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Original Article

Thin HSIL of the Cervix: Detecting a Variant of High-grade Squamous Intraepithelial Lesions With a p16^{INK4a} Antibody

Olaf Reich, M.D. and Sigrid Regauer, M.D.

Summary: The WHO defines thin high-grade squamous intraepithelial lesions (HSIL) as a high-grade intraepithelial lesion of the cervix that is usually $\leq$9 cells thick. These lesions usually develop in early metaplastic squamous epithelium without anteceding low-grade squamous intraepithelial lesions (LSIL). The prevalence of thin HSIL is not well documented. We evaluated different characteristics of thin HSIL at time of treatment. We studied 25 formalin-fixed and paraffin-embedded conization specimens processed as step-serial sections. HSIL$\leq$9 cells thick were classified as thin HSIL. HSIL$\geq$10 cells thick were classified as classic HSIL. Immunohistochemical p16^{INK4a} staining was used to confirm lesions of thin HSIL. Overall, 19 (76%) specimens contained both thin HSIL and classic HSIL, 4 (16%) contained thin HSIL only, 1 (4%) contained classic-type HSIL only, and 1 (4%) contained thin HSIL and LSIL. Thin HSILs developed in both the columnar surface epithelium and deep cervical glandular epithelium. Most thin HSILs were 5 cells thick. All HSILs (thin and classic) were located inside the transformation zone and had a median horizontal extension of 8 mm (range, 0.3 to 21 mm). Our findings suggest that thin HSILs are frequent findings, that they coexist with classic HSIL, and preferably arise in the exposed parts of the transformation zone including the glandular crypts. **Key Words:** Cervix—Carcinogenesis—Thin High-grade squamous Intraepithelial Lesions.

Cervical squamous cell cancer and its precursors develop predominately in metaplastic squamous epithelium inside the transformation zone (TZ). In the original squamous epithelium cervical cancer and its precursors are rare (1,2). The concept of transformation (squamous metaplasia) is central to the understanding of the pathogenesis of squamous cell carcinomas and its precursors because the distribution and extension of cervical cancer precursors largely correlate with the extent of the TZ (1–3). Inside the TZ there are at least 2 types of high-grade squamous intraepithelial lesions (HSIL). One is the well-described classic HSIL, which develops through low-grade squamous intraepithelial lesions (LSIL) after high-risk human papilloma virus (HR-HPV) infection of mature stratified metaplastic squamous epithelium (1,2,4). In classic HSIL, cellular atypia and atypical mitotic figures are present in all layers of the squamous epithelium to an extent and degree that exceeds those of LSIL (1). In the second pathway, HSIL develops in early metaplastic squamous epithelium without anteceding LSIL (2,5). These lesions develop in nonstratified or very thin immature squamous epithelium and are defined as thin HSIL. Koiloytes are typically absent. The WHO defines thin HSIL as a variant of HSIL of the cervix that is usually $\leq$9 cells thick (6). Immature basal cells occupy the entire epithelium and can be covered by a

From the Department of Obstetrics & Gynecology (O.R.); and Institute for Pathology (S.R.), Medical University of Graz, Graz, Austria.

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Address correspondence and reprint requests to Olaf Reich, MD, Department of Obstetrics & Gynecology, Medical University of Graz, Auenbruggerplatz 14, A-8036 Graz, Austria. E-mail: olaf.reich@medunigraz.at.

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layer of columnar cells. Thin HSIL typically show continuous, strong, diffuse staining with $p16^{INK4a}$ that allow a definitive diagnosis (6,7). The prevalence of thin HSIL and association with classic HSIL is unclear. This study addresses different characteristics of classic HSIL and thin HSIL at time of treatment.

MATERIALS AND METHODS

We studied 25 consecutive patients with biopsy-confirmed HSIL of the cervix who underwent large loop excision of the TZ at our institution between March 2015 and May 2015. The formalin-fixed and paraffin-embedded conization specimens were processed as step-serial sections. Immunohistochemical $p16^{INK4a}$ overexpression was used to separate immature metaplastic lesions from lesions of thin HSIL or classic-type HSIL and staining was performed specifically for this study. HSIL $\leq$ 9 cells thick were classified as thin HSIL. HSIL $\geq$ 10 cells thick were classified as classic-type HSIL. The classification was done by 2 pathologists (S.R. and O.R.). Four locations of HSILs were distinguished (Fig. 1): ectocervical in the original squamous epithelium, ectocervical between the external os and the last gland, deep in the cervical glands, and in the cervical canal. The last gland is a landmark separating the TZ from the original squamous epithelium of the cervix (2,3). The last gland, the estimated position of the external os, and the site and extent of the entire lesion was noted and marked on the histologic slides.

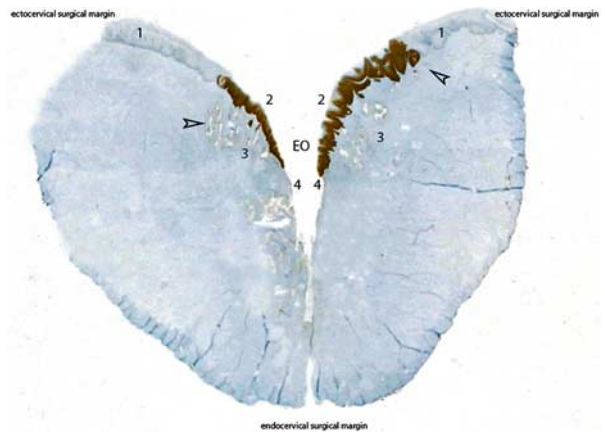

FIG. 1. Schematic illustration of locations of HSIL in a cone specimen: open arrow = last cervical gland (on the right side last gland is involved by SIL; on the left side last gland is without SIL); EO = external os; (1) ectocervix in the original squamous epithelium, (2) ectocervix between the external os and the last gland, (3) deep cervical glands, (4) endocervical canal. HSIL indicates high-grade squamous intraepithelial lesions.

RESULTS

The median age of patients was 36 years (range, 22 to 51 y). All HSIL (thin and classic) showed $p16^{INK4a}$ overexpression in all atypical cells. Among the 25 cone specimens, 19 (76%) contained both, thin HSIL and classic HSIL, 4 (16%) contained thin HSIL only, 1 (4%) contained classic-type HSIL only, and 1 (4%) contained thin HSIL and LSIL (Table 1). Thin HSIL were 1 to 9 cells thick, with the majority being only 5 cells thick (Fig. 2). Eighteen cone specimens (72%) contained multifocal thin HSIL affecting both (44%) or 1 lip (56%) of the cervix. The median size of the entire lesion of HSIL (thin and classic) was 8 mm (range, 0.3 to 21 mm; 8 lesions were $<$ 5 mm, 8 lesions were 5 to 10 mm, and 9 lesions were $>$ 10 mm) (Table 1). The anatomic location of classic HSIL corresponded to that of thin HSIL. Twenty-two of 25 patients had thin HSILs on the surface of the cervix inside the TZ, i.e., all thin HSIL were located between the external os and the last gland. Of the 22 patients with thin HSIL on the surface of the cervix, 19 patients had concomitant thin HSILs deep in cervical glands. Eight of 25 patients had a thin HSIL located within the endocervical canal. All 8 patients had synchronous thin HSILs deep in cervical glands and 6 of 8 patients had simultaneous thin HSILs in the ectocervical surface epithelium. No thin HSIL was observed in the original squamous epithelium.

DISCUSSION

In 2014, the WHO described thin HSIL as a variant of cervical HSIL that is usually $\leq$9 cells thick (6). In clinical practice, thin HSIL is subject to a high level of interobserver variability and has been a diagnostic problem if immunohistochemistry is not used. Formerly lesions now classified as thin HSIL

TABLE 1. *Characteristics of HSIL (Thin and Classic) in 25 Cone Specimen*

HSIL	N (%)
Thin and classic HSIL	19 (76)
Thin HSIL only	4 (16)
Classic HSIL only	1 (4)
Thin HSIL and LSIL	1 (4)
Thin HSIL multifocal lesions	18 (72)
Thin HSIL both lips	11 (44)
Thin HSIL 1 lip only	14 (56)
Thin HSIL singular lesion	2 (28)
Lesion $<$ 5 mm (classic and thin)	8 (32)
Lesion 5-10 mm (classic and thin)	8 (32)
Lesion $>$ 10 mm (classic and thin)	9 (36)

HSIL indicates high-grade squamous intraepithelial lesions.

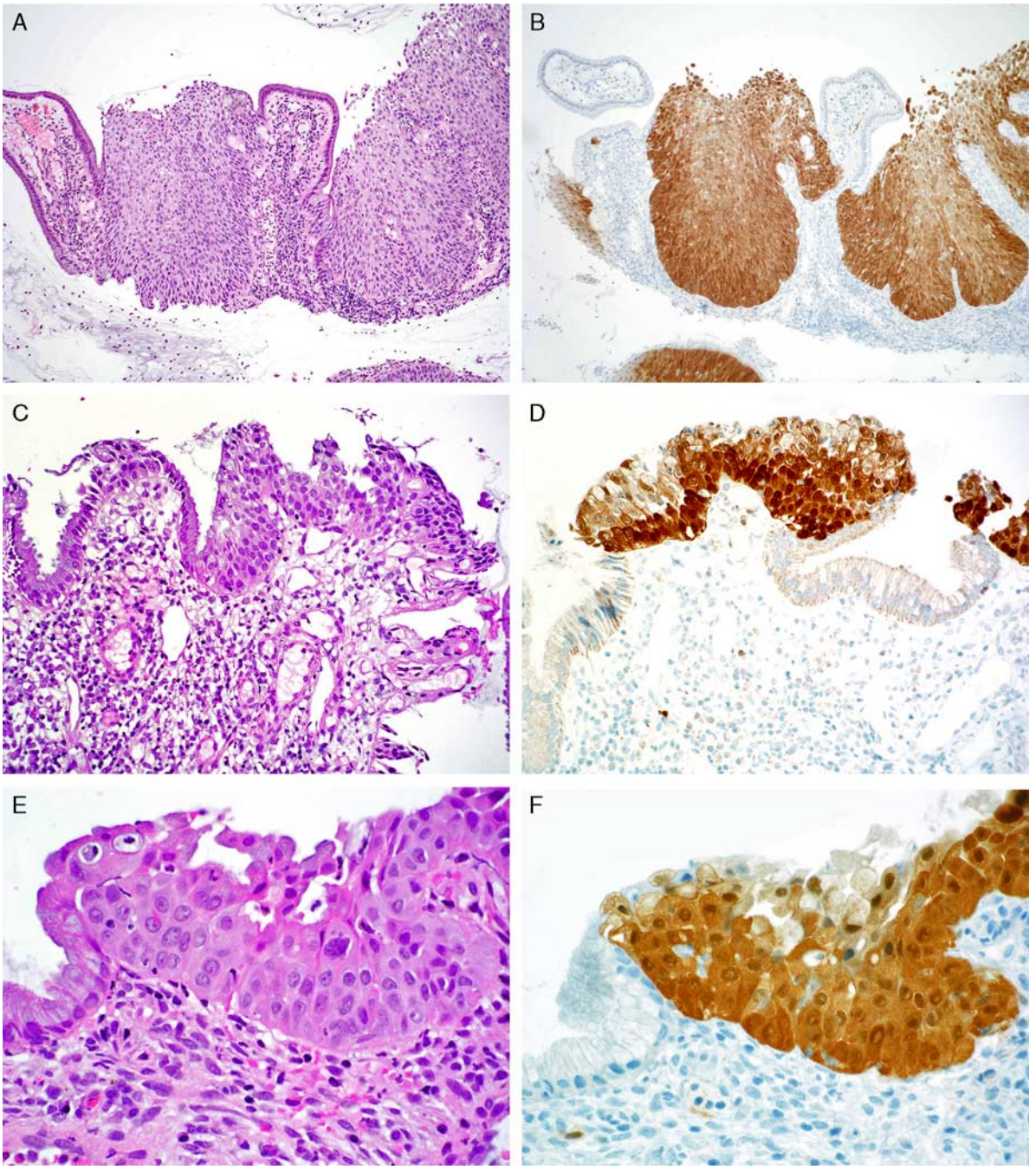

FIG. 2. A and B, Classic HSIL. A, HE stain showing sharply outlined classic HSIL of ≥ 10 cell layers flanked by nondysplastic single-layered mucinous columnar epithelium. B, Corresponding immunohistochemical stain with antibody to $p16^{INK4a}$ illustrates overexpression with a continuous uniform staining of all atypical cells beginning at and including all basal keratinocytes. C–F, Thin HSIL. C, HE stain shows a thin HSIL arising on the surface of the transformation zone underneath persisting columnar epithelium. HSIL consists of < 5 cell layers and is bordered by nondysplastic single-layered columnar epithelium. D, Corresponding immunohistochemical stain with antibody to $p16^{INK4a}$ illustrates overexpression with uniform staining of all atypical cells beginning at and including all basal keratinocytes. The overlaying columnar mucinous cells show no reaction. E, HE stain shows a focus of thin HSIL in an endocervical gland consisting of 3 to 5 cell layers with sharp transition to nondysplastic single-layered columnar epithelium. F, Corresponding immunohistochemical stain with antibody to $p16^{INK4a}$ illustrates overexpression with uniform staining of all atypical cells. Intraepithelial inflammatory cells and overlaying columnar mucinous cells show no reaction. HSIL indicates high-grade squamous intraepithelial lesions.

were likely grouped into categories such as atypical immature metaplasia (Table 2). Demonstration of $p16^{INK4a}$ overexpression significantly improves the interpretation of H&E slides (7,15).

This study systematically examined conization specimens to better define the characteristics of thin HSIL. Most specimens in our study showed both classic HSIL and thin HSIL (76%). A minority of specimens showed classic HSIL only, thin HSIL only, or thin HSIL occurring together with LSIL. A majority of thin HSIL (72%) occurred multifocally affecting both (44%) or 1 lip (56%) of the cervix (Table 1). Thin HSIL involved the surface of the cervix and also the endocervical glands. Only 8 patients had thin HSIL in the endocervical canal, where the epithelium may be less exposed to HR-HPV than at the ectocervix. Thin HSIL were seen independently of the size of the entire lesion (Table 1).

Although the WHO defines thin HSIL as lesions of 1 to 9 cell layers (6), the majority of thin HSIL in our series were only 5 cell layers thick (Fig. 2). This suggests that the WHO definition is rather cautious, as full-thickness lesion consisting of 6 to 9 cell layers do not typically pose a diagnostic problem. The difficult cases are those with ≤ 5 cell layers, often within a gland, without concomitant classic HSIL in biopsies.

Surface and glandular involvement by thin HSIL can be explained in different ways. One explanation would be lateral spread of cells from a classic HSIL undermining the adjacent columnar epithelium. Alternatively, thin HSIL could be a direct result of HR-HPV infection with subsequent neoplastic transformation of early metaplastic immature squamous epithelium (1,5).

TABLE 2. *Historical Terms Used to Describe Atypical Immature Squamous Epithelium of the Cervix Including Thin SIL*

References	Term
Howard et al (8)	Squamous metaplasia with atypia
Stoddard (9)	Atypical reserve cell proliferation
Johnson et al (10)	Anaplasia of the subcolumnar or reserve cells
Haam and Old (11)	Atypical reserve cell hyperplasia, atypical squamous metaplasia
Coppleson and Reid (12)	Atypical metaplasia
Burghardt (3)	Atypical squamous metaplasia
Crum et al (13)	Atypical immature squmous metaplasia
Park et al (14)	Atypical immature metaplastic-like proliferation
Regauer and Reich (7)	CIN III/HSIL ($\times$)

($\times$) positive for $p16^{INK4a}$.
HSIL indicates high-grade squamous intraepithelial lesions.

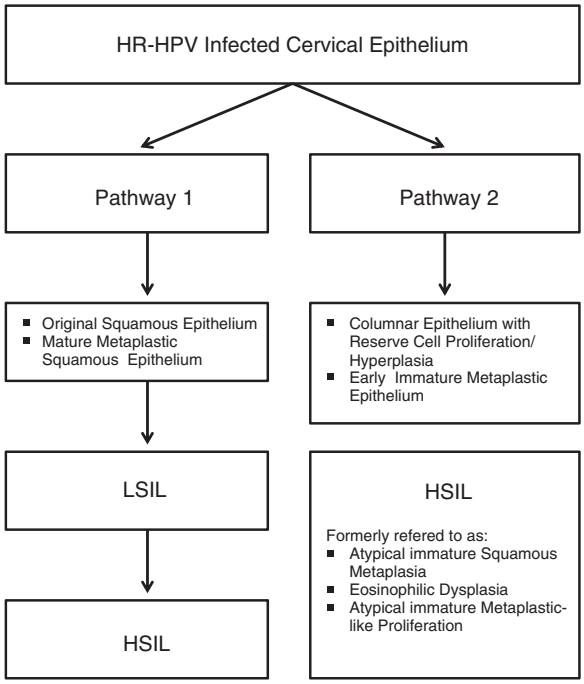

FIG. 3. Schematic illustration of 2 types of pathogenesis of HSIL (classic and thin). HSIL indicates high-grade squamous intraepithelial lesions; LSIL, low-grade squamous intraepithelial lesions.

Metaplasia denotes the gradual transformation of columnar epithelium into squamous epithelium under the influence of increased estrogen levels and growth of vaginal bacterial flora. Metaplasia is preceded by the appearance of so-called subcolumnar reserve cells beneath the columnar epithelium (3). Subcolumnar reserve cells are probably derived from a specific cell embryonic cell population (16,17). Reserve cell proliferation and subcolumnar reserve cell hyperplasia is followed by progressive maturation with development of multilayered squamous epithelium (1–3).

Our view is that there are 2 pathways of development of HSIL (Fig. 3) and that thin HSIL is the result of HR-HPV infection of reserve cell proliferation/ hyperplasia and immature metaplastic epithelium. Because reserve cell hyperplasia and immature metaplasia lacks a protective layer of terminally differentiating cells, it may be more susceptible to HR-HPV infection than mature squamous epithelium.

In summary, we demonstrate that thin HSIL is a common finding, if looked for. Thin HSIL was more common in the exposed parts of the cervix, that is,

the surface epithelium of the ectocervix inside the TZ. Most thin HSIL coexist with classic HSIL. These data suggest that the phenotypic presentation of HSIL depends on the degree of maturation of the epithelium at the time of HR-HPV infection.

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