

Low-Grade Squamous Intraepithelial Lesion Diagnosed by Colposcopy-Directed Biopsy with More Severe Lesions Undetected

Fengyi Xiao¹, Long Sui¹¹Obstetrics and Gynecology Hospital, Fudan University, Shanghai, P. R. China

Summary

Objective: We aimed to explore the factors involved in high-grade squamous intraepithelial lesion (HSIL) or more severe lesions undetected by colposcopy-directed biopsy (CDB). **Materials and Methods:** We retrospectively reviewed 2,427 patients diagnosed with low-grade squamous intraepithelial lesion (LSIL) by CDB. After undergoing the loop electrosurgical excision procedure (LEEP), 2,023 patients were classified as having LSIL (group A), 393 as having HSIL (group B), 6 as having squamous cell carcinoma (group C), and 5 as having adenocarcinoma in situ and adenocarcinoma (group D). **Results:** The patients in groups D (47.6 ± 4.0) and B (39.7 ± 8.2) were significantly older ($p < 0.01$) than those in group A (37.6 ± 8.4). The proportion of multicentricity detected by CDB was significantly higher in group B (13.2%) than in group A (8.6%) ($p = 0.003$). CDB detected glandular involvement (GI) in 0.35% of the patients in group A, 0.51% in group B, and none in groups C and D ($p = 0.964$). The circumference, width, and length of LEEP specimens were significantly higher ($p = 0.000$, $p = 0.011$, and $p = 0.000$, respectively) in group B (2.8 ± 0.8 cm, 0.75 ± 0.24 cm, and 1.23 ± 0.29 cm, respectively) than in group A (2.6 ± 0.9 cm, 0.72 ± 0.23 cm, and 1.13 ± 0.31 cm, respectively), which are indicative of the presence of larger lesions in group B. **Conclusion:** Older age, but not GI, in patients diagnosed with LSIL by CDB is indicative of HSIL+ lesions. CDB-diagnosed LSIL patients with large lesion sizes and multicentric lesions may have occult HSIL.

Key words: Colposcopy; Age; LSIL; Multicentricity; Glandular involvement.

Introduction

Low-grade squamous intraepithelial lesion (LSIL) is defined as the cytopathic effect of transient human papillomavirus (HPV) infection known as koilocytosis and mild dysplasia or cervical intraepithelial neoplasia 1 (CIN1) [1]. It was first introduced in the 2001 Bethesda System [2]. Since LSIL frequently regresses, it is generally treated with more conservative measures or even just follow-up [3, 4]. However, a high proportion of women with persistent LSIL had HSIL on post-LEEP histopathology was reported [5]. The risk of occult CIN3+ among women with CIN1 at colposcopic biopsy was reported to be associated with the risk conveyed by prior cytology [6]. Women with CIN1 after LSIL or HPV-positive atypical squamous cells of undetermined significance (ASC-US) had a 5-year risk of CIN3+ of 3.8%, while those with CIN1 after HSIL had a 5-year risk of CIN3+ of 15% [7]. However, features of colposcopic biopsy findings that may be indicative of occult HSIL+ lesions have not been fully explored. In the present study, we focused on the factors, apart from cytology and HPV testing results before colposcopy, which are related to occult HSIL+ lesions.

Cervical lesions are sometimes multicentric; therefore, more severe lesions could possibly be undetected by colposcopy-directed biopsy (CDB). The loop electrosurgi-

cal excision procedure (LEEP) is a treatment option for LSIL. For women with LSIL cytology, cryotherapy and conisation/LEEP decreased the subsequent risk of CIN3+ lesions, especially in women with biopsy-proven LSIL [8].

Glandular involvement (GI) is defined as the involvement of the endocervical glands by a squamous lesion [9]. Endocervical glandular involvement, positive endocervical surgical margin, and multicentricity detected by LEEP or cold knife conisation have been reported to be more often associated with high-grade squamous intraepithelial lesion (HSIL) than LSIL [10, 11]. GI has also been reported to be related with the reappearance of CIN after loop excision with clear margins [12]. Nevertheless, the detection of GI and multicentricity by CDB and their possible association with HSIL+ or LSIL have not been explored to the best of our knowledge.

In this study, we retrospectively analysed LSIL patients who subsequently underwent LEEP and aimed to explore factors such as age, GI, or multicentricity, which were detected by CDB and may be indicative of HSIL+ lesions in CDB-diagnosed LSIL.

Materials and Methods

We performed a retrospective cohort study of women with CDB-diagnosed LSIL in the Obstetrics and Gynecology Hospital of the Fudan University from December 2006

Table 1. — *GI and multicentricity detected by CDB in the four groups*

	LEEP diagnosis				<i>p</i>
	LSIL	HSIL	SCC	AIS and Adenocarcinoma	
GI, n (%)					
Absent	2016 (99.7)	391(99.5)	6 (100)	5 (100)	0.964
Present	7 (0.3)	2 (0.5)	0 (0)	0 (0)	
Multicentricity, n (%)					
No	1850 (91.4)	341 (86.8)	5 (83.3)	5 (100)	0.024
Yes	173 (8.6)	52 (13.2)	1 (16.7)	0 (0)	

CDB: colposcopy-directed biopsy; GI: glandular involvement; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; SCC: squamous cell carcinoma; AIS: adenocarcinoma in situ; LEEP: loop electrosurgical excision procedure.

Table 2. — *GI and multicentricity detected by LEEP in the four groups*

	LEEP diagnosis				<i>p</i>
	LSIL	HSIL	SCC	AIS and Adenocarcinoma	
GI, n (%)					
Absent	1982 (98.0)	176 (44.8)	4 (66.7)	0 (0)	0
Present	41 (2.0)	217 (55.2)	2 (33.3)	5 (100)	
Multicentricity, n (%)					
No	1837 (90.8)	170 (43.3)	2 (33.3)	4 (80)	0
Yes	186 (9.2)	223 (56.7)	4 (66.7)	1 (20)	

CDB: colposcopy-directed biopsy; GI: glandular involvement; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; SCC: squamous cell carcinoma; AIS: adenocarcinoma in situ; LEEP: loop electrosurgical excision procedure.

to November 2010. This hospital has a specific protocol established to recommend the three-step conventional strategy. Abstracted data from patients' medical records were identified. This study was approved by the Ethics Committee of the Obstetrics and Gynecology Hospital of the Fudan University.

Although the study patients were diagnosed between 2006 and 2010, the new terminology described in 2012 by the Lower Anogenital Squamous Terminology Project in reporting histopathological and cytological results of all HPV-related lesions was adopted to ensure conformity [13]. CIN1 lesions were reported as LSIL, while CIN2 and CIN3 lesions were reported as HSIL.

Colposcopy was indicated in patients with the following cytology results as previously reported: ASC-US with persistent HPV infection; ASC, cannot exclude high-grade squamous intraepithelial neoplasia (ASC-H); LSIL; HSIL; squamous cell carcinoma (SCC); atypical glandular cell (AGC); adenocarcinoma in situ (AIS); and adenocarcinoma [14]. CDB was performed by attending physicians according to a standard protocol [15] in the Department of Diagnosis and Treatment of Cervical Disease of our hospital. The first biopsy was taken from the most suspicious site and was usually followed by two or more biopsies. Endocervical curettage was performed for patients with unsatisfactory colposcopy.

Indications for LEEP in CDB-diagnosed patients with LSIL include the following: ASC-H, AGC, or HSIL on cytology; patients with persistent ASC-US or LSIL on cytology when HSIL could not be excluded based on clinical features or colposcopy; inadequate colposcopic examination wherein more severe lesions could not be excluded; and if the patient was considered poorly compliant to follow-up. LEEP was performed in the outpatient department by attending physicians in the Department of Diagnosis and Treatment of Cervical Disease of our hospital using a standard procedure, as previously reported [16]. Before LEEP, acetic acid and Lugol's iodine solution were applied under colposcopy to determine the size of the cervical lesions alongside the circumference and width of excision. The length of excision varied according to the transformation zone: < 1 cm for type 1, 1–1.5 cm for type 2, and 1.5–2.5 cm for type 3. Before fixation with paraformaldehyde, every LEEP specimen was measured to verify its circumference, length, and width. All histopathology reports were confirmed by two pathologists including at least one senior pathologist.

LSIL patients who were diagnosed by CDB and subsequently underwent LEEP were included. We excluded women if they lacked biopsy data in colposcopy, or showed HSIL+ lesions in CDB. We retrospectively reviewed and analysed 2,427 patients diagnosed with LSIL by CDB. Af-

ter LEEP, the diagnosis of LSIL was confirmed in 2,023 patients (group A), while 393 patients were subsequently diagnosed with HSIL (group B), 6 with SCC (group C), and 5 with AIS (1 patient) and adenocarcinoma (group D) by LEEP. Patients' age; circumference, length, and width of the LEEP specimen; multicentricity; and GI of the lesions detected by CDB or LEEP were compared between the four groups. The presence of uninvolved squamous and/or endocervical glandular epithelium in between the dysplastic areas was considered to represent a multicentric lesion as previously reported [10].

Statistical Analysis

All the statistical analyses were performed using the Statistical Package for the Social Sciences software version 22.0 (2013; Armonk, NY, USA). Continuous variables were compared between multiple groups using the Kruskal-Wallis test and between two groups using the t-test or Mann-Whitney U test. Pearson χ^2 test was performed to assess the association between categorical variables. $p < 0.05$ was considered statistically significant. Quantitative variables were presented as mean \pm standard deviation.

Results

Patients' ages were significantly greater in groups D (47.6 ± 4.0 years) ($p = 0.005$) and B (39.7 ± 8.2 years) ($p = 0.000$) than in group A (37.6 ± 8.4 years). The mean patient age in group C (41.5 ± 4.9 years) was higher than those of group A and group B, but the difference was not significant, which may be partly explained by the small size of group C (Figure 1).

The circumferences of LEEP specimens were significantly larger ($p = 0.000$) in group B (2.8 ± 0.8 cm) than in group A (2.6 ± 0.9 cm). The mean circumferences of LEEP specimens in groups C (2.8 ± 0.6 cm) and D (2.8 ± 0.7 cm) were higher than in group A, but the differences were not significant.

The widths of LEEP specimens were significantly greater ($p = 0.011$) in group B (0.75 ± 0.24 cm) than in group A (0.72 ± 0.23 cm). The mean width of LEEP specimens in group C (0.88 ± 0.24 cm) was greater than that of group A, though the difference was not significant. The mean width of LEEP specimens in group D (0.72 ± 0.22 cm) was comparable to that of group A.

The lengths of LEEP specimens were significantly greater ($p = 0.000$) in group B (1.23 ± 0.29 cm) than in group A (1.13 ± 0.31 cm). The mean length of LEEP specimen in group C (1.25 ± 0.25 cm) was greater than that in group A, but the difference was not significant. The mean length of LEEP specimen in group D (1.18 ± 0.20 cm) was comparable to that in group A (Figure 2).

In group A, CDB detected GI in 0.4% (7/2023) of patients; in group B, CDB detected GI in 0.5% (2/393) of patients; and in group C and D, CDB was not able to detect GI in any patient ($P = 0.964$). CDB diagnosed 8.6% (173/2023) of patients had lesion multicentricity in group

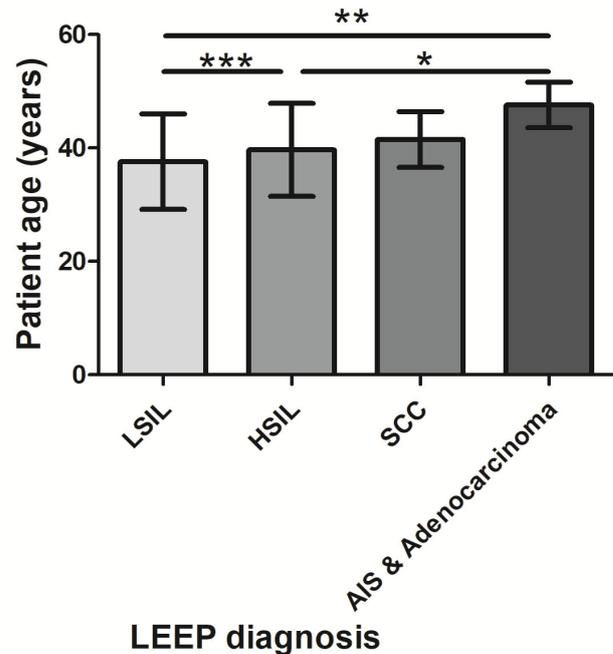


Figure 1. — Comparison between groups on patient age. The values show the means \pm standard deviation. *, **, and *** indicate the value for the difference between the 2 groups compared is $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. Only differences with statistical significance are indicated. LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; SCC: squamous cell carcinoma; AIS: adenocarcinoma in situ; LEEP: loop electrosurgical excision procedure.

A, 13.2% (52/393) of patients had lesion multicentricity in group B, 16.7% (1/6) of patients had lesion multicentricity in group C, and none of the patients had lesion multicentricity in group D ($p = 0.024$) (Table 1). The proportion of multicentricity was significantly greater in group B than in group A ($p = 0.003$), while between other groups, there were no significant differences.

LEEP found that 2% (41/2023) of patients had GI in group A, which was significantly less ($p = 0.000$) than the 55.2% (217/393) of patients in group B with GI, 33.3% (2/6) of patients in group C with GI, and 100% (5/5) of patients in group D with GI. LEEP detected 9.2% (186/2023) of patients had lesion multicentricity in group A, which was significantly less ($p = 0.000$) than the 56.7% (223/393) of patients in group B, 66.7% (4/6) of patients in group C, and 20% (1/5) of patients in group D (Table 2).

Discussion

LSIL patients are generally treated with more conservative measures. However, some of them may harbour HSIL+ lesions. We retrospectively analysed CDB-diagnosed LSIL patients who subsequently underwent LEEP and found that older age, large lesion size, and multicentricity of lesions detected by CDB may be indicative of HSIL or HSIL+ lesions in CDB-diagnosed LSIL.

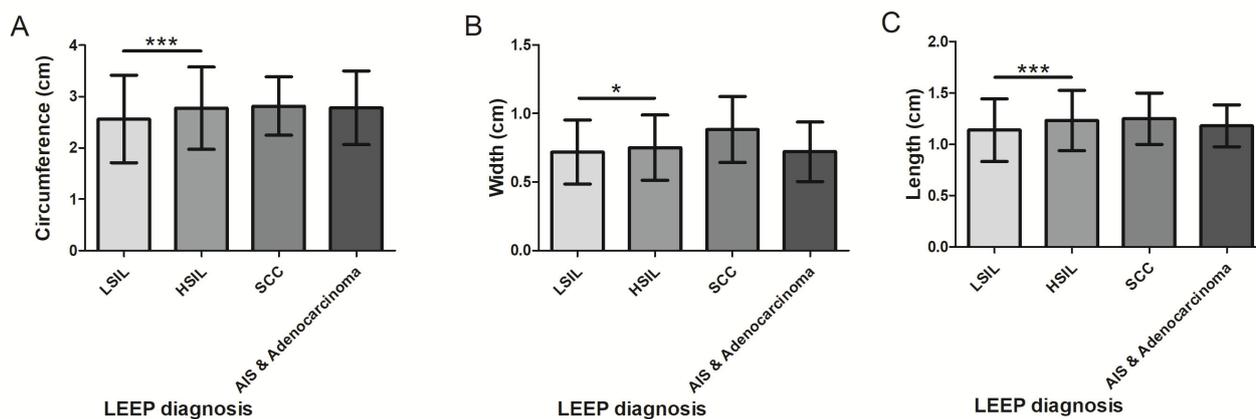


Figure 2. — The circumference (A), width (B) and length (C) of LEEP specimens in the 4 groups. The values show the means \pm standard deviation. *, **, and *** indicate the value for the difference between the 2 groups compared is $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. Only differences with statistical significance are indicated. LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; SCC: squamous cell carcinoma; AIS: adenocarcinoma in situ; LEEP: loop electro-surgical excision procedure.

The risk of high-grade cytology was reported to increase with age [17]. As age increased, the risk of missed invasive carcinoma under the microscope in CDB-diagnosed HSIL also increased [18]. In our study, older age was proven to be a factor which predisposes to HSIL+ lesions, especially in patients with adenocarcinoma. It was reported that the rate of accuracy of CDB depends on the type of transformation zone and the patient's age [19]. As women get older, the chances of the transformation zone to be completely invisible during colposcopy could be higher [20], and this would prevent the detection of cervical lesions.

The proportion of multicentricity of the lesions detected by CDB was significantly higher in the HSIL group than in the LSIL group. The proportion of multicentricity detected by CDB was also higher in the SCC group, but the difference was not significant, which was partly due to the small sample size of the SCC group. However, no case of multicentricity was detected by CDB in the AIS and adenocarcinoma group, which was partly due to the small size of this group or the comparative deficiency of CDB in the diagnosis of lesions from the glandular epithelium [14].

GI detection rate by CDB was quite low in all four groups. However, the detection rate of GI by LEEP in HSIL+ lesions was significantly higher than LSIL. GI has been reported to increase the risk of LEEP surgical margin positivity and higher grade of abnormality [21]. Therefore, GI may be frequently present in HSIL+ lesions as identified by LEEP, but the detection rate of GI by CDB was generally too low to show a difference between the different groups.

With respect to the size of LEEP specimens, the mean circumference, width, and length in the HSIL+ groups were greater than those in the LSIL group, but the difference was only significant between the HSIL and LSIL groups, which may be due to the small sample size in the SCC and AIS and adenocarcinoma groups. The size of a LEEP cone generally

varies with the size of the lesion detectable during the procedure. Hence, a larger cone indicates that the lesion was larger in the three groups than in the LSIL group.

In this study, we retrospectively analysed a large cohort of patients with CDB-diagnosed LSIL who subsequently underwent LEEP in a single centre. One limitation of this study was that rather than being a randomised controlled study, our study was based on a retrospective analysis of data from one tertiary hospital. Another limitation was the lack of information on some of the colposcopic findings, which were not recorded in our database.

In light of the findings of our study, CDB-diagnosed LSIL patients with older age, but not GI, are more prone to harbour HSIL+ lesions, and those with large lesion size and multicentric lesions may have occult HSIL.

Ethics approval and consent to participate

This study was approved by our hospital's Institutional Review Board. All patients in this study gave informed consent and patient anonymity is preserved.

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Conflict of interest

To the best of our knowledge, neither of the authors have any relevant personal or financial conflicts of interest to disclose.

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Corresponding Author:

LONG SUI, M.D., Ph.D.

Obstetrics and Gynecology Hospital of Fudan University, Shanghai, China. Address: No. 419 Fangxie Road, Huangpu District, Shanghai 200011 (China)

email: suilong01@sohu.com