67 is consistent with nondysplastic interpretation.

We randomly selected 67 atrophic Pap smears with HGI along with biopsies over the past 10 years. Of 67, only 36 cases were positive for HGD with the PPV of only 54% (36/67). Thirty-one cases had a negative biopsy for HGD. Ancillary role of hrHPVT was stronger in favoring lack of HGD in cases with negative hrHPVT. In contrast to Pap smears of the patients in non-ACP category, hrHPVT was more specific with lower sensitivity [Table 2]. In suspicious ACP cases, reflex hrHPVT may be helpful as ancillary test with higher NPV to rule out HGI in cases with negative hrHPVT [Table 2]. However, seven cases with negative hrHPVT were positive for HGD in biopsy with obvious risk of false negativity and liability if depended on negative hrHPVT alone [Table 2].

In ACP group, most of HGI were interpreted as ASC-H, whereas in non-ACP group, interpretations included ASC-H, LSIL-H, and HSIL [Table 1]. The findings demonstrated higher chances of gray-zone interpretations in ACP group.

The false-positive cases with HGI in the background with ACP were predominantly due to overlap of HCG with parabasal/basal cells [Figure 2]. Crushing artifact and degenerative changes/poor preservation added to the possible reasons for overinterpretation. The superficial resemblance of atypical squamous metaplasia with cytomorphology overlapping with HSIL is an additional pitfall. Some cases with additional many HCG in the background may lead to upgrading to LSIL-H. Many of these pitfalls are discussed previously in the literature.

Because the reversal of ACP-related changes/artifacts after topical estrogen cream or oral estrogen would improve the interpretation, it is recommended to repeat Pap test in all ACP cases with HGI after ET as clinically indicated. As applicable to other studies, this study is limited by lack of prospective follow-up with ET. Due to this, the hypothetical improvement could not be evaluated. Further studies evaluating the beneficial role of ET in reducing the false negatives are indicated.

In suspicious cases, reflex hrHPVT may be helpful as an ancillary test with higher NPV to rule out HGI in cases with negative hrHPVT. However, a few cases with negative hrHPVT showed biopsy positive for HGI [Table 2]. Application of p16 (with or without Ki67) may be valuable if cell blocks (made from residual liquid-based specimen) are available for further evaluation. Cell block making from residual liquid-based specimen or from parallelly collected brush sample directly in 10% formalin may be equated with brush biopsy. This would prevent unindicated surgical interventions with potential morbidity and prevent wasteful higher cost.

CONCLUSION

In conclusion, the cytopathological interpretation of Pap smear with ACP may be challenging. Due to higher chances of overinterpretation with higher false positives, the interpretation criteria should be applied conservatively while interpreting HGI with ACP. Ancillary tests including hrHPVT and p16 may be applied as indicated. Repeating the Pap smear after topical estrogen cream or oral estrogen is simple option to nullify ACP-related interference.
COMPETING INTERESTS STATEMENT BY ALL AUTHORS

The authors declare that they have no competing interests.

AUTHORSHIP STATEMENT BY ALL AUTHORS

All other co-authors reviewed this article before sharing it.

ETHICS STATEMENT BY ALL AUTHORS

This study was conducted with approval from the Institutional Review Board at Detroit Medical Center/Wayne State University, Detroit, Michigan.

LIST OF ABBREVIATIONS (In alphabetic order)

ACP – Atrophic cellular pattern
ET – Estrogen therapy
HCG – Atypical squamous cells-cannot exclude HSIL
HCG – Hyperchromatic crowded groups
HGI – Higher than LSIL (ASC-H, LSIL-H, and HSIL) interpretations
HGD – Higher than cervical intraepithelial neoplasm (CIN2) dysplasia
HSIL – High-grade squamous intraepithelial lesion
hrHPVT – High-risk HPV test
LEEP – Loop Electrosurgical Excision Procedure
LSIL-H – Low-grade Squamous Intraepithelial Lesions-cannot exclude HSIL
N/C – Nuclei-to-cytoplasm ratio
Non-ACP – Without ACP.

EDITORIAL/PEER-REVIEW STATEMENT

To ensure the integrity and highest quality of CytoJournal publications, the review process of this manuscript was conducted under a double-blind model (the authors are blinded for reviewers and vice versa) through automatic online system.

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