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Original Article

Negative loop electrosurgical excision procedure (LEEP) following cervical biopsy diagnosis of high grade squamous intraepithelial lesion

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Abstract: Context: Loop Electrosurgical Excision Procedure (LEEP) is commonly performed after cervical biopsy diagnosis of high grade squamous intraepithelial lesion (HSIL/CIN2 or CIN 3). Histological and immunohistochemical assessments are made to differentiate reactive and metaplastic changes from dysplastic changes. A Human Papillomavirus (HPV) test is used for prognostic assessment after conization. Objective: We retrospectively reviewed cases where the cervical biopsy showed HSIL but the LEEP specimen was negative for high grade dysplasia. Our aim was to determine the cause of miscorrelation. Data: IRB approval was obtained and a search was made of all LEEP specimens received during 2018. We reviewed 25 of 137 LEEP specimens that did not correlate with the diagnosis of HSIL rendered on the cervical biopsy. These were from women between 25 to 54 years. All cases had positive high-risk HPV with 80% being non16/18 subtype. On review, 8/25 had HSIL with the remainder of cases falling short of HSIL diagnosis. Follow up cytology with HPV test after the LEEP procedure was negative in all but one case of LSIL with persistent non-16/18 HPV. Conclusion: The study highlights the diagnostic difficulties of distinguishing HSIL from immature squamous metaplasia. The practical implication is that in cases with non-16/18 high risk HPV which have thin epithelium and fall short of definite morphologic criteria of HSIL, presence of immature squamous metaplasia should be carefully evaluated. The specific role of CK7 and CK17 which highlight squamocolumnar junctional cells and metaplastic cells, respectively, needs to be explored in these cases.

Keywords: High grade squamous intraepithelial lesion (HSIL), immature squamous metaplasia, atypical immature squamous metaplasia, Loop electrosurgical excision procedure (LEEP), Human Papilloma Virus (HPV)

Introduction

Cervical carcinoma is amenable to early detection by screening cervical cytology, Human Papilloma Virus (HPV) testing and co-testing [1]. The US FDA has approved primary testing by HPV for the Cobas HPV and BD Onclarity with specific reporting of HPV 16 and 18 presence [2]. This test is approved for women 25 years and older with cytology only being the preferred method of screening those women under 25 years of age.

The pros of screening are obvious and include early detection of treatable lesions with reduction in incidence and mortality from cervical cancer. The cons of screening include economic health costs, psychosocial effects, false positive results with unnecessary procedures and associated negative effects of reproductive outcome [3, 4].

In our institution, patients are screened for cervical cancer by cytology and HPV testing using the 2019 American Society for Colposcopy and Cervical Pathology (ASCCP) risk based management consensus guidelines [5]. Briefly, the individual patient's risk estimate of developing CIN3 and above is based on previous screening history and current cytology/HPV results. In step with the recommendations, colposcopic biopsy diagnosis of CIN2 and CIN3 lead to treatment by LEEP and post treatment combined cytology and HPV surveillance. Post-treatment HPV based testing is more sensitive than cytology alone in detecting persistent/recurrent CIN.

From the foregoing, the colposcopic biopsy results of CIN2 and CIN3 trigger LEEP treatment. The aim of our study was to review the cases whereby the LEEP specimen did not show evidence of high-grade squamous lesions. Factors considered included overinterpretation of the biopsy, a small high-grade lesion that was completely removed by the biopsy and/or endocervical curetting and a lesion that was not captured by the LEEP.

Methods

Institutional Board Review (IRB) approval was obtained and a search was made of all LEEP specimens received during 2018. All cases with LEEP diagnosis of negative for dysplasia, reactive changes and low-grade dysplasia (LSIL/CIN I) were retrieved along with their previous cervical biopsy specimens.

Cervical biopsies were reviewed by two pathologists who were also boarded in cytopathology (FZA and JK). The reviewed biopsy results were recorded and if there was disagreement, then a third pathologist (RK) reviewed the slides as a tie breaker.

High grade squamous intraepithelial lesion (HSIL) was diagnosed when there was high nuclear density often with nuclear overlapping and hyperchromasia, anisonucleosis, and mitoses in the upper two thirds of the epithelium. When these morphologic criteria of HSIL were not met, and, in the presence of metaplasia, the lesion was denoted as atypical immature squamous metaplasia. The commonest scenario was when mitoses were notably absent in a lesion that would otherwise be consistent with HSIL. Immature squamous metaplasia was diagnosed when the epithelium was composed of a mixture of squamous and endocervical cells. Examples of these are illustrated in **Figure 3**.

The lesion thickness was estimated by counting the number of cell layers of the lesion and dividing it by the cell layer of the unaffected epithelium which was included in the biopsy. P16 was assessed as diffuse, block like positivity or patchy positivity. Ki-67 was scored as increased if it appeared in cells within the upper two thirds of the epithelium.

The LEEP specimen slides and accompanying endocervical curettage were reviewed. Per departmental protocol the LEEP specimen was entirely submitted for microscopic examination. Each cassette contained no more than two pieces of tissue and three levels were obtained upfront. The number of blocks of the LEEP specimen was recorded. All LEEP specimens were accompanied by endocervical curettage.

The preceding cytology and HPV status of the subjects was recorded. The test for high risk HPV DNA genotyping was performed using the Cobas® HPV test. The test utilizes amplification of target DNA by the real time Polymerase Chain Reaction (PCR) and nucleic acid hybridization for the detection of 14 high-risk (HR) HPV types in a single analysis. The test specifically identifies types HPV 16 and HPV 18 while concurrently detecting the rest of the high-risk HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).

Results

Of a total of 137 LEEP cases, we reviewed 25 specimens (18%) that did not correlate with the diagnosis of HSIL rendered on the cervical biopsy (**Figure 1**). This cohort was composed of 6 patients who were current smokers (4 with confirmed biopsy results of high-grade lesions), 5 former smokers (>4 years ago) and 14 non-smokers. No subjects had a history of immune system disorder.

The women were between the ages of 25 to 54 (average age 35.9 years; median 34 years). Only one subject was menopausal and three had preceding HSIL cytology result (two with confirmed HSIL on biopsy and one with immature squamous metaplasia). All cases had positive high-risk HPV with approximately 80% being non 16/18 subtype. Colposcopy appearance prior to cervical biopsy was suspicious for HSIL in only one case.

The LEEP specimens were processed as per standard protocol which dictated entire submission of the specimen with no more than 2 sections per block and three H&E levels provided on each block. Review of all LEEP specimens and the accompanying endocervical curettage showed no evidence of HSIL. The transformation zone was present in in at least

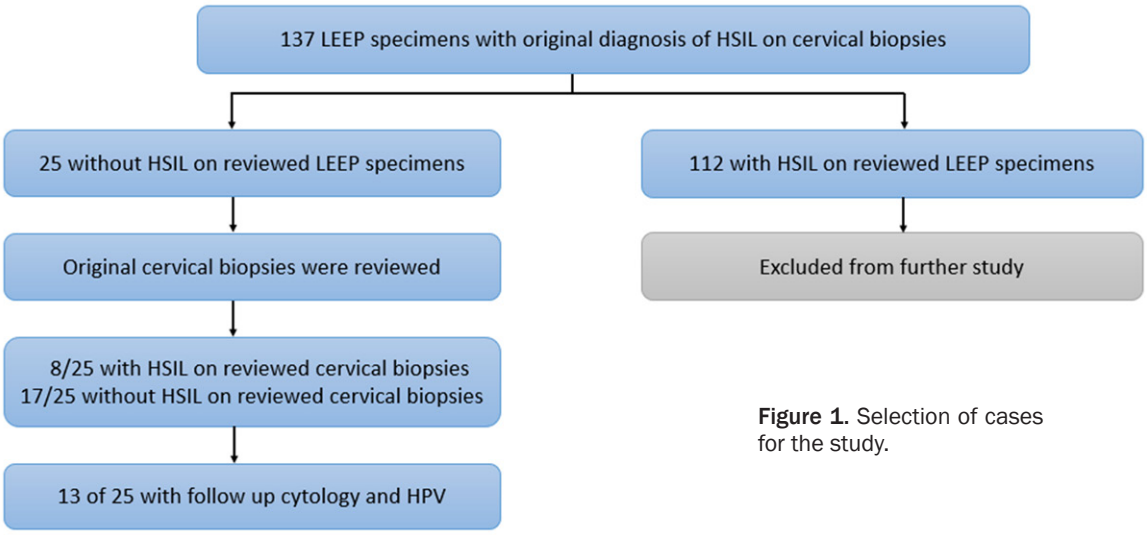

Figure 1. Selection of cases for the study.

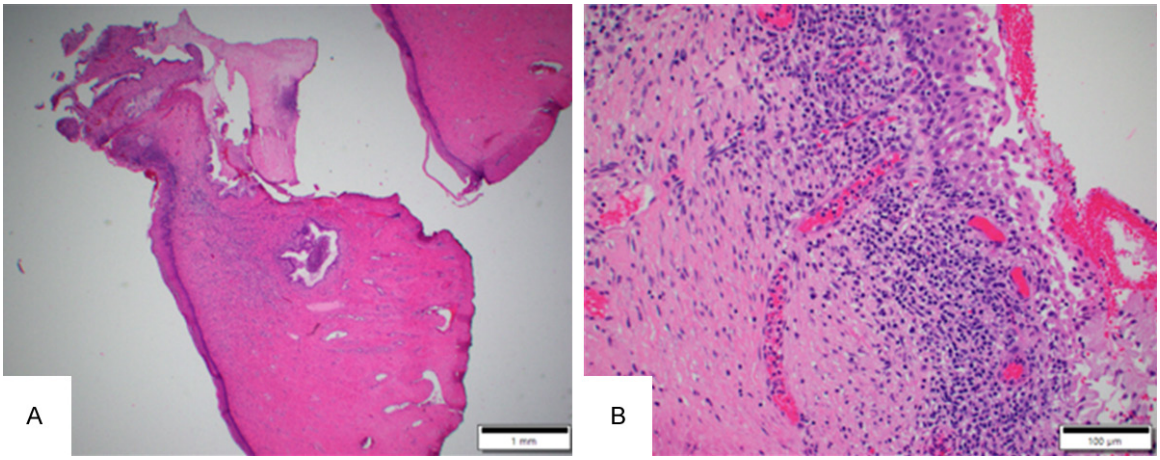

Figure 2. Example of a LEEP specimen. A shows a LEEP containing the transformation zone with focal chronic inflammation at the biopsy site (H&E ×20). B shows reparative cellular changes and associated chronic inflammation at the previous cervical biopsy site (H&E ×200).

one block of all LEEP specimens (**Figure 2**). The overall average number of blocks was 5.4 (median 5). For specimens with confirmed HSIL on the biopsy, the average number of blocks was 5.9 (median 4.5).

On review of the cervical biopsies, 8 of the total of 25 had HSIL including the one from the menopausal woman (**Table 1**). The remainder of cases fell short of morphologic HSIL diagnosis in that they displayed lack of mitoses in the suprabasal upper two-thirds of the epithelium (**Figure 3**). These were diagnosed on review as negative, atypical immature squamous metaplasia, immature squamous metaplasia, or CIN 1.

The size of the lesion on which the initial interpretation of HSIL was made (cervical biopsy specimen) was measured and ranged from 0.23 and 2.13 mm (average 0.81 mm and median of 0.62 mm). The average thickness of the lesions was 11.25 cells (or 59.7% of normal thickness). There was no difference between immature squamous metaplasia, atypical immature squamous metaplasia, or HSIL lesions with respect to size or thickness of the lesion. In other words, all the lesions were similarly thin with regard to number of layers of cells. Of those that had P16 performed (n=22), all but three showed diffuse full thickness positivity. Concurrent Ki-67 was performed on a fraction

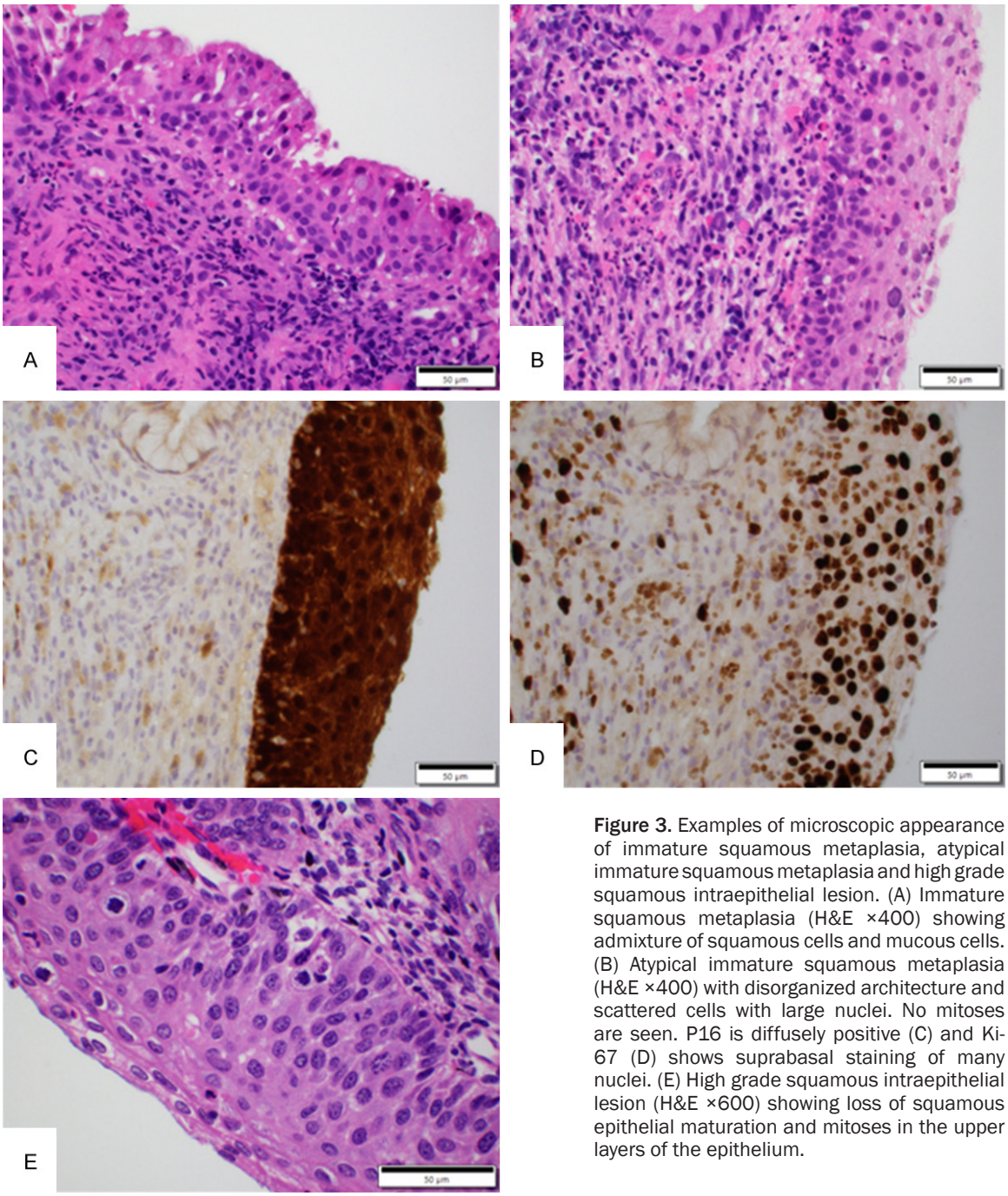

Figure 3. Examples of microscopic appearance of immature squamous metaplasia, atypical immature squamous metaplasia and high grade squamous intraepithelial lesion. (A) Immature squamous metaplasia (H&E ×400) showing admixture of squamous cells and mucous cells. (B) Atypical immature squamous metaplasia (H&E ×400) with disorganized architecture and scattered cells with large nuclei. No mitoses are seen. P16 is diffusely positive (C) and Ki-67 (D) shows suprabasal staining of many nuclei. (E) High grade squamous intraepithelial lesion (H&E ×600) showing loss of squamous epithelial maturation and mitoses in the upper layers of the epithelium.

of these (n=7; 28%) and all showed positive cells above the basal third of the epithelium.

Follow up data on HPV and cytology was available in twelve subjects with an additional subject having undergone total hysterectomy. Ten of the twelve subjects had negative high-risk HPV on subsequent follow up at 6 months. Two had persistent non-16/18 and one had corresponding LSIL on follow up cytology. None developed high grade SIL on follow up (≥24 months).

Interestingly, three subjects had received HPV vaccination including one who was partially vaccinated. One had the quadrivalent HPV vaccine and the other two did not have type of vaccine documented in the clinical charts. The prior cytology result of these subjects was positive for HSIL in two and all had high risk HPV.

Table 1. Results of 25 reviewed cervical biopsies previously interpreted as HSIL

Review of cervical biopsy	HPV 16	HPV 18	HR HPV+	Non 16/18	Not done	Grand Total
Atypical immature squamous metaplasia				3		3
CIN I				1	1	2
CIN II	3	1		1	1	6
CIN III				1	1	2
Immature squamous metaplasia	1	1	2	6	1	11
Negative				1		1
Grand Total	4	2	2	13	4	25

HSIL High grade squamous intraepithelial lesion; CIN Cervical Intraepithelial Neoplasia; HPV Human Papillomavirus.

Two had non-16/18 and one who was partially vaccinated had HPV 16 isolated on their cytology specimens.

Discussion

Biopsy diagnosis of HSIL is normally followed by LEEP. A significant minority of LEEPs did not have HSIL in our study. Our negative correlation rate of 18% is well within that reported in literature which ranges between 14-34% [6-11]. Factors considered included overinterpretation of the biopsy, a small high-grade lesion that was completely removed by the biopsy and/or endocervical curettage and a lesion that was not captured by LEEP.

We found that immature squamous metaplasia (with or without atypia) was frequently over interpreted as high grade SIL with resulting unnecessary treatment of the patient. The p16 diffuse staining of immature squamous metaplasia was confounding in these cases. The addition of Ki-67 in some of the cases did not discriminate between high grade SIL and immature squamous metaplasia. Similar findings are reported by another study which found 10% of atypical squamous metaplastic epithelium showed band like p16 and Ki67 positivity [12]. Van der Marel et al. [13] demonstrated a high rate of HPV positivity in both atypical immature metaplastic as well as immature metaplastic squamous foci. They suggest that transformation of immature metaplastic cells by high risk HPV is a possible pathway for the development of CIN and cervical carcinoma in which atypical immature squamous metaplasia is a critical step.

It is possible that some cases of miscorrelation are due to complete ablation by the cervical biopsy procedure. The five of the eight cases of HSIL on biopsy were most likely early lesions that were completely removed by the biopsy procedure since follow up HPV testing (more sensitive than cytology) was negative [14]. The remaining three cases were lost to follow up and hence did not have HPV tests.

On the other hand, it is unlikely that the lesion was not included or sampled in the LEEP procedure. All our LEEP specimens were at least partly surfaced by epithelium that could be assessed for HSIL and included the transformation zone. There was no difference in extent of sampling as the number of blocks was similar in those with reviewed biopsy diagnosis of HSIL or without HSIL. Additionally, all LEEPs were accompanied by endocervical curettage, none of which contained atypical cells.

A limitation of our study is that follow up data (at least 24 month) were available for only 13 of the 25 patients. All showed no evidence of high-risk HPV except for 2 who harbored non-16/18 HPV at follow up. Previous studies have reported no difference in the recurrence of HSIL subsequent to negative LEEP [8, 15]. Interestingly, one study showed that the risk of developing persistent/recurrent disease after treatment was significantly lower in patients with negative high-risk HPV test or a low viral load than in patients with a high viral load [7].

The FDA has approved three vaccines shown to be effective at preventing HPV infection: a bivalent vaccine (HPV-16 and HPV-18), a quadrivalent vaccine (HPV-6, HPV-11, HPV 16 and HPV 18) and a 9-valent vaccine approved in 2014, which covers an additional five high-risk HPV genotypes [16]. One of our patients had the quadrivalent vaccine and we did not have information on the type of vaccine for the second patient or the third partially vaccinated patient.

Interestingly, the partially vaccinated patient harbored HPV 16. It seems that HPV vaccination does show some protection against HPV 16 and 18. On the other hand, smoking is associated with HSIL as seen in the six patients who smoked, five of whom had HSIL. The association of smoking with cervical dysplasia and cancer has been previously reported [17]. The proposed mechanism is that the nicotine adducts damage the DNA [18] and may also depress the immune reaction to HPV infections [19] culminating in transformation of cervical cells into high grade dysplasia and invasive carcinoma. Notably, none of our patients had a history of immunosuppression.

In this context, the study by Regauer [20] using p16 and CK17 to distinguish immature squamous metaplasia from high grade SIL is intriguing. Although the authors did not specify their morphologic criteria for distinguishing HSIL from immature squamous metaplasia, they found that CK17 (a marker for cervical reserve stem cells) was positive in immature squamous metaplasia but negative in HSIL. In another study [21], CK7 highlighted squamocolumnar junctional cells which are expanded in SIL but not in metaplastic epithelium. The proposed mechanism is that metaplastic transformation is preceded by the appearance of subcolumnar reserve cells, initially as a single layer. With progressive proliferation depicted by extensive CK17 staining, immature and mature squamous metaplasia become apparent. The expression of CK7 heralds dysplastic change at the transformation zone. The utility of CK7 and CK17 are the focus of our future study.

In conclusion, we propose that a thin lesion on a cervical biopsy in a non-smoker who has non-HPV16/18 should be carefully scrutinized for the presence of immature squamous metaplasia before considering the diagnosis of high grade SIL. Ki-67 and P16 should not be relied on to confirm the presence of high grade SIL if the lesion does not meet morphologic criteria for high grade SIL. In such cases it is prudent to follow up in six months with combined cytology and HPV. In cases where excision is performed, a shallow LEEP would lower the risk of cervical stenosis or incompetence [3]. These measures would go some way towards minimizing the negative consequences of overtreatment by LEEP. Discovery of specific biomarkers allowing histologic distinction of HSIL from its mimickers would be of great value in this context.

Disclosure of conflict of interest

None.

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