Over diagnosis of Epithelial Abnormalities in Atrophic Cervical Pap Smears

Unmasking Over diagnosis in Atrophic Pap smears

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Abstract:

Background: Cervical cancer has seen a significant decline in death rates due to early diagnosis and treatment. The Pap test, remains valuable but exhibits limitations such as false-positives and false-negatives, the former being represented by Atrophy-related changes. This article aims to bring attention to cervical carcinoma screening, focusing on the interpretation of atrophy-related changes in Pap smears and minimise intervention.

Methods: This retrospective study, conducted in a tertiary care centre evaluated cases with intra-epithelial abnormalities or malignancies in Pap smears. A total of 11680 cervical cytology smears received in the department of Pathology, Vydehi Institute of Medical Sciences and Research Centre, Bengaluru for 7.5 years from 1st January 2016 to 30th June 2023 were reviewed.

Results: 56 cases had epithelial abnormalities and were reported as the following 4 categories - 1. Atypical squamous cells of undetermined significance (ASCUS), 2. Low grade squamous intra epithelial lesion (LSIL), 3. High grade squamous intra epithelial lesion (HSIL) and 4. Malignancy. Out of 56 smears, 40 smears (71%) had co- existent atrophy. Biopsies were available in 22 smears, of which Atrophy and Epithelial abnormalities co-existed in 16 (16/56), 28% cases. Only 8 cases out of these 16 cases, (50%) were found to have higher than Cervical Intra-epithelial Neoplasia (CIN) II Dysplasia, which made the positive predictive value of cervical smear cytology to detect epithelial abnormalities in smears with co-existing atrophy related changes is only 50%.

Conclusion: The cytology of atrophic Pap smears may depict cells with nucleomegaly, hyperchromasia, overcrowding with granular debris in the background. This resembles neoplastic changes in most of the cases, in the absence of atypia on histology.

Keywords: Atrophy, Cervical cancer, Cervical dysplasia, Pap smear, Screening.

Introduction:

Worldwide, cervical carcinoma is the fourth most common cancer in women.(1) Fifty years ago, carcinoma of the cervix was the first cause of cancer mortality in women in the United States, eventually the death rate has declined by 75% and it has become the thirteenth cause of cancer mortality. No other form of cancer has shown the remarkable benefits of effective screening, early diagnosis and treatment than cervical carcinoma (2). It is because of the effectiveness of the Pap test in detecting the precursor lesions, some of which would have progressed to carcinoma if not treated; Also, the Pap test can help in detecting these at an early stage and hence can be treated effectively.(1)

The Pap test along with the Bethesda terminology have helped in the interpretation of these precursor lesions.(1)The classification of these precursor lesions has been updated over time and now as the decision of treatment is either observation or surgery, the older three-tier classification system has been recently simplified to a two-tiered system, with Cervical Intraepithelial Neoplasia (CIN) I renamed low-grade squamous intraepithelial lesion (LSIL) and CIN Il and CIN Ill combined into one category referred to as high-grade squamous intraepithelial lesion (HSIL)(2) as represented in Table 1.

The Pap smear test being the standard screening test for cervical dysplasia, still has its false-positives and false-negatives. One of the reasons of these false-positives would be a Pap smear associated with atrophy related changes. Atrophy is a normal aging phenomenon associated with lack of hormonal stimulation that leads to thinned out epithelium that only consists of immature basal/parabasal cells.

The changes that will be noted in Atrophic smears would be flat, monolayer sheets of parabasal-like cells with preserved nuclear polarity and little nuclear overlap. Dispersed parabasal-type cells may predominate, and may have mild hyper-chromasia and tend to have more elongated nuclei. Generalised nuclear enlargement may be seen (Figure 1). Autolysis may result in the presence of stripped nuclei. An abundant inflammatory exudate and basophilic granular background that resembles tumour diathesis may be present in examples of extreme atrophy (Figure 2). (3)

We aim to highlight the importance of the knowledge of mimickers of intraepithelial abnormalities and/or malignancy in Pap smears of which co-existing atrophy forms a great majority.

Methods:

In this retrospective study conducted in the department of pathology, Vydehi Institute of Medical Sciences and Research Centre, Bengaluru, a total of 11680 cases were received over a period of 7 years and 6 months from 1st January 2016 to 30th June 2023. The cervical cytology was performed as a part of cervical carcinoma screening guidelines at the discretion of the clinician after obtaining an informed consent. All the Pap smears were prepared by the Sure path (U-prep, Liquid based cytology).We reviewed 56 cases that were interpreted as having intra-epithelial abnormality or malignancy in cervical cytology Pap smears, out of these 56 cases, 40 cases (71%) were also found to have Atrophy related changes. 22 cases were followed up by cervical biopsy and histopathological evaluation in our hospital. Both cervical cytology Pap smears and biopsies were reviewed. The ethical clearance was obtained from the institutional ethics committee.

Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions.

The sensitivity, specificity, positive and negative predictive values were calculated.

Statistical software**:** MS Excel**,** SPSS version 22 **(**IBM SPSS Statistics, Somers NY, USA) was used to analyze data.

Results:

The mean age of subjects was 50.30 ± 10.9 years (Table 2).

Out of 56 cases interpreted as intra-epithelial abnormality or malignancy in the cervical Pap smears, 40 smears (71%) also had atrophy related cytological changes as shown in Figure 1.

22 cases which were reported as intra-epithelial abnormality or malignancy were followed by Cervical Biopsy and Histopathological evaluation for confirmation.

16 (40%) of these 40 smears with co-existent epithelial abnormality and atrophy smears had follow-up biopsy reports available. 8 out of these 16 biopsies (50%) confirmed the presence of higher than CIN Il dysplasia.

As represented in the Table 3, the total number of smears with co-existent epithelial abnormality and atrophy were 40.

Out of these ,10 smears (25%) were interpreted as Atypical Squamous cells of undetermined Significance (ASCUS), 11 smears (27%) were interpreted as Low grade Intraepithelial lesion (LSIL), 17 smears (42%) were interpreted as High Grade Intra-epithelial lesion (HSIL) and 2 smears (5%) were interpreted as having carcinoma. Most of the cervical pap smears with atrophy related changes were in the HSIL group.

16 cases interpreted as having Intra-epithelial abnormality by cervical pap smears did not have Atrophic changes and 8 smears (50%) were found to have ASCUS, 3smears (19%) were interpreted as LSIL and 5 smears (31%) were found to have HSIL.

Most of the cases that were interpreted as having intra-epithelial abnormality by cervical Pap smears with co-existent atrophy related changes were not actually found to have high grade dysplasia on follow-up biopsies as shown in Figure 3, which is shown by the data in Table 4.

Out of the total 40 cases with intra-epithelial abnormality and co-existent atrophy related changes, follow-up biopsy results were available in 16 cases, out which 8 smears (50%) were confirmed to have higher than CIN II dysplasia on biopsy (Table 4). This shows the **positive predictive value of cervical pap smear in the setting of co-existing atrophy related changes is only (8/16) 50%**.Unfortunately, the number of follow-up biopsies was very low.

Discussion:

In our study, out of 56 cases interpreted as intra-epithelial abnormality or malignancy in the cervical Pap smears, 40 smears (71%) also had co-existing atrophy related cytological changes. Majority of these 40% (17/40) were reported as HSIL. This was not the case in the study done by Li et al (1), were majority of the cases belonged to the ASCUS category in both atrophic and non-atrophic smears. The positive predictive value of cervical Pap smear in the setting of Atrophy related changes to detect higher than CIN II dysplasia in the present study was 50%, which was in concordance with study conducted by Li et al (1) who had a PPV of 54%.

Patton et al (4), reviewed Pap tests for a period of 3 years and 10 months from March 2003 to December 2006 that were diagnosed as ASC-H and divided them into postmenopausal, pregnant, postpartum, and contraceptive-use categories. Correlation was made with results from tissue specimens and/or from Digene Hybrid Capture II for human papilloma virus (HPV) .A total of 195 cases were retrieved. 135 cases (69.2%) had histologic follow-up. The frequency of high-grade follow-up in the postmenopausal category was compared with the frequency of high-grade follow-up in the other patient groups using the chi-square test. There was a statistically significant difference between the frequency of subsequent high-grade follow-up in the postmenopausal group compared with the other patient groups.

Bulten et al (5), propose that due to reduced levels of oestrogen in postmenopausal women, atrophic squamous epithelium of the cervix shows substantially diminished maturation which mimics dysplastic epithelium of high-grade cervical intraepithelial neoplasia (CIN 2 and 3). This “atypical” atrophic Pap smear in postmenopausal women is repeated after a course of systemic or locally applied estrogens, which allows the atrophic epithelium, in contrast to

dysplastic epithelium, to mature into normal squamous epithelium. In the study, Pap smears of postmenopausal women with an atypical atrophic Pap smear who underwent a second Pap smear after oestrogen treatment for definite diagnosis were used for the MIB1 restaining procedure. The Proliferative Activity Index (PAI) values were measured in MIB1 restained Pap smears obtained before and after oestrogen therapy. They concluded that majority of women with cervical atrophy had PAI < 0.17 and all patients with high-grade CIN had PAI >0.17. They concluded that PAI can be a cost-effective method to obtain a considerable reduction of additional diagnostic procedures in postmenopausal women with an atypical pap smear where atrophy can be a differential diagnosis. High-grade CIN can be referred correctly, without any delay, to the gynaecologist for further treatment.

Tabrizi (6) states in their review article that atrophic Pap smears may be misinterpreted as having epithelial abnormality in postmenopausal women. This is because of the abundance of parabasal cells that have relatively less amount of cytoplasm and a high nuclear/cytoplasmic ratio, condensed nuclear chromatin that leads to nuclear hyperchromasia and the presence of parakeratotic cells that are also noted in LSIL. The inflammatory cell debris clinging to cytoplasmic borders should not be interpreted as tumor diathesis.

Crothers BA et al (7) describe that when estrogen levels decrease in women, cervical and vaginal epithelium thins. The epithelium ceases to produce superficial and intermediate squamous cells, leaving only parabasal and basal cell populations. Initially, these cells are exfoliated as single cells or small groups of metaplastic-appearing cells, but with advanced atrophy, the epithelium becomes so thin that large surface fragments of parabasal cells exfoliate and may predominate. In their study they reviewed 18302 participant responses to 717 Pap slides from 2000 to 2009. For purposes of selection into the program, a slide would not be submitted as both atrophic vaginitis and NILM. They describe the smears that were overcalled as HSIL on an atrophic vaginitis slide had more degenerating parabasal cells, necrotic background, and pseudo parakeratotic cells, as well as had more inflammation and stripped nuclei and/or nuclear streaks.

Conclusion:

The cytology of atrophic Pap smears may depict cells with nucleomegaly, hyperchromasia, overcrowding with granular debris in the background. This resembles neoplastic changes in most of the cases, in the absence of atypia on histology. These can be followed up by p16 Immunohistochemistry and high risk human papilloma virus (hrHPV) testing which can be an area of further research. So, we need to be cautious and not overdiagnose epithelial abnormality in Pap smears with atrophy related changes.

The limitations of the present study, are that the number of cases that had follow-up biopsy report available after an interpretation of epithelial abnormality or malignancy in the Pap smears was less. The cervical biopsies were not followed by newer diagnostic entities like high risk human papilloma virus (hrHPV) testing, p16 Immunohistochemistry (IHC) or Proliferation Index markers.

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Not Applicable.

Ethics statement

The Ethical clearance was obtained from the Vydehi Institutional Ethics committee.

Registration number - ECR/747/Inst/KA/2015/RR21.

Conflicts of interest

The authors declare that they have no competing interests.

Author's contribution

FJU analysed and interpreted the patient data including the interpretation of pap smears and follow up cervical Biopsy, SL performed the cytological examination of pap smears and PS performed the histopathological examination of cervical Biopsy reports. All the authors read and approved the final manuscript.

Highlights:

Cervical pap smears are very instrumental in diagnosis of epithelial abnormality. They have reduced the morbidity and mortality related to cervical cancer significantly worldwide.

However, having the knowledge about the false positive interpretation of cervical Pap smears in presence of atrophy can help to avoid unnecessary intervention and help alleviate apprehension in patients.

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Tables and figures:

Table 1: Classification of precursor lesions of cervical carcinoma

Dysplasia/Carcinoma in situ	CIN	SIL
Mild dysplasia	CIN I	Low grade SIL
Moderate dysplasia	CIN II	High grade SIL
Severe dysplasia	CIN III	High grade SIL
Carcinoma in situ	CIN III	High grade SIL

CIN Cervical Intraepithelial Neoplasia, SIL Squamous Intraepithelial lesion

Table 2: Age distribution of patients

Age	Count	Percentage
<40 years	10	17.9%
41 to 50 years	20	35.7%
51 to 60 years	18	32.1%
61 to 70 years	5	8.9%
>70 years	3	5.4%
Total	56	100.0%

Mean age of subjects was 50.30 ± 10.9 years.

Table 3: Tabular Representation of Interpretation of Cervical Pap smears

Pap smears with	ASCUS	LSIL	HSIL
Atrophy	10(25%)	11(27%)	17(42%)
Non atrophy	8(50%)	3(18%)	5(31%)

Table 4: Tabular representation of comparison of results of pap smears and follow-up biopsy

Cases	IEL/M BY PAP	Available biopsies	Confirmed higher than CIN II on biopsy reported
Total	56 (100%)	22 (39%)	10
With atrophy	40 (71%)	16/22 (72%)	8 /16 (50%)
Without atrophy	16 (28%)	6/22 (27%)	2 /6 (33%)

Figures:

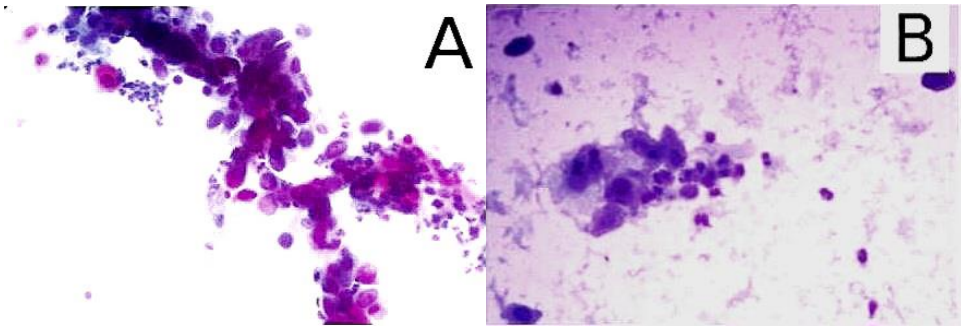
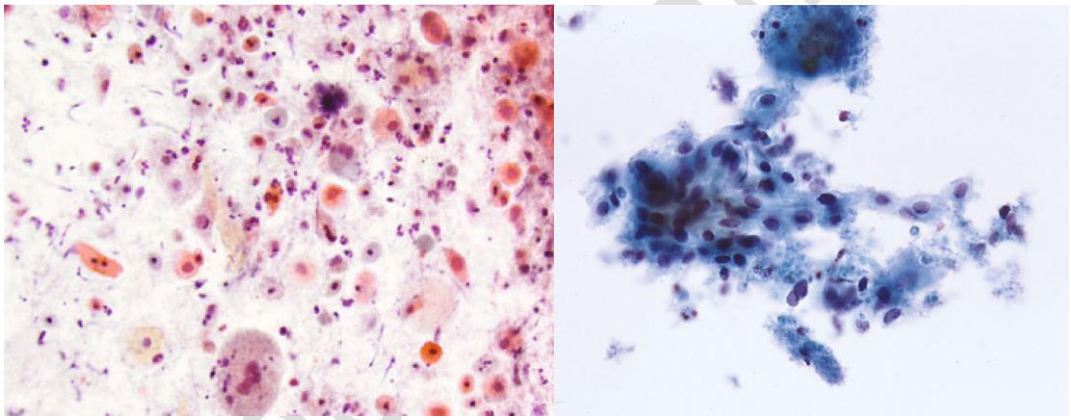


Figure 1A (Left) - Routine cervical cytology in a perimenopausal women showing cluster of cells with nuclear hyperchromasia and crowding.

Figure 1B (Right) - Liquid based cytology: group of hyperchromatic and degenerated nuclei against a background of atrophy with lysed cells and debris.



Atrophy with inflammation Liquid Based Cytology (LBC)

Figure 2A (Left) - Clumped granular debris clinging to atrophic cell clusters resembling tumor preparation.

Figure 2B (Right) - Degenerating parabasal cells, polymorphonuclear leucocytes in a granular debris.

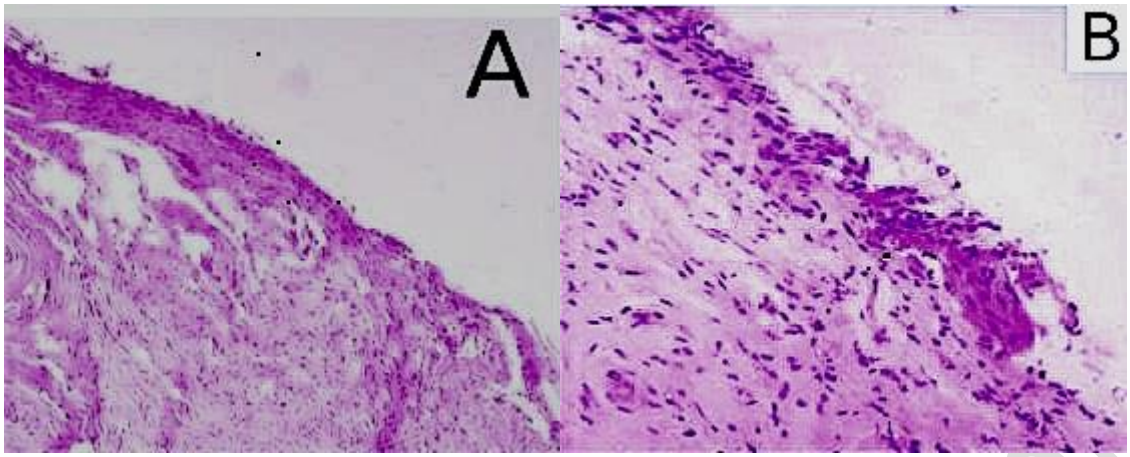


Figure 3A (Left) H&E 4x magnification.

3B. (Right) H &E Follow up cervical Biopsy. It shows superficial atrophic squamous epithelium.

From,

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To,

The Editor General,

Medical Laboratory Journal

Subject: Regarding publication of our study titled "Overdiagnosis of epithelial abnormality in Atrophic Cervical Pap smears "

Respected sir/Madam,

I Dr.Fasahath Jahan Uzma (Post graduate), Dr.Shilpa L (Associate Professor), Dr.Prathima S (HOD and Professor),Department of Pathology , Vydehi institute of medical sciences and research centre, Bengaluru declare that our study

Titled "Over diagnosis of epithelial abnormality in Atrophic Cervical Pap smears "

1. It has been carried out in Department of pathology, Vydehi institute of medical sciences and research centre, Whitefield ,Bengaluru, Karnataka
2. It has not been published earlier.
3. The correponding author is Dr.Fasahath Jahan Uzma,Post graduate ,ORCID ID 0009-0003-0554-0890.

Kindly consider our study for publication and do the needful.

Thanking you

Yours Faithfully

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Date: **07/07/2023**

CERTIFICATE OF ETHICAL CLEARANCE

This is to certify that the study titled "OVERDIAGNOSIS OF EPITHELIAL CELL ABNORMALITY IN CERVICAL PAP SMEARS WITH ATROPHY RELATED CHANGES" submitted by DR. FASAHATH JAHAN U/MA Post Graduate, Department of PATHOLOGY under the guidance of DR. SHILPA L , Associate Professor, Department of PATHOLOGY, Vydehi Institute of Medical Sciences & Research Center, Bangalore is approved by Vydehi Institutional Ethics Committee(VIEC)

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