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ANATOMICAL PATHOLOGY

High-grade squamous intraepithelial lesion (HSIL) of the cervix with bizarre cytological appearances ('pleomorphic HSIL'): a review of 19 cases

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Summary

Cervical high-grade squamous intraepithelial lesions (HSILs) are typically characterised by a proliferation of immature basaloid cells with relatively uniform hyperchromatic nuclei. In this report we describe 19 cases of HSIL exhibiting focal but very marked nuclear atypia often associated with multinucleation ('pleomorphic HSIL'). The bizarre cytological changes mainly involved the basal epithelium particularly in endocervical crypts where the neoplastic cells undermined the native glandular epithelial cells. Superficially invasive squamous cell carcinoma (SISCCA) was present in three cases (16%) and while this was more common than in a comparative series of 40 'conventional' HSIL excision specimens (5%), the difference was not statistically significant. All three invasive cases demonstrated additional histological features that have been associated with increased risk of SISCAA (expansile crypt involvement by HSIL, luminal necrosis, and/or intraepithelial squamous maturation), and the invasive foci were associated microanatomically with conventional-type rather than pleomorphic HSIL. The bizarre cells expressed p16 and p63 proteins but usually lacked mitotic activity and showed less Ki-67 labelling than adjacent conventional HSIL. These findings suggest that pleomorphic epithelial changes in HSIL do not necessarily indicate more aggressive biological behaviour and may, in some cases, represent a degenerative phenomenon.

Key words: Cervix; HSIL; pleomorphic; cervical intraepithelial neoplasia; invasion; immunohistochemistry; bizarre.

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INTRODUCTION

Squamous cell carcinoma (SCC) of the cervix remains one of the most common cancers worldwide and has a well-established causative association with HPV infection.[1] However, in countries with effective screening programs the majority of neoplastic squamous lesions are detected at the pre-invasive stage, histologically represented by squamous intraepithelial lesions also referred to as cervical intraepithelial neoplasia (CIN).[2] It is widely accepted that high-grade squamous intraepithelial lesions (HSILs)/CIN 3 are the precursor of cervical SCC although not all cases progress to invasive carcinoma.[3,4]

The characteristic histological features of HSIL are familiar to all surgical pathologists who examine cervical biopsy or hysterectomy specimens. Typically, there is replacement of the native cervical epithelium by a relatively monomorphic population of crowded and disorganised keratinocytes that exhibit basaloid or undifferentiated appearances with hyperchromatic nuclei and scant cytoplasm. Mitotic and apoptotic figures are conspicuous and usually present in all cell layers.[5–7] Occasionally, however, HSILs include larger, more differentiated appearing squamous cells with vesicular nuclei, prominent nucleoli and more abundant cytoplasm, and sometimes keratinisation may be present.[8,9] These features have been termed intraepithelial squamous maturation (ISM),[8] and they are seen most commonly in superficial cells close to the mucosal surface where they may coexist with HPV-associated morphological changes. Extensive surface and expansile endocervical crypt involvement by HSIL, luminal necrosis within endocervical crypts, and ISM have been associated with increased risk of associated stromal invasion and therefore their presence is an indicator for thorough histological sampling.[8]

In recent years we have encountered cases of HSIL in which there was focal but striking nuclear pleomorphism, often associated with multinucleation, sufficiently obvious to be evident at scanning magnification. Although these changes overlap to some extent with those of ISM and HPV effect, the pleomorphic cells were more frequently seen within endocervical crypts and distributed along the basal layer of the epithelium, often undermining residual glandular epithelial cells. On occasion, there was difficulty distinguishing these changes from those of superficially invasive squamous cell carcinoma (SISCCA), particularly when apparently detached clusters of cells with highly atypical nuclei were present in the stroma. To our knowledge the nature and significance of these bizarre cytological changes in HSIL ('pleomorphic HSIL') is not established. Therefore, in the current study we have reviewed the clinicopathological features of a series of such cases, including immunohistochemical analysis in a subgroup of lesions.

METHODS

The study group comprised 19 consecutive cases of pleomorphic HSIL (as defined below) encountered by the author over the 5-year period 2012–2016. Thirteen cases were accessioned at the histopathology department of King Edward Memorial Hospital (KEMH), Perth, and six were seen in consultation. All patients were asymptomatic and had undergone colposcopy following abnormal findings on cervical cytology screening. The index

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cytology was reported to be low-grade squamous intraepithelial lesion/CIN 1 in two cases, possible HSIL in four cases, and definite HSIL in 13 cases: in two of the latter the cytological changes raised the possibility of invasion and this was confirmed histologically in one case. All patients had colposcopic changes consistent with HSIL, and in one pregnant patient colposcopy was concerning for invasive malignancy but this was not confirmed histologically.

Two patients underwent only cervical punch biopsy, 16 had punch biopsy followed by a large loop excision of the transformation zone (LLETZ) or cone biopsy, and one had a cone biopsy as her primary diagnostic procedure. Four of the patients who had a LLETZ procedure later underwent cone biopsy and one of these patients also had a subsequent hysterectomy; all of these specimens were negative for residual neoplasia. All histological specimens were reviewed.

For inclusion in this study at least one biopsy specimen from each patient demonstrated HSIL of 'conventional' (basaloid) type, and at least one specimen showed pleomorphic HSIL. The latter was characterised by keratinocytes exhibiting marked nuclear enlargement, hyperchromasia and pleomorphism, often accompanied by multinucleation. Nuclear diameter was at least 4–5 times that of normal basal cells,[9] and the degree of nucleomegaly and atypia was sufficient to be recognisable at scanning magnification (×40). Representative examples are illustrated in Figs. 1 and 2. The distribution of the pleomorphic cells (surface versus endocervical crypt epithelium, and basal versus superficial/luminal location) was noted. The excisional biopsy specimens (LLETZ and cone biopsies) were also reviewed for the presence of stromal invasion and for additional microscopic features that have been associated with invasion, namely extensive and expansile endocervical crypt involvement by HSIL, luminal necrosis, and ISM.[8] To determine whether cases of pleomorphic HSIL were more frequently associated with SISCCA, the results were compared with a separate consecutive series of 40 excisional procedures (LLETZ or cone biopsies) performed for presumptive HSIL during the study period.

Immunohistochemistry

Immunostaining for p16 protein, Ki-67 and p63 was performed in 14, 14 and six cases, respectively, during initial diagnostic assessment. Cyclin D1 expression was subsequently investigated in the six cases that showed the most extensive pleomorphic HSIL since cyclin D1 immunoreactivity may be a marker of superficial stromal invasion in early stage cervical SCC.[10]

RESULTS

The mean and median ages were 33.2 years and 28 years, respectively (range 20–71 years). Sixteen patients had a final diagnosis of HSIL while three (15.8%) had SISCCA. One of the latter cases showed two separate foci of stromal invasion while invasion was unifocal in the remaining two cases. All invasive foci measured <2 mm in lateral extent and <1 mm in depth, and they were associated with adjacent conventional-type rather than pleomorphic HSIL (Fig. 3). Lymphovascular space invasion was not identified in any case. Two specimens (5%) in the comparative series of 40 LLETZ/cone biopsies showed SISCCAA. The difference in the incidence of invasion was not statistically significant ($p = 0.16$).

Follow-up cervical cytology was available in 14 patients ranging from 9 months to 30 months (mean 21 months) after the excisional procedures, and this was negative in all cases. Eight of these patients also had negative HPV testing during follow-up. Four of the remaining cases were recent (<6 months) while no additional follow-up was available in the single patient who had a negative cone biopsy and hysterectomy after her LLETZ procedure.

HSIL involved the surface epithelium alone in three cases and both the surface epithelium and endocervical crypts in 15 cases. In the remaining case HSIL was identified only in endocervical crypts with atrophic and inflammatory changes in the overlying squamous epithelium (Fig. 4); however, this patient's LLETZ specimen was stripped of surface epithelium in many areas and therefore involvement by HSIL may have been missed. Seven cases showed 'expansile' crypt involvement by HSIL and five of these cases, including all

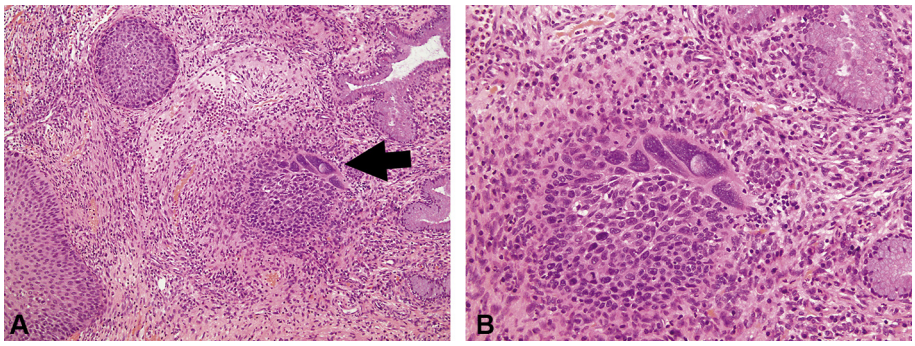

Fig. 1 Pleomorphic high-grade squamous intraepithelial lesion (HSIL). (A) Low magnification demonstrating HSIL involving endocervical crypts. Focal marked cytological atypia is present at the basal aspect of one crypt (arrow). (B) Higher magnification showing marked nucleomegaly compared with adjacent basaloid HSIL.

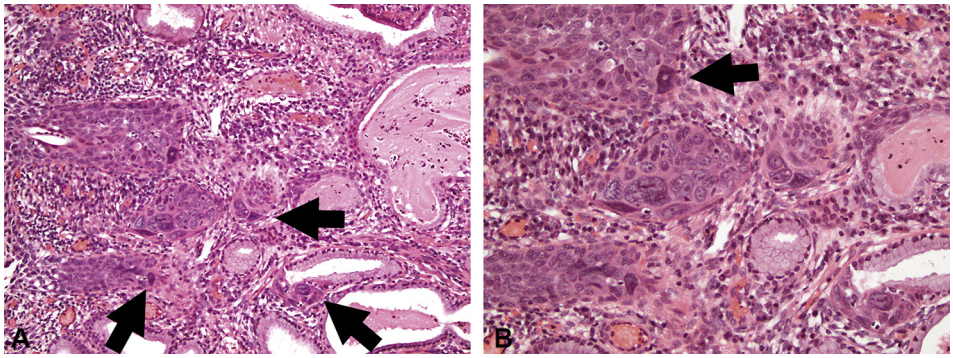

Fig. 2 Pleomorphic high-grade squamous intraepithelial lesion (HSIL). (A) HSIL involving endocervical crypts with multifocal marked cytological atypia (arrows). (B) Higher magnification showing degenerative nuclear appearances of some of the atypical cells (arrow).