

Spatial Distribution of Cervical Intraepithelial Lesions and Diagnostic Performance of Colposcopy-Directed versus Random Biopsies

Servikal İntraepitelyal Lezyonların Anatomik Dağılımı ve Kolposkopi Yönlendirmeli Biyopsiler ile Rastgele Biyopsilerin Tanısal Performansı

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ABSTRACT

Aim: Optimal biopsy site selection during colposcopy remains controversial, and evidence regarding the anatomical distribution of cervical intraepithelial lesions is limited. We investigated the spatial distribution of cervical lesions and compared the diagnostic yield of colposcopy-directed and random four-quadrant biopsies.

Materials and Methods: This retrospective cross-sectional study included 514 women who underwent colposcopy for HPV positivity and/or abnormal cervical cytology between January 2022 and March 2026. A total of 1,415 cervical biopsy specimens were analyzed. Lesion distribution was evaluated using chi-square goodness-of-fit testing and circular statistical methods, including Rayleigh and axial Rayleigh analyses. Generalized estimating equation models were applied to account for multiple biopsies obtained from the same patient.

Results: HPV16 infection was independently associated with both LSIL+ and HSIL+ lesions. Cervical lesions were not clustered around a single clock position but demonstrated a significant anterior-posterior anatomical distribution (axial Rayleigh test, both $p < 0.001$). In multivariable GEE analysis, anterior-posterior lesion localization independently increased the likelihood of HSIL+ (adjusted OR 1.50, 95% CI 1.12–2.01), while HPV16 remained an independent predictor (adjusted OR 2.79, 95% CI 1.56–4.99). At the patient level, detection rates of LSIL+ and HSIL+ were comparable between targeted and random biopsy strategies. However, lesion-level analysis demonstrated superior diagnostic efficiency for colposcopy-directed biopsy, with higher detection of LSIL+ (adjusted OR 1.44, 95% CI 1.06–1.96) and HSIL+ (adjusted OR 1.58, 95% CI 1.02–2.47). Targeted biopsy also required substantially fewer biopsies to detect one clinically significant lesion.

Conclusions: Cervical intraepithelial lesions exhibit a preferential anterior-posterior anatomical distribution rather than clustering at isolated clock positions. Although colposcopy-directed biopsy provides greater diagnostic efficiency for individual lesions, random biopsy contributes additional patient-level case detection in selected high-risk women. Integrating lesion morphology, HPV genotype, and anatomical lesion distribution may optimize individualized biopsy strategies and improve the diagnostic yield of colposcopy.

Keywords: Cervical intraepithelial neoplasia; Colposcopy; Cervical biopsy; HPV16; Spatial distribution; Circular statistics

ÖZ

Amaç: Kolposkopi sırasında en uygun biyopsi bölgesinin belirlenmesi halen tartışmalıdır ve servikal intraepitelyal lezyonların anatomik dağılımına ilişkin kanıtlar sınırlıdır. Bu çalışmada servikal lezyonların anatomik dağılımının belirlenmesi ve kolposkopi eşliğinde hedefe yönelik biyopsi ile rastgele dört kadran biyopsisinin tanısal performansının karşılaştırılması amaçlandı.

Gereç ve Yöntemler: Ocak 2022–Mart 2026 tarihleri arasında HPV pozitifliği ve/veya anormal servikal sitoloji nedeniyle kolposkopi uygulanan 514 kadının verileri retrospektif olarak değerlendirildi. Toplam 1415 servikal biyopsi örneği analiz edildi. Lezyonların anatomik dağılımı ki-kare uygunluk testi ile birlikte dairesel istatistik yöntemleri (Rayleigh ve aksiyel Rayleigh analizleri) kullanılarak incelendi. Aynı hastadan alınan çoklu biyopsiler arasındaki bağımlılığı dikkate almak amacıyla Genelleştirilmiş Tahmin Denklemleri (GEE) modeli kullanıldı.

Bulgular: HPV16 pozitifliği hem LSIL+ hem de HSIL+ lezyonları bağımsız olarak öngördü. Servikal lezyonlar belirli bir saat pozisyonunda kümelenmemekle birlikte anterior-posterior eksen boyunca anlamlı anatomik dağılım gösterdi (aksiyel Rayleigh testi, her iki analiz için $p < 0.001$). Çok değişkenli GEE analizinde anterior-posterior yerleşim HSIL+ olasılığını bağımsız olarak artırırken (düzeltilmiş OR=1,50; %95 GA:1,12–2,01), HPV16 da bağımsız bir belirleyici olarak saptandı (düzeltilmiş OR=2,79; %95 GA:1,56–4,99). Hasta düzeyinde hedefe yönelik ve rastgele biyopsiler arasında LSIL+ ve HSIL+ saptama oranları benzer bulundu. Buna karşın lezyon düzeyinde hedefe yönelik biyopsiler hem LSIL+ hem de HSIL+ lezyonların saptanmasında anlamlı derecede daha yüksek tanısal verimlilik sağladı ve klinik olarak anlamlı bir lezyonun saptanması için daha az biyopsi gerektirdi.

Sonuç: Servikal intraepitelyal lezyonlar izole saat pozisyonlarında değil, ağırlıklı olarak anterior-posterior anatomik eksen boyunca yerleşim göstermektedir. Kolposkopi eşliğinde hedefe yönelik biyopsiler lezyon düzeyinde daha yüksek tanısal verimlilik sağlarken, rastgele biyopsiler seçilmiş yüksek riskli hastalarda ek tanısal katkı sunmaktadır. Lezyon morfolojisi, HPV genotipi ve anatomik dağılımın birlikte değerlendirilmesi, kolposkopik biyopsi stratejilerinin bireyselleştirilmesine ve tanısal doğruluğun artırılmasına katkı sağlayabilir.

Anahtar Kelimeler: Servikal intraepitelyal neoplazi, kolposkopi, servikal biyopsi, HPV16, anatomik dağılım, dairesel istatistik

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INTRODUCTION

A significant percentage of cervical cancer cases are related to infection with oncogenic subtypes of human papillomavirus (HPV). Persistent HPV infection of the cervix can result in precancerous lesions, such as cervical intraepithelial neoplasia, and is cause of 95% of cervical malignancies (1). Traditionally, the Pap smear was the most commonly performed screening test for cervical cancer globally. Currently, HPV-based screening programs are extensively implemented. Since 2014, women aged 30 to 65 in Türkiye have received cervical cancer screening performed with HPV testing every five years (2).

An abnormal Pap smear or HPV testing necessitates a colposcopic evaluation accompanied by biopsies of the atypical cervical tissue. The diagnostic procedure for cervical intraepithelial lesions is based on cytology, colposcopy, and histopathology. Colposcopy-directed or random biopsy samples represent the conclusive phase in the diagnostic process (3,4). Debate continues regarding the methodology of cervical tissue sampling. This results from disparities in expertise and training among physicians performing colposcopy. For example, should colposcopy be performed by gynecologists or gynecologic oncologists? Training programs are being implemented and guidelines are being developed in numerous countries to enhance the standardization and efficacy of colposcopic evaluation (5). Moreover, numerous studies suggest that sampling cervical tissue that appears normal upon colposcopy, together with performing endocervical curettage, enhances the probability of diagnosis (6-8).

This study also aims to determine whether there is a cervical region with a higher prevalence of lesions. We observed that there are very few studies on this topic in the literature. Certain researches indicated a preference of histologically verified cervical intraepithelial neoplasia (CIN) sites for the anterior and posterior cervical os. A different study indicates that CIN lesions are not randomly distributed; rather, they are concentrated in the 12-, 8-, and 7-o'clock positions on the cervix. (3,4). Clarifying this issue may benefit physicians performing colposcopy in determining the appropriate biopsy site. In cases where lesions are not clearly identifiable, their preference may alter for biopsy site if a random biopsy is performed.

MATERIALS AND METHOD

This study was designed as a retrospective cross-sectional study. The research received clearance from the Kütahya Health Sciences University Ethics Committee (approval no. 2025/13-12). The

medical documentation of patients who underwent colposcopy and had samples obtained for pathological analysis between January 2022 and March 2026 have been analyzed. A total of 514 patients who had colposcopy and cervical biopsy due to HPV positive and/or abnormal smear results, with complete data available, were included in the study. The colposcopy procedures for the included patients, between 19 and 75 years old (Including pregnant women, those with a history of prior cervical excisional/destructive surgery [LEEP, conization, cryotherapy], and immunosuppressed or HIV-positive patients), were performed by faculty members or specialists in gynecological oncology at the clinic. Unlike other patients, endocervical curettage (ECC) was not performed on pregnant women undergoing colposcopy. Patients who had previously undergone procedures such as LEEP or conization were excluded from the study if the transformation zone was not visualized.

While conventional four-quadrant biopsies (12, 3, 6, and 9 o'clock positions) were routinely performed during colposcopies conducted by specialists in obstetrics and gynecology, lesion-directed biopsies were obtained during colposcopies performed by gynecologic oncology surgeons on patients referred to the gynecologic oncology department. Endocervical curettage was routinely performed during colposcopies conducted by gynecologic oncology surgeons. The comparison of both methods for diagnostic efficacy was carried out through statistical analysis. In patients from whom specific biopsies were taken, a colposcopic examination, standard practice for gynecological oncology specialists in identifying suspicious areas, was performed. This procedure entails initially cleansing the cervix with physiological saline, thereafter inspecting it under green light using a colposcope, and the application of a 3% acetic acid solution; an assessment was then performed one minute after application to identify areas exhibiting "acetowhite" changes (whitening caused by the acetic acid) and atypical vascular patterns. The colposcopist recorded the existence and specifics of the acetowhite lesion, including observations such as coarse punctate or mosaic patterns, on a standardized form. Patients in whom the transformation zone could not be clearly visualized were considered to have an inadequate colposcopy and were not included in the study. Colposcopists subsequently mapped the lesions, rendered a colposcopic assessment, and specified the sites from which colposcopically directed samples were obtained. No further evaluation with Lugol's solution was performed after the assessment with acetic acid. Cervical biopsies were then obtained from the most suspicious region, however multiple biopsy sites were allowed in patients with multifocal lesions. The study additionally analyzed the anatomical alignment in which low-grade and high-grade intraepithelial lesions are most commonly detected in the cervix. Patients with pathology results indicating a clockwise positioning were investigated for this purpose.

Statistical analyses were performed using R software (R Foundation for Statistical Computing, Vienna, Austria; version 4.4.1). Categorical variables were presented as numbers and percentages [n (%)]. The associations between HPV genotypes and histopathological results (LSIL+ and HSIL+) were evaluated using the Pearson chi-square test. Fisher's exact test was used for 2×2 tables where the expected cell frequency was below 5, while the Fisher–Freeman–Halton exact test was used for larger contingency tables. The magnitude of the associations was expressed using odds ratios (OR) and 95% confidence intervals (CI).

The distribution of cervical lesions according to clockwise was examined utilizing the chi-square goodness-of-fit test. Circular statistical analyses were performed using the 'circular' package in R to evaluate the directional distribution of lesions in targeted biopsies obtained under colposcopic guidance. Unidirectional clustering was evaluated by the Rayleigh test, and axial clustering along the anterior-posterior axis was analyzed using the axial Rayleigh test. Generalized Estimating Equations (GEE) were used in the lesion-level analyses to account for the dependency between observations resulting from obtaining multiple biopsies from the same patient. The models were constructed using an exchangeable correlation structure, and patient ID was defined as the clustering variable. While the presence of a high-grade lesion (HSIL+) was considered the dependent variable, lesion localization, HPV-16, HPV-18, other

high-risk HPV types, and the biopsy approach (targeted or random four-quadrant biopsy) were included in the model as independent variables. Findings were reported as adjusted odds ratios (aOR) with 95% confidence intervals. All analyses were performed using a two-tailed approach, with a p-value of < 0.05 being acceptable for statistical significance.

RESULTS

A total of 514 women were included in the study, and 1,415 cervical biopsy specimens obtained from these participants were analyzed. Random four-quadrant biopsies were performed in 39.5% of the patients, whereas 60.3% underwent colposcopy-guided targeted biopsies. HPV-16, HPV-18, and other high-risk HPV genotypes were detected in 34.6%, 12.3%, and 58.9% of the study population, respectively. Cytological assessment revealed negative for intraepithelial lesion or malignancy (NILM) in more than half of the women (51.9%). Histopathological examination most frequently demonstrated benign findings (45.9%), followed by low-grade squamous intraepithelial lesions (LSIL, 33.7%), high-grade squamous intraepithelial lesions (HSIL, 19.6%), and carcinoma (0.8%) (Table 1).

The association between HPV genotypes and histopathological outcomes is presented in Table 2. Women with HPV-16 infection had

Table 1. Distribution of biopsy approach, HPV genotypes, cytology, lesion histology, and lesion focality (n = 514 women; 1,415 biopsies)

Variable		n	%
Groups	Random four-quadrant biopsy (3, 6, 9, and 12 o'clock positions)	203	39.5
	Targeted (colposcopy-guided) biopsy	310	60.3
	No biopsy performed	1	0.2
HPV-16	Positive	178	34.6
	Negative	336	65.4
HPV-18	Positive	63	12.3
	Negative	451	87.7
Other high-risk HPV	Positive	303	58.9
	Negative	211	41.1
Any high-risk HPV	Positive	476	92.6
	Negative	38	7.4
Cytology (smear)	NILM	267	51.9
	ASC-US	196	38.1
	LSIL	35	6.8
	HSIL	8	1.6
	ASC-H	8	1.6
Worst histology	Negative (Benign/Cervicitis)	236	45.9
	LSIL	173	33.7
	HSIL	101	19.6
	Carcinoma	4	0.8
Lesion focality	No lesion	236	45.9
	Unifocal	142	27.6
	Multifocal	136	26.5

ASC-H, atypical squamous cells cannot exclude HSIL; ASC-US, atypical squamous cells of undetermined significance; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy.

Table 2. Association of HPV-16, HPV-18, and other high-risk HPV genotypes with LSIL+ and HSIL+ lesions (n = 514)

HPV genotype	Status	n	LSIL+, n (%)	OR (LSIL+)	p-value	HSIL+, n (%)	OR (HSIL+)	p-value
HPV-16	Positive	178	111 (62.4)	1.68 (1.16–2.43)	0.008	55 (30.9)	2.56 (1.65–3.96)	0.001
	Negative	336	167 (49.7)			50 (14.9)		
HPV-18	Positive	63	36 (57.1)	1.15 (0.68–1.96)	0.700	14 (22.2)	1.13 (0.60–2.14)	0.833
	Negative	451	242 (53.7)			91 (20.2)		
Other HR-HPV	Positive	303	152 (50.2)	0.68 (0.48–0.97)	0.041	53 (17.5)	0.65 (0.42–1.00)	0.062
	Negative	211	126 (59.7)			52 (24.6)		

CI, confidence interval; LSIL, low-grade squamous intraepithelial lesion; HR-HPV, high-risk human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; OR, odds ratio. Each genotype was analyzed as positive versus negative; the reference group for "other HR-HPV" therefore includes HPV-16- and HPV-18-positive women, which accounts for the inverse association observed. Comparisons by Pearson chi-square test; ORs by the Woolf (logit) method.

significantly higher frequencies of both LSIL+ and HSIL+ lesions. HPV-16 positivity was associated with a 1.68-fold increased likelihood of LSIL+ lesions (OR = 1.68, 95% CI: 1.16–2.43; $p = 0.008$) and a 2.56-fold increased likelihood of HSIL+ lesions (OR = 2.56, 95% CI: 1.65–3.96; $p = 0.001$). In contrast, HPV-18 positivity showed no significant association with either LSIL+ or HSIL+ lesions. Other high-risk HPV genotypes were inversely associated with LSIL+ lesions (OR = 0.68, 95% CI: 0.48–0.97; $p = 0.041$), whereas no significant association was observed with HSIL+ lesions.

Evaluation of lesion distribution across the cervix demonstrated significant variation in the detection rates of both CIN1+ and CIN2+ according to clockwise position ($p = 0.011$ and $p = 0.003$, respectively). When the cervix was divided into four anatomical

quadrants, a significant difference was observed only for the distribution of CIN2+ lesions ($p = 0.022$). Among women who underwent random four-quadrant biopsy, the detection rate of HSIL+ varied significantly according to the biopsy location ($p = 0.008$), whereas no significant positional difference was found for CIN1+ lesions (Table 3, Figure 1).

Circular analyses of targeted biopsy specimens demonstrated that cervical lesions were not clustered around a single clockwise position. However, both LSIL+ and HSIL+ lesions exhibited a significant axial distribution along the anterior–posterior axis (axial Rayleigh test for both analyses, $p < 0.001$). Regional analysis further showed that lesions in both categories were most frequently located on the anterior cervix (Table 4, Figure 2).

Table 3. Lesion Detection Rates According to Cervical Clock Position

Clock Position	Cervical Quadrant	Biopsies Sampled, n	CIN1+ n (%)	CIN2+ n (%)
1 o'clock	Anterior	45	14 (31.1)	7 (15.6)
2 o'clock	Right	24	16 (66.7)	7 (29.2)
3 o'clock	Right	289	92 (31.8)	19 (6.6)
4 o'clock	Right	19	7 (36.8)	4 (21.1)
5 o'clock	Posterior	26	10 (38.5)	2 (7.7)
6 o'clock	Posterior	304	108 (35.5)	33 (10.9)
7 o'clock	Posterior	33	16 (48.5)	7 (21.2)
8 o'clock	Left	8	1 (12.5)	0 (0.0)
9 o'clock	Left	291	97 (33.3)	28 (9.6)
10 o'clock	Left	8	4 (50.0)	1 (12.5)
11 o'clock	Anterior	56	27 (48.2)	10 (17.9)
12 o'clock	Anterior	312	127 (40.7)	47 (15.1)
Total		1,415	519 (36.7)	165 (11.7)
Comparison				
Across all 12 clock positions			$\chi^2=24.42$, df=11, p=0.011	$\chi^2=28.03$, df=11, p=0.003
Across 4 quadrants			$\chi^2=5.02$, df=3, p=0.170	$\chi^2=9.58$, df=3, p=0.022
Across standard positions only (3-6-9-12) (n=203)			$\chi^2=5.99$, df=3, p=0.112	$\chi^2=11.80$, df=3, p=0.008

CIN, cervical intraepithelial neoplasia; HSIL, high-grade squamous intraepithelial lesion.

Rates are expressed per biopsy sampled at that position. Comparisons test whether detection rates differ across positions and apply to the grouped rows indicated, not to individual rows.

Positions other than 3, 6, 9, and 12 o'clock were sampled almost exclusively when a lesion was colposcopically suspected; this comparison is therefore susceptible to work-up bias and is shown for completeness only. For HSIL+, 6 of 24 expected counts were below five.

Restricted to the 203 women sampled with the random four-quadrant protocol, in whom all four positions were biopsied irrespective of colposcopic findings; this comparison is free of work-up bias.

Comparisons are made at the biopsy level and do not account for clustering of multiple biopsies within the same woman; the patient-clustered model in Table 5 addresses this and yields concordant results.

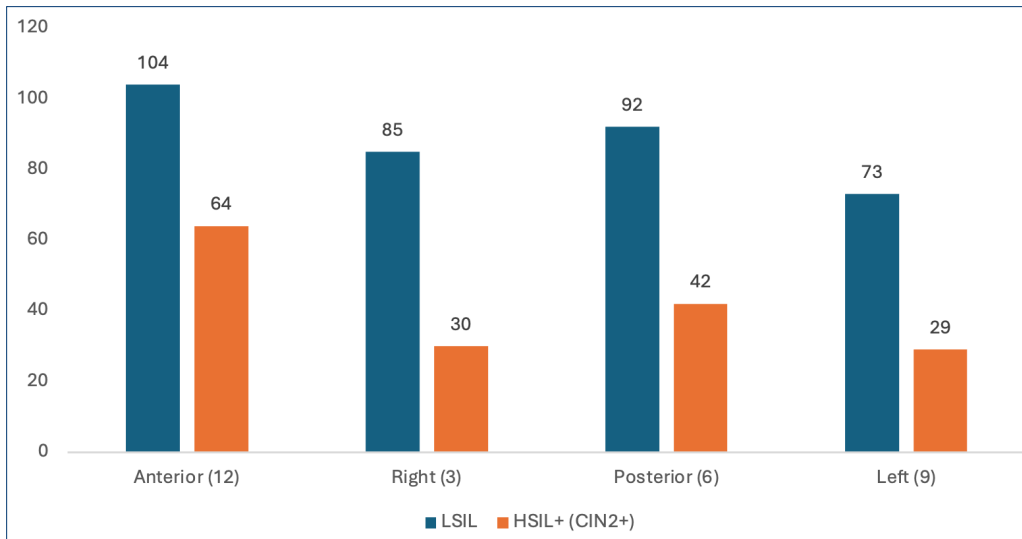


Figure 1. Distribution of LSIL and HSIL (CIN2+) According to Colposcopically Identified Lesion Location

In lesion-level multivariable generalized estimating equation (GEE) analysis, lesions located along the anterior–posterior axis were independently associated with a higher probability of being high grade (adjusted OR [aOR] = 1.50, 95% CI: 1.12–2.01; $p = 0.007$).

HPV-16 positivity also emerged as an independent predictor of high-grade lesions (aOR = 2.79, 95% CI: 1.56–4.99; $p = 0.001$). In contrast, HPV-18 positivity, other high-risk HPV genotypes, and biopsy approach did not independently contribute to the model.

Table 4. Regional distribution and circular statistical analysis of lesions in targeted (colposcopy-directed) biopsies

Cervical location	LSIL+, n (%) (n = 258)	HSIL+, n (%) (n = 90)
Regional distribution		
Anterior (11–12–1)	96 (37.2)	39 (43.3)
Posterior (5–6–7)	73 (28.3)	27 (30.0)
Right (2–3–4)	53 (20.5)	16 (17.8)
Left (8–9–10)	36 (14.0)	8 (8.9)
Circular statistics		
Unimodal Rayleigh test	Z = 2.92, $p = 0.054$	Z = 1.94, $p = 0.144$
Axial Rayleigh test (12–6 vs 3–9 axis)	Z = 14.19, $p < 0.001$	Z = 13.74, $p < 0.001$

LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion.

Analysis was restricted to targeted biopsies; random four-quadrant biopsies were excluded to remove protocol-related sampling bias. Recorded lesion locations clustered at the main clock positions (12, 3, 6, and 9 o'clock; 63% of CIN1+ lesions and 58% of HSIL+ lesions, compared to an expected 33%); therefore, zonal summaries were used for interpretation, and the precise distribution at the clock-position level is shown in Figure 1. Lesions of both grades were concentrated along the anterior–posterior (12–6 o'clock) axis rather than in a single clock direction: axial clustering was highly significant for both grades, whereas unimodal Rayleigh testing did not identify a dominant directional peak.

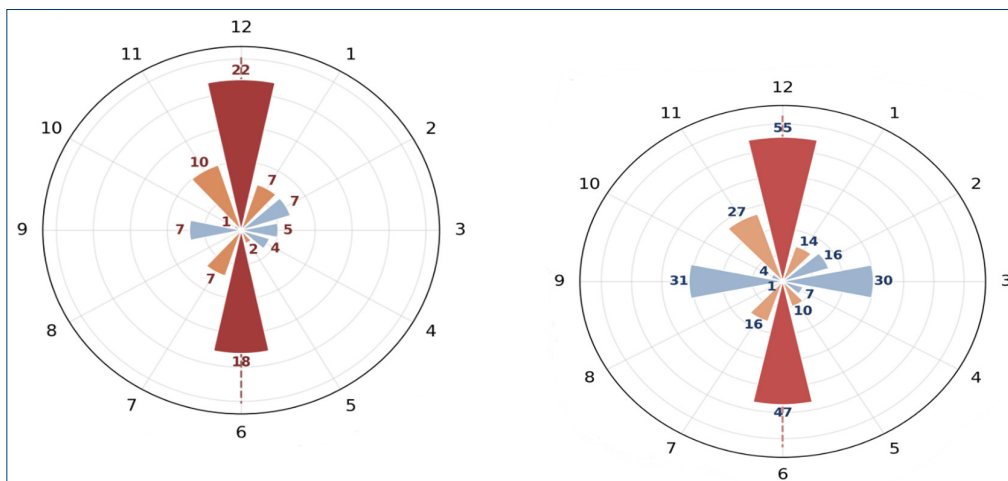


Figure 2. Polar distribution of cervical lesions in targeted (colposcopy-directed) biopsies.

Clock-position distribution of (A) LSIL+ lesions (n = 258) and (B) HSIL+ lesions (n = 90). Bars represent the number of lesions recorded at each clock position; the dashed line indicates the anterior–posterior (12–6 o'clock) axis. Lesions of both grades clustered along this axis (axial Rayleigh test, $p < 0.001$ for both), whereas no single dominant clock direction was identified (unimodal Rayleigh test, $p = 0.054$ and $p = 0.144$, respectively). LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion.

When lesion location was modeled using four separate cervical sectors rather than the anterior–posterior axis, none of the sectors reached statistical significance (Table 5, Figure 3).

Patient-level comparisons between random four-quadrant biopsy and targeted biopsy showed no significant differences in the detection rates of either LSIL+ or HSIL+ lesions ($p = 0.652$ and $p = 0.663$, respectively). These findings remained unchanged after

adjustment for cytology and HPV-16 status. In contrast, biopsy-level analysis demonstrated superior diagnostic yield with targeted biopsy. After adjustment for potential confounders, targeted biopsy was associated with a 44% higher likelihood of detecting LSIL+ lesions (aOR = 1.44, 95% CI: 1.06–1.96; $p = 0.020$) and a 58% higher likelihood of detecting HSIL+ lesions (aOR = 1.58, 95% CI: 1.02–2.47; $p = 0.042$). Furthermore, the number of biopsies required to identify one case of LSIL+ was nearly half that of

Table 5. Multivariable GEE logistic regression of predictors of high-grade lesion (HSIL+ vs LSIL+) at the lesion level

Variable	Adjusted OR (95% CI)	p-value
Model 1. Lesion location represented as anterior–posterior axis		
Anterior–posterior axis (12–6 region)	1.50 (1.12–2.01)	0.007
HPV-16	2.79 (1.56–4.99)	0.001
HPV-18	1.48 (0.71–3.12)	0.299
Other high-risk HPV	1.19 (0.66–2.13)	0.568
Targeted biopsy (vs random biopsy)	1.11 (0.68–1.80)	0.687
Model 2. Lesion location represented as cervical sector		
Right sector (vs left sector)	0.76 (0.45–1.26)	0.283
Posterior sector (vs left sector)	1.11 (0.71–1.73)	0.656
Anterior sector (vs left sector)	1.47 (0.92–2.34)	0.104
HPV-16	2.70 (1.50–4.84)	0.001
HPV-18	1.44 (0.68–3.05)	0.339
Other high-risk HPV	1.16 (0.64–2.10)	0.621
Targeted biopsy (vs random biopsy)	1.10 (0.68–1.80)	0.692

CI, confidence interval; GEE, generalized estimating equations; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; OR, odds ratio.

Analysis included all 519 CIN1+ lesions from 278 women. Models were fitted with GEE (exchangeable working correlation, woman as the subject variable) to account for multiple biopsies per patient. The anterior–posterior axis variable denotes lesions located at the 11-, 12-, 1-, 5-, 6-, or 7-o'clock positions.

No individual sector reached significance in Model 2 because the effect is bipolar (involving both the 12- and 6-o'clock regions) and is diluted when these are assigned to separate categories; the axis parameterization in Model 1 captures it more efficiently.

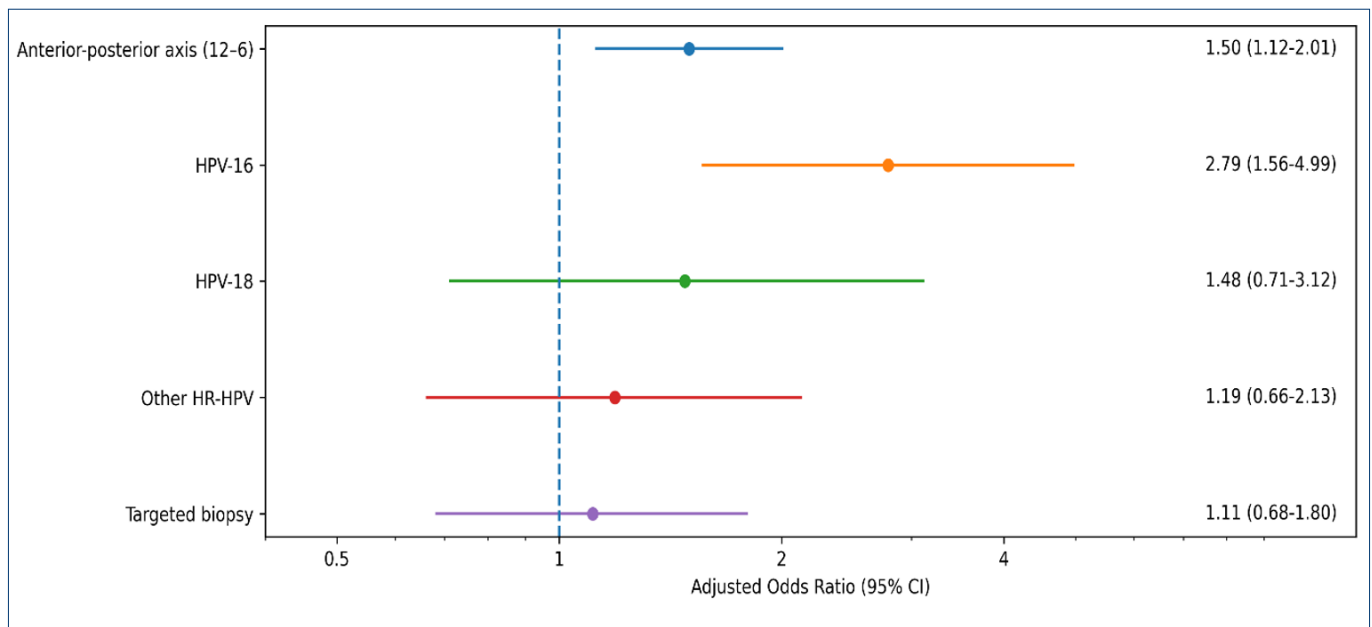


Figure 3. Forest plot of independent predictors of high-grade lesion (HSIL+ vs LSIL).

Adjusted odds ratios and 95% confidence intervals derived from the multivariable GEE logistic regression model (Model 1, Table 5), fitted at the lesion level with the woman as the clustering unit ($n = 519$ lesions from 278 women). Anterior–posterior axial location and HPV-16 positivity were independently associated with high-grade disease. CI, confidence interval; GEE, generalized estimating equations; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

Table 6. Comparison of random four-quadrant and targeted (colposcopy-directed) cervical biopsy: baseline characteristics, lesion detection, and diagnostic yield

Variable	Random four-quadrant (n = 203)	Targeted (n = 310)	p	Effect size (95% CI)
Sampling				
Total biopsies, n	812	603	—	—
Biopsies per patient, mean	4.0	1.9	—	—
Baseline characteristics				
Cytology, n (%)			0.001	—
NILM	132 (65.0)	135 (43.5)		
ASC-US	59 (29.1)	136 (43.9)		
LSIL	9 (4.4)	26 (8.4)		
ASC-H	2 (1.0)	6 (1.9)		
HSIL	1 (0.5)	7 (2.3)		
HPV-16 positive, n (%)	65 (32.0)	112 (36.1)	0.388	—
HPV-18 positive, n (%)	20 (9.9)	43 (13.9)	0.223	—
Other HR-HPV positive, n (%)	126 (62.1)	176 (56.8)	0.271	—
Any HR-HPV positive, n (%)	191 (94.1)	284 (91.6)	0.382	—
Per-patient detection				
LSIL+, n (%)	113 (55.7)	165 (53.2)	0.652	—
Adjusted OR ^a	Reference	—	0.278	0.81 (0.56–1.18)
HSIL+, n (%)	44 (21.7)	61 (19.7)	0.663	—
Adjusted OR ^a	Reference	—	0.238	0.76 (0.48–1.20)
Per-biopsy yield				
LSIL+, n (%)	261/812 (32.1)	258/603 (42.8)	—	—
Unadjusted OR ^b	Reference	—	0.003	1.57 (1.16–2.13)
Adjusted OR ^{b,c}	Reference	—	0.020	1.44 (1.06–1.96)
HSIL+, n (%)	75/812 (9.2)	90/603 (14.9)	—	—
Unadjusted OR ^b	Reference	—	0.014	1.74 (1.12–2.71)
Adjusted OR ^{b,c}	Reference	—	0.042	1.58 (1.02–2.47)
Efficiency				
Biopsies per LSIL+, case detected	7.2	3.7	—	—
Biopsies per HSIL+ case detected	18.5	9.9	—	—

ASC-H, atypical squamous cells cannot exclude HSIL; ASC-US, atypical squamous cells of undetermined significance; CI, confidence interval; GEE, generalized estimating equations; HR-HPV, high-risk human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy; OR, odds ratio.

Biopsy approach was classified from the sampling pattern: random four-quadrant, biopsies obtained at exactly the 3-, 6-, 9-, and 12-o'clock positions irrespective of colposcopic findings; targeted, biopsies directed at colposcopically suspicious sites. No woman underwent both approaches. One woman without any biopsy was excluded.

^a Binary logistic regression at the patient level, adjusted for cytology and HPV-16 status; random four-quadrant as reference.

^b Logistic regression fitted with GEE (exchangeable working correlation, woman as the subject variable) to account for multiple biopsies per patient; random four-quadrant as reference.

^c Adjusted for cytology and HPV-16 status.

The two approaches were not randomly allocated and differed at baseline in cytology, indicating confounding by indication; adjusted estimates are therefore interpreted. No reference standard (excisional histology) was available, so diagnostic sensitivity could not be estimated. Per-biopsy yield reflects the efficiency of colposcopic targeting rather than superior diagnostic accuracy, as targeted biopsies are by definition obtained from visually suspicious areas.

random four-quadrant biopsy (3.7 vs. 7.2). Similarly, fewer targeted biopsies were required to detect one HSIL+ lesion compared with the random four-quadrant approach (9.9 vs. 18.5) (Table 6).

DISCUSSION

HPV-based screening, early colposcopic assessment, and treatment of cervical precancerous lesions prevent cervical cancer. Optimal biopsy technique during colposcopy is contentious since diagnostic accuracy depends on lesion visibility and biopsy site selection. In this context, our study provides several clinically relevant findings.

First, HPV16 independently predicted both LSIL+ and HSIL+, confirming its dominant oncogenic role. Second, cervical lesions demonstrated a significant anterior–posterior spatial orientation rather than clustering around a single clockwise position. Third, although colposcopy-directed biopsies showed a higher diagnostic yield at the lesion level, patient-level detection rates were comparable between targeted and random biopsy strategies. Collectively, these findings suggest that optimizing biopsy location may be as important as increasing biopsy number.

The strong association between HPV16 and both LSIL+ and HSIL+ is consistent with extensive evidence demonstrating the superior

oncogenic potential of this genotype. Accordingly, HPV16 remained an independent predictor of lesion severity after adjustment for lesion location and biopsy strategy. In contrast, HPV18 was not independently associated with HSIL+ in our cohort. Beyond viral genotype, our findings indicate that cervical anatomy itself contributes to lesion detection. Colposcopy is inherently dependent on lesion visibility and specialist experience, and previous studies have consistently shown that visually guided assessment alone may overlook clinically significant lesions (9-11). Consequently, improving biopsy site selection remains an important strategy for enhancing diagnostic accuracy (12,13).

One of the principal contributions of the present study is the demonstration that cervical intraepithelial lesions are preferentially distributed along the anterior–posterior axis rather than concentrated at isolated clockwise positions. Although lesion location has traditionally been described using the 12-clock cervical map, this approach treats biopsy sites as independent anatomical sectors despite the circular structure of the cervix. Our findings suggest that cervical lesion distribution is better interpreted as a continuous spatial phenomenon than as a series of discrete clock positions. The clustering of biopsy sites at the 12 and 6 o'clock positions should not necessarily be interpreted as a consequence of rounding or preferential clock-face documentation by colposcopists. In our cohort, this pattern was largely attributable to two distinct sampling approaches. Obstetricians and gynecologists frequently used a routine four-quadrant biopsy strategy, with specimens obtained at the 3, 6, 9, and 12 o'clock positions irrespective of whether a discrete lesion was identified. In contrast, gynecologic oncology surgeons generally performed lesion-directed biopsies and documented the precise clock-face position of the visible abnormality. Within this targeted-biopsy group, lesions were also more frequently documented at the 12 and 6 o'clock positions. Thus, the observed distribution most likely reflects the combined influence of systematic quadrant-based sampling and the anatomical distribution of visible lesions, rather than an artifact introduced by rounding of biopsy locations. Previous investigations have also suggested that cervical lesions are not randomly distributed. Allard et al. first reported preferential localization around the anterior and posterior cervix, whereas He et al. subsequently observed a predominance of lesions within the posterior cervical lip (3,14). Zhao et al., analyzing more than 53,000 cervical specimens, similarly demonstrated increased frequencies of CIN2+ in posterior cervical quadrants (4). Rather than contradicting one another, these apparently different observations may reflect the same underlying anatomical organization viewed through conventional clockwise analyses.

Our study extends these observations by applying circular statistical methodology together with axial Rayleigh analysis, allowing lesion distribution to be evaluated while respecting the circular anatomy of the cervix. Although lesions did not demonstrate clustering toward a single angular direction, both LSIL+ and HSIL+ showed a significant anterior–posterior orientation. This finding suggests that cervical lesions preferentially develop along an anatomical axis crossing the cervical os instead of arising from isolated clock positions. Such an interpretation provides a biologically plausible explanation for why previous studies have variably identified increased lesion frequencies at the 4-, 6-, 7-, 8-, or 12-o'clock positions. The mechanisms underlying this preferential spatial organization remain uncertain. Regional differences in transformation-zone maturation, epithelial remodeling, hormonal influences, local immune responses, or persistent HPV infection may contribute to the observed distribution.

Our findings indicate that evaluating the lesion by predicting its anteroposterior orientation, rather than relying on routine clock-position biopsies, can improve colposcopy results. Previous studies consistently demonstrated that increasing biopsy numbers improves the overall detection of CIN2+, particularly in women with low-grade cytology, normal colposcopic impressions, or small lesions that may escape visual recognition. However, most of these investigations evaluated patient-level case detection, whereas relatively few distinguished this outcome from the diagnostic efficiency of individual biopsy specimens (7,12,15). Our findings help reconcile these apparently conflicting perspectives. Targeted biopsy was significantly more likely to detect both LSIL+ and HSIL+, confirming that visually suspicious acetowhite lesions remain the most efficient biopsy targets. In contrast, detection rates were comparable between targeted and random biopsy approaches, indicating that random biopsy primarily contributes by identifying occult lesions that are not recognized during colposcopy rather than by increasing the diagnostic yield of each biopsy. This interpretation is broadly consistent with previous reports. Pretorius, Nam, Baasland, Chu, Jespersen, and colleagues demonstrated that systematic or random biopsies increase the overall detection of CIN2+, whereas Wentzensen et al. showed that the greatest improvement in diagnostic sensitivity resulted from obtaining multiple biopsies from separate acetowhite lesions rather than from visually normal cervical epithelium (5,7,12,13,15,16). Together with these studies, our results suggest that careful colposcopy-directed biopsy remains the most efficient method for sampling visible lesions, whereas complementary random biopsies may be particularly valuable in women with persistent HPV16 infection, high-grade cytology, equivocal colposcopic findings, or extensive transformation zones where clinically significant lesions may remain occult.

An important strength of this study is its analytical approach. Unlike previous investigations that primarily relied on frequency comparisons or conventional regression analyses, we combined circular statistics, axial Rayleigh analysis, and generalized estimating equation (GEE) modeling to evaluate both the spatial organization of cervical lesions and the correlation among multiple biopsies obtained from the same patient. This approach provides a more robust assessment of lesion topography while reflecting routine clinical practice, where several biopsy specimens are commonly collected from an individual. Furthermore, by evaluating both lesion-level and patient-level outcomes, our study offers complementary perspectives on biopsy performance that have rarely been examined simultaneously.

Several limitations should also be acknowledged. First, the retrospective single-center design may have introduced selection bias and limits the generalizability of our findings. Second, despite standardized colposcopic procedures, interobserver variability in lesion recognition and biopsy selection cannot be completely excluded because colposcopy remains an operator-dependent examination. Third, information regarding transformation-zone type, lesion size, HPV viral load, and biomarkers such as p16/Ki-67 was unavailable. Fourth, diagnostic sensitivity and specificity could not be determined because an excisional specimen (LEEP/conization) serving as the reference standard was not available for all patients. Accordingly, the reported case detection rates and diagnostic yields should not be interpreted as measures of diagnostic accuracy but rather as indicators of lesion detection within the biopsied population. Finally, the absence of longitudinal follow-up prevented evaluation of lesion persistence, progression, or regression over time. These factors should be considered when interpreting our findings and warrant further investigation in prospective multicenter studies. Despite these limitations, our findings have several clinically relevant implications. The demonstration of a preferential anterior–posterior distribution suggests that cervical lesion localization should be interpreted as a spatial pattern rather than as isolated clockwise positions. This concept may assist colposcopists in selecting biopsy sites, particularly when abnormalities are subtle or multifocal.

The application of circular statistical methods also highlights the potential value of incorporating spatial analysis into future colposcopic research. As artificial intelligence-assisted colposcopy and digital image analysis continue to evolve, anatomical lesion mapping may become an integral component of individualized biopsy planning. Future multicenter prospective studies integrating HPV genotype, colposcopic image analysis, and spatial statistical modeling may further improve diagnostic accuracy while reducing unnecessary biopsies.

CONCLUSIONS

Cervical intraepithelial lesions demonstrated a preferential anterior–posterior spatial distribution rather than clustering at individual clockwise positions. HPV16 infection and anterior–posterior lesion localization independently predicted histologically significant cervical disease, emphasizing the combined contribution of viral oncogenicity and cervical anatomy. Although colposcopy-directed biopsy achieved greater diagnostic efficiency at the lesion level, patient-level detection remained comparable between targeted and random biopsy strategies, indicating that these approaches address different aspects of diagnostic yield. Rather than supporting indiscriminate increases in biopsy number, our findings suggest that biopsy selection should become more anatomically informed and individualized by integrating lesion morphology, HPV genotype, and spatial lesion distribution. This strategy may improve diagnostic precision and contribute to more efficient, risk-adapted colposcopic practice.

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