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Original Article

A study on the diagnostic value of PAX1 methylation gene testing in cervical lesions with incomplete visibility of the squamocolumnar junction

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Abstract: Objective: To evaluate the effectiveness of PAX1 methylation gene testing in cervical lesions where the squamocolumnar junction (SCJ) is incompletely visible. Methods: For patients with TCT ≥ ASCUS or HR-HPV positivity, whose colposcopy results indicate incomplete visibility of the SCJ, cervical exfoliated cells were collected for PAX1 gene methylation testing. Using histopathology results as the gold standard, the study calculated the positive rate of PAX1 methylation testing across various lesion grades, assessing the sensitivity, specificity, positive predictive value, negative predictive value, and positive concordance rate with histopathology for high-grade cervical lesions. Results: The PAX1 positivity rates in the cervicitis, LSIL, HSIL, and CC groups were 30% (3/10), 30.43% (7/23), 51.28% (20/39), and 71.42% (5/7), respectively. The positivity rate in the LSIL group (cervicitis and LSIL), at 29.72% (11/37), was significantly lower than in the HSIL+ group (HSIL and CC), which was 54.76% (23/42) ($P < 0.05$). For HSIL+ lesions, PAX1 methylation testing showed a sensitivity of 54.76%, specificity of 70.27%, positive predictive value of 67.65%, negative predictive value of 57.78%, positive likelihood ratio of 1.84, and negative likelihood ratio of 0.22. In comparison, HPV testing for HSIL+ showed a sensitivity of 100% and specificity of 13.51%; TCT testing for HSIL+ had a sensitivity of 35.71% and specificity of 94.59%. The positive concordance rate with histopathology was 97.1% for the PAX1 group, 95.9% for the high-risk HPV group, and 100% for the TCT group. Conclusion: The overall efficacy of PAX1 in diagnosing high-grade cervical lesions is relatively high, enhancing diagnostic accuracy to a certain extent and making it suitable for further clinical application and promotion.

Keywords: PAX1 gene methylation, cervical lesions, high-grade squamous intraepithelial lesion, cervical cancer diagnosis, sensitivity and specificity, colposcopy

Introduction

Cervical cancer (CC) is among the top three most prevalent cancers affecting women worldwide [1]. As of 2022, China was estimated to have 4,796,996 new cancer cases, with cervical cancer accounting for 111,820 of these cases, approximately 2.3%, and the estimated number of cervical cancer deaths was 61,579, making up about 1.9%. Both the incidence and mortality rates of cervical cancer in women are on the rise [2]. Accurate staging and assessment of prognostic factors before treatment are crucial for determining the appropriate treatment approach for cervical cancer in China [3, 4]. Co-testing with HPV and cytology is the most recommended screening method is when medical resources are ample. In the absence of sufficient resources, HPV screening alone is recommended as the primary method, with cytology as a secondary option [5]. Liquid-based thin-layer cytology (TCT) is the first choice for cervical cancer screening, as the accuracy of examination can reach 100% in theory [6]. In low-income regions, limited medical resources and a lack of well-trained gynecologists and cytologists for conducting TCT contribute to high rates of false negatives and false positives in cervical screening. As a result, reducing the incidence and mortality of cervical cancer in underdeveloped countries remains an unresolved challenge [7]. HPV testing is

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more sensitive than cytology but has relatively low specificity, as most HPV infections are subclinical, transient, and non-cancerous. An HPV-positive result may lead to unnecessary patient anxiety and excessive follow-up testing, such as an increased colposcopy referral rate due to a high number of transient HPV infections.

Inadequate colposcopy results make conventional screening methods prone to misdiagnosis and underdiagnosis, especially in cases where the squamocolumnar junction (squamocolumnar junction, SCJ) is incompletely visible. The squamocolumnar junction (SCJ) refers to the transitional zone between the squamous epithelium and columnar epithelium of the uterine cervix. Clinical studies indicate that inadequate visualization of the SCJ significantly increases the risk of diagnostic errors, including both misdiagnosis and missed diagnosis [8]. Applying specific gene hypermethylation testing of Paired Box Gene 1 (PAX1) during the pre-invasive and invasive stages of cervical cancer in women with cytological abnormalities and HPV positivity may serve as an effective method for triage, post-treatment follow-up, and assessing long-term prognosis. This study explores the use of PAX1 hypermethylation testing in cases where SCJ is incompletely visible under colposcopy, particularly for patients with completely invisible SCJ, to determine the effectiveness of this method. Clarifying whether PAX1 methylation testing can reduce misdiagnosis and underdiagnosis in SCJ-invisible cases, while also reducing unnecessary diagnostic conization rates and preserving fertility, is of critical importance for clinical treatment and preventive strategies.

Our study aims to evaluate the diagnostic accuracy of HPV testing, TCT, and PAX1 methylation testing in patients presenting with incompletely visible squamocolumnar junctions (SCJ) under colposcopy. By comparing the results from these screening methods with postoperative or biopsy histopathological findings, we pursue to better understand their diagnostic value and potential clinical effectiveness.

Materials and methods

General information

From July 2020 to February 2021, cervical exfoliated cytology samples were collected from 79 patients at the Fourth Affiliated Hospital of Guangxi Medical University's Gynecology Department. Inclusion Criteria: ① Age between 21 and 65 years. ② Abnormal ThinPrep cytologic test (TCT) result (≥ ASCUS) or positive high-risk human papillomavirus (HR-HPV) test. ③ Incomplete visualization of the SCJ. ④ Full understanding of the study's benefits and risks, willingness to participate, and signed informed consent. Exclusion Criteria: ① History of cervical surgery. ② Prior hysterectomy. ③ History of malignancy or receipt of radiotherapy/chemotherapy within the past 5 years. ④ Active urinary or reproductive tract infection. ⑤ Pregnant or lactating women. ⑥ Presence of other organ/system dysfunctions constituting a surgical contraindication. ⑦ History of uncontrolled epilepsy, central nervous system disorders, or psychiatric conditions that, in the investigator's judgment, may compromise clinical research compliance due to their severity. ⑧ Patients or legally authorized representatives who refuse participation. These patients had undergone cervical cancer screening with results of TCT ≥ ASCUS or HR-HPV positivity and were found to have incomplete visibility of the SCJ on colposcopy. All patients underwent cervical biopsy, LEEP, or cervical conization, with biopsy or postoperative histopathology serving as the gold standard. The average age of patients was 43.33 years (range: 23-66 years). Pathological diagnoses included 10 cases of cervicitis, 23 cases of LSIL, 39 cases of HSIL, and 7 cases of cervical cancer. For analysis, cervicitis and LSIL cases were grouped as LSIL, while HSIL and cervical cancer cases were grouped as HSIL+. With postoperative or biopsy pathology as the "gold standard", there were 42 HSIL cases and 37 LSIL cases. The average age of the LSIL group (42.97 years) and the HSIL+ group (43.64 years) showed no statistically significant difference ($P = 0.767 < 0.05$). All included patients were free from pregnancy, history of malignancies at other sites, history of cervical surgery, and any other organ or system dysfunction.

Methods

Cytology testing method: The hospital used TCT kits produced by Hubei Kangqiang Biotechnology Co., Ltd. for cytology testing.

Sample Collection Method: Cervical exfoliated cell samples were collected by professional gynecologists using standard methods. Sampling procedure: A specialized cytology cervical

brush was inserted approximately 1 cm into the cervical canal and rotated clockwise for 4-6 turns. The cervical brush with the collected exfoliated cells was immediately placed into a vial containing cell preservation solution, sealed, and sent for testing.

Testing Results: Following the 2001 NCI-revised Bethesda System (TBS) reporting system, results were categorized as follows: NILM (negative for intraepithelial lesion or malignancy), ASC-US (atypical squamous cells of undetermined significance), ASC-H (atypical squamous cells cannot exclude HSIL), LSIL (low-grade squamous intraepithelial lesion), HSIL (high-grade squamous intraepithelial lesion), SCC (squamous cell carcinoma), AGC (atypical glandular cells), AIS (adenocarcinoma in situ), and AC (adenocarcinoma).

PAX1 gene methylation testing: Cervical exfoliated cells were collected using standard sampling methods, labeled, and stored at -20°C for no longer than 4 months. After pretreatment of the cervical exfoliated cells, DNA was extracted, and 2 μl of solution was used to assess nucleic acid concentration and integrity. Nucleic acid samples with concentrations ≥ 4 ng/μl underwent bisulfite conversion. The PAX1 gene methylation detection kit, provided by Macroya Gene Technology (Hunan) Co., Ltd., was used following the manufacturer's instructions. The bisulfite-converted DNA, gene primers, and DNA polymerase were placed in an LC480 real-time fluorescent quantitative PCR instrument [Roche, Switzerland] to amplify the target fragment. Reaction conditions were as follows: 95°C pre-denaturation for 10 minutes; 95°C for 10 seconds, and 60°C for 40 seconds, for a total of 50 cycles. The PCR instrument displayed the Cp values for the PAX1 gene and internal reference gene, with ΔCp = sample Cp value - internal reference Cp value. A ΔCp ≤ 11 was determined as methylation positive.

Diagnostic criteria: The highest pathological result from biopsy or surgery (CKC or LEEP) was used as the final diagnosis for each patient, with all histopathological results reported by pathologists in our hospital. Cervical Intraepithelial Neoplasia (CIN), also referred to as Squamous Intraepithelial Lesion (SIL), is classified into Low-Grade Squamous Intraepithelial Lesion (LSIL) and High-Grade Squamous Intraepithelial Lesion (HSIL). LSIL encompasses proliferative squamous epithelial lesions associated with Human Papillomavirus (HPV) infection, including Cervical Intraepithelial Neoplasia grade 1 (CIN1), CIN1 with flat condyloma, flat condyloma, genital warts, and pathological conditions characterized by clear cell proliferation during the early or late stages of HPV infection, where the recovery of koilocytes is atypical. These lesions exhibit squamous or metaplastic epithelial cell proliferation with abnormal nuclear features, such as nuclear enlargement, irregular nuclear membranes, and an increased nuclear-to-cytoplasmic ratio. The lower third of the epithelium demonstrates immature cells, while maturation begins in the middle third, with relatively normal-appearing cells in the upper third. Mitotic activity is confined to the lower third of the epithelium. HSIL represents a category of HPV-associated squamous epithelial lesions with a higher risk of malignant progression, including CIN2, CIN3, squamous cell carcinoma in situ, and papillary squamous cell carcinoma in situ. These lesions are characterized by squamous or metaplastic epithelial cell proliferation with pronounced nuclear abnormalities, including nuclear enlargement, irregular nuclear membranes, and an increased nuclear-to-cytoplasmic ratio, accompanied by mitotic figures. The middle and superficial two-thirds of the epithelium show minimal or absent cytoplasmic differentiation. Mitotic activity extends beyond the basal third, occurring in the middle and/or superficial thirds of the epithelium [9]. Cervical invasive carcinoma: Tumor cells breach the basement membrane and invade the stromal tissue with a depth of infiltration measuring ≥ 5 mm [10]. Positive pathology was defined as a diagnosis of LSIL (CINI) or above.

Statistical analysis was conducted using SPSS 26.0 and MedCalc software. Measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$) and compared using the t-test. Categorical data were expressed as percentages (%) and compared using the χ^2 test. For 2×2 tables where $n \geq 40$ but $1 < T < 5$, a continuity-corrected χ^2 value was calculated; if $T \leq 1$, Fisher's exact test was applied. The positive rates for each level of lesion across the three testing methods were calculated, and diagnostic results were described using sensitivity, specificity, positive predictive value, negative predictive value, and positive concordance

Table 1. Basic characteristics and relevant data of study results

Variable	$\bar{X}$±SD	Range	
Age	43.33±9.925	23-66	
Body Mass Index	24.21±3.804	16.42-34.89	
Number of Pregnancies	3.44±1.439	0-8	
Number of Births	1.99±1.068	0-8	
	Group	Count	Percentage (%)
SCJ	Partially Visible	46	58.2
	Completely Invisible	33	41.8
High-risk HPV	Positive	74	93.7
	Negative	5	6.3
TCT	NILM	19	24.1
	ASC-US	24	30.4
	ASC-H	6	7.6
	LSIL	13	16.5
	HSIL	15	19.0
	Cervical cancer	2	2.5
PAX1	Positive	34	43.0
	Negative	45	57.0
Pathology Type	Cervicitis	10	12.6
	LSIL	23	29.1
	HSIL	39	49.4
	Cervical Cancer	7	8.9
Pathological source	Biopsy	11	13.9
	CKC	52	65.8
	LEEP	16	20.3

rate. Receiver Operating Characteristic (ROC) curves were plotted for different groups, with the area under the ROC curve used for comparison. The significance level was set at $\alpha = 0.05$, with $P < 0.05$ indicating statistically significant differences. The cutoff value for a positive PAX1 methylation result was also explored.

Results

General patient information

The 79 patients had an average age of 43.33 years (range: 23-66 years). Pathological examination revealed 10 cases of cervicitis, 23 cases of LSIL, 39 cases of HSIL, and 7 cases of cervical cancer. For analysis purposes, cervicitis and LSIL were grouped as the LSIL group, and HSIL and cervical cancer (CC) were grouped as the HSIL+ group. Using postoperative or biopsy pathology as the "gold standard", there were 42 HSIL cases and 37 LSIL cases. The average age of the LSIL group (42.97 years) and the HSIL+ group (43.64 years) showed no statistically significant difference ($P = 0.767 < 0.05$). Detailed study characteristics and relevant data are provided in **Table 1**.

Comparison of the positivity rates of high-risk HPV, TCT, and PAX1 for diagnosing HSIL+

Among the patients, 43.0% (34/79) tested positive for PAX1, and 93.7% (74/79) tested positive for high-risk HPV, with HPV16 detected in 49%, HPV18 in 27%, and other high-risk HPV types in 63%. TCT results were categorized into LSIL and HSIL+ groups, where LSIL included cervicitis and LSIL cases, and HSIL+ included HSIL and cervical cancer (CC) cases. HSIL+ was considered positive for TCT testing, while LSIL was considered negative. TCT positivity was observed in 21.52% (17/79) of patients.

Using biopsy or postoperative pathology as the gold standard, the PAX1 positivity rates for the cervicitis, LSIL, HSIL, and CC groups were 30% (3/10), 30.43% (7/23), 51.28% (20/39), and 71.42% (5/7), respectively. The PAX1 positivity

Table 2. Comparison of positivity rates in HSIL+ and LSIL groups among the three groups

Detection Method	Pathology Level	Positive (Cases)	Negative (Cases)	Positive Rate (%)	Chi-Square Value	*P*
PAX1	HSIL+	23	19	54.76	5.03	0.025
	LSIL	11	26	29.72		
HPV	HSIL+	42	0	100	-	0.019
	LSIL	32	5	86.49		
TCT	HSIL+	15	27	35.71	8.98	0.003
	LSIL	2	35	5.41		

Table 3. Diagnostic efficacy of three detection methods for HSIL+ in the population with partially visible SCJ

Detection method	HSIL+							
	Sen (%)	Spe (%)	PV+ (%)	PV- (%)	+LR	-LR	AUC (%)	AUC (95% CI)
PAX1	54.76	70.27	67.65	57.78	1.84	0.22	65.80	0.543 to 0.761
HPV	100	13.51	56.76	100	1.16	0	56.80	0.451 to 0.679
TCT	35.71	94.59	88.24	16.13	6.6	0.67	65.20	0.536 to 0.755

Note: Sen: Sensitivity; Spe: Specificity; PV+: Positive Predictive Value; PV-: Negative Predictive Value; +LR: Positive Likelihood Ratio; -LR: Negative Likelihood Ratio; AUC: Area Under the Curve.

rate for the LSIL group (cervicitis and LSIL) was 29.72% (11/37), significantly lower than the HSIL+ group (HSIL and CC) at 54.76% (23/42) ($P < 0.05$). HPV positivity rates were 80% (8/10), 91.30% (21/23), 100% (39/39), and 85.71% (6/7) for the cervicitis, LSIL, HSIL, and CC groups, respectively. The HPV positivity rate for the LSIL group was 86.49% (32/37), significantly lower than the HSIL+ group at 100% (42/42) ($P < 0.05$). TCT positivity rates were 80% (8/10), 0% (0/23), 30.77% (12/39), and 57.14% (4/7) for the cervicitis, LSIL, HSIL, and CC groups, respectively. The TCT positivity rate for the LSIL group was 5.41% (2/37), significantly lower than the HSIL+ group at 35.71% (15/42) ($P = 0.003 < 0.05$) (**Table 2**).

Analysis of diagnostic efficacy of the three testing methods for detecting HSIL+ in populations with incomplete SCJ visibility

In the population with incomplete SCJ visibility, the diagnostic performance for detecting HSIL+ was as follows: HPV testing had a sensitivity of 100.00%, specificity of 13.51%, positive predictive value of 56.76%, negative predictive value of 100%, positive likelihood ratio of 1.16, and negative likelihood ratio of 0. For PAX1 methylation testing, the sensitivity was 54.76%, specificity was 70.27%, positive predictive value was 67.65%, negative predictive value was 57.78%, positive likelihood ratio was 1.84, and negative likelihood ratio was 0.22. TCT testing had a sensitivity of 35.71%, specificity of 94.59%, positive predictive value of 88.24%, negative predictive value of 16.13%, positive likelihood ratio of 6.6, and negative likelihood ratio of 0.67 (**Table 3**).

The sensitivity of PAX1 for diagnosing HSIL+ (54.76%) was lower than that of high-risk HPV (100%) ($P < 0.05$). However, the specificity of PAX1 for diagnosing HSIL+ (70.27%) was higher than that of high-risk HPV (13.51%) ($P < 0.05$). When using high-risk HPV positivity for triage, the colposcopy referral rate was 93.67% (74/79), whereas using PAX1 positivity for triage reduced the colposcopy referral rate to 43.04% (34/79), representing a 50.63% decrease in referrals compared to high-risk HPV.

The areas under the ROC curve (AUC) for detecting HSIL+ were 65.8%, 56.8%, and 65.2% for PAX1 methylation, HPV, and TCT, respectively (**Table 4**). Pairwise comparisons of the ROC curve AUCs for the three methods showed no significant differences (all $P > 0.05$) (**Tables 5** and **6**). When comparing the AUCs for detecting HSIL+ in populations with incomplete SCJ visibility using ROC curves, PAX1 methylation testing had the largest AUC (AUC = 65.8%) (**Figure 1**).

Table 4. AUC of the three testing methods for detecting HSIL+ in populations with incomplete SCJ visibility

Detection Method	AUC	Standard Error	Z Value	*P* Value	95% CI	
					Lower limit	Upper limit
HPV	0.568	0.0285	2.372	0.0177	0.451	0.679
TCT	0.652	0.0419	3.617	0.0003	0.536	0.755
PAX1	0.658	0.0624	2.537	0.0112	0.543	0.761

Table 5. Comparison of the AUC for HPV and PAX1 in diagnosing HSIL, P > 0.05

Group	AUC	SE	95% CI	Z Value	P
HPV	0.568	0.0285	0.451 to 0.679	1.345	0.1785
PAX1	0.658	0.0624	0.543 to 0.761		

Table 6. Comparison of the AUC for TCT and PAX1 in diagnosing HSIL, P > 0.05

Group	AUC	SE	95% CI	Z Value	P
TCT	0.652	0.0419	0.536 to 0.755	0.112	0.9108
PAX1	0.658	0.0624	0.543 to 0.761		

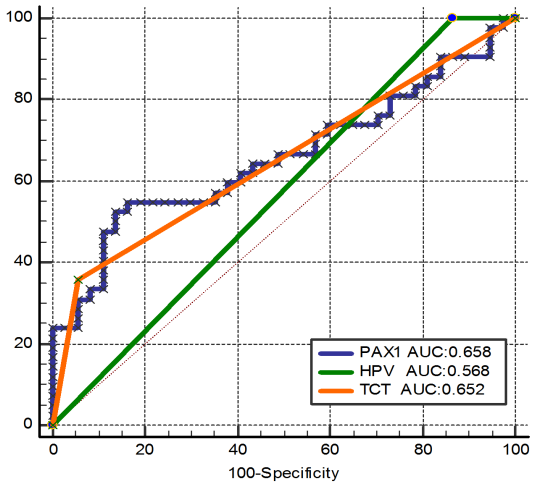

Figure 1. Area under the ROC curve (AUC) of the three detection methods.

Positive concordance rate between positive cases in the high-risk HPV group, TCT group, and PAX1 group and pathology results

Among 74 high-risk HPV-positive cases, the positivity detection rate was 93.6%, with 71 cases confirmed positive by pathology, resulting in a positive concordance rate of 95.9%. In the TCT-positive group, 30 cases were detected as positive, with a detection rate of 37.9%, and all 30 cases were confirmed positive by pathology, achieving a positive concordance rate of 100%. In the PAX1 group, 34 cases were detected as positive, with a detection rate of 43.0%, and 33 cases were confirmed positive by pathology, resulting in a positive concordance rate of 97.1%.

Exploration of cutoff value

Through ROC curve analysis in populations with invisible SCJ, when the AUC was maximized, the Youden Index was 0.389. The optimal threshold for PAX1 methylation in detecting HSIL+ was 9.23. When a PAX1 methylation level of 9.23 was used as the cutoff, the sensitivity was 86.5%, and the specificity was 52.4% (**Figure 2**). When the ΔCP value of PAX1 is below 9.23, the risk of cancer increases in patients with invisible SCJ. This roughly aligns with the predefined standard for methylation positivity (PAX1 cutoff value of 11).

Discussion

Since its invention by German scholar Hans Hinselman in 1925, colposcopy, in combination with cervical cancer screening and treatment of precancerous lesions, has played a crucial role in reducing the incidence and mortality rates of cervical cancer. Compared to TCT and HPV testing, colposcopy has a higher sensitivity for detecting cervical squamous intraepithelial lesions (SIL). Colposcopy uses magnification to

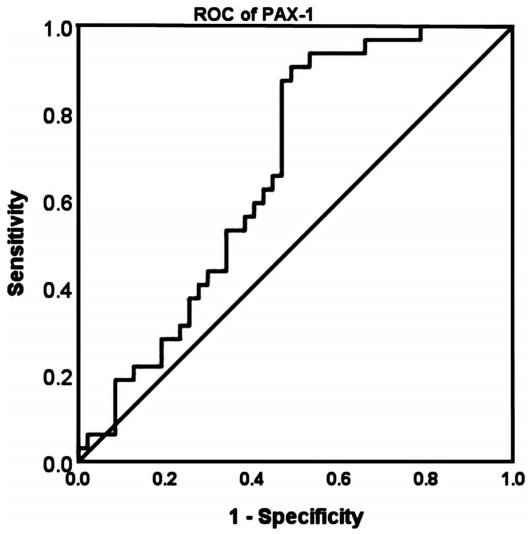

Figure 2. Area under ROC curve of Pax1 gene detection.

observe and assess potential cervical lesions. When screening results indicate a high-risk lesion, such as a high-grade squamous intraepithelial lesion (HSIL), atypical squamous cells cannot exclude HSIL (ASC-H), atypical glandular cells of undetermined significance (AGC), HPV types 16 or 18 positivity, or acetowhite changes on the cervix during colposcopy, multiple targeted biopsies should be performed to improve biopsy positivity rates and diagnostic accuracy. However, colposcopy has limitations in accurately evaluating lesions within the cervical canal. With age, particularly in postmenopausal women, the transformation zone migrates upwards and the new SCJ recedes into the cervical canal, making sample collection more challenging and increasing the likelihood of missed diagnoses.

The transformation zone (TZ) significantly impacts the colposcopic diagnosis of SIL and the accuracy of colposcopy-directed biopsies. Experts from the American Society for Colposcopy and Cervical Pathology (ASCCP) have noted that the effectiveness of colposcopy is limited by the lack of standardized terminology, relevant guidelines, and quality assurance measures [11]. Consequently, at the 2017 conference of the International Federation for Cervical Pathology and Colposcopy (IFCPC), ASCCP released updated colposcopy standards, further standardizing colposcopy terminology. Regarding the transformation zone or squamous column junction (SCJ), the 2017 ASCCP terminology replaced the 2011 IFCPC TZ types (1, 2, 3) with a description of SCJ visibility, categorizing it simply as either fully or incompletely visible [12, 13]. An incompletely visible SCJ, due to inadequate exposure under colposcopy, is more likely to result in missed diagnoses than a fully visible SCJ. Studies have shown that when cytology results indicate ASCUS or LSIL and the TZ is partially visible, the detection rate of HSIL+ after endocervical curettage (ECC) is 13%; when the TZ is fully visible, this rate drops to < 5% following ECC [14, 15]. Moreover, research indicates that among LSIL patients, those with a type III TZ have a significantly higher HSIL+ detection rate post-LEEP procedure compared to patients with type I/II TZ, with type III TZ being an independent risk factor for pathological progression to HSIL+ after LEEP [16].

The dysregulation of Pax gene expression is closely related to the development of cancer. The proteins encoded by the Pax gene family are crucial transcriptional regulators whose regulatory functions span from the growth and development of arthropods like fruit flies to vertebrates like humans. Their main roles include regulating gene transcription, promoting cell proliferation, inhibiting apoptosis, and guiding cell differentiation. The Pax gene family in vertebrates consists of nine members (Pax1-Pax9), which play vital roles in the development of the skeletal system, thymus and thyroid, urogenital system, immune system, nervous system, and digestive system. Among them, the Pax1 gene (paired box 1) is considered a key tumor suppressor gene involved in regulating cell differentiation and maturation. Abnormal methylation of PAX1 is closely linked to the initiation and progression of cancer.

In 2008, Taiwanese scholar Lai and colleagues first discovered abnormal methylation of the PAX1 gene in cervical cancer, reporting a high abnormal methylation frequency of 94.4% in cervical cancer tissues. Their study showed that combining PAX1 methylation testing with HPV detection could increase clinical detection sensitivity by approximately 14%. Compared to TCT and HPV, PAX1 gene methylation testing significantly improved cervical cancer screening sensitivity (85%) and specificity (92%) [17, 18]. Subsequently, numerous studies demon-

strated that PAX1 has high diagnostic performance in cervical cancer screening. According to Yingnan Lu's research, the PAX1 methylation level may be useful for tailoring treatment decisions on ECC for patients infected with HPV 16/18, particularly when low-grade cytological abnormalities are present [19]. Furthermore, Karen KL Chan demonstrated that PAX1 detected CIN2+ more sensitively than cytology and HPV16/18 genotyping and that in women with HPV, PAX1 triage referral to colposcopy may be better than cytology and HPV16/18 genotyping [20]. An area under the ROC curve of 0.948 (95% CI: 0.895-0.99) was then found for PAX1 in identifying CIN2+ lesions in a related study conducted by Meiyuan Huan in non-16/18 hrHPV-positive women. PAX1 demonstrated comparable sensitivity and negative predictive value to cytology. Still, it decreased the colposcopy referral rate from cytology alone from 47.7% to 25.6% with PAX1, demonstrating superior specificity and positive predictive value across age groups [21]. Similarly, when compared to liquid-based cytology ≥ ASCUS, Xiaojing Chen's study in non-16/18 hrHPV-positive women showed that PAX1/JAM3 was better than cytology for the diagnosis of CIN3+ and decreased the frequency of mass referrals for colposcopy without sacrificing diagnostic sensitivity [22].

In this study, cervical cancer screening was conducted on 79 patients with incompletely visible SCJ using three detection methods - PAX1, HPV, and TCT - followed by postoperative or biopsy histopathology as the gold standard. The sensitivity, specificity, and AUC of each method were compared. Results showed that PAX1 methylation testing for HSIL+ had a sensitivity of 54.76% and specificity of 70.27%; HPV testing for HSIL+ showed a sensitivity of 100.00% and specificity of 13.51%; and TCT testing for HSIL+ demonstrated a sensitivity of 35.71% and specificity of 94.59%. The AUC for PAX1 methylation testing of HSIL+ was 65.8%, higher than HPV (56.8%) and TCT (65.2%). These findings indicate that HPV testing has exceptionally high sensitivity but low specificity; conversely, TCT testing has high specificity but low sensitivity. In this study, PAX1 testing demonstrated higher sensitivity than TCT with acceptable specificity and a larger AUC than both HPV and TCT, compensating to some extent for the higher false positive and false negative rates of HPV and TCT, in alignment with previous studies [23, 24].

Extensive research has shown that PAX1 has higher diagnostic efficacy than HPV and TCT. Compared to previously reported findings, the sensitivity, specificity, and AUC for PAX1 testing of HSIL in this study were lower. A meta-analysis by Christos Nikolaidis in 2015, which included 7 studies with a total of 1,385 patients, showed that the combined sensitivity and specificity of PAX1 methylation for diagnosing HSIL were 0.66 and 0.92, respectively, with an AUC of 0.923 [25, 26]. Similarly, a 2016 meta-analysis by Yan Chen on PAX1 methylation for diagnosing cervical precancerous lesions found a sensitivity of 0.73, a combined specificity of 0.87, and an AUC as high as 0.91 for HSIL diagnosis [27, 28]. While sensitivity was not exceptionally high in these meta-analyses, the AUC values were above 0.9, indicating a high overall diagnostic accuracy for PAX1 in diagnosing HSIL. In recent years, correlational studies have been published successively. Research by Li Yang et al. indicates that PAX1 levels increase with the severity of cytological and histopathological findings. For cervical intraepithelial neoplasia (CIN), specifically CIN2+ and CIN3+, the areas under the curve were both 0.87. The specificity and positive predictive value of PAX1 were higher than those of abnormal cytology [29]. Meiyuan Huang's research demonstrates that PAX1 exhibited an AUC of 0.948 (95% confidence interval [CI]: 0.895-0.990) for identifying cervical intraepithelial neoplasia grade 2 or worse (CIN2+) lesions. PAX1 showed comparable sensitivity and negative predictive value to conventional cytology, while significantly reducing the colposcopy referral rate from 47.7% (with cytology alone) to 25.6% (with PAX1 testing) [30]. Meng-Meng Chen's study reports that PAX1 gene methylation detection achieved AUC values of 0.934 for diagnosing CIN2+ lesions (with 93.49% sensitivity and 93.24% specificity) and 0.875 for CIN3+ lesions (with 95.31% sensitivity and 79.77% specificity) [31].

Furthermore, this study explored the positive concordance rate between PAX1 test results and histopathology, with the PAX1 group achieving a high positive concordance rate of 97.1%, consistent with the high positive concordance rates of established screening methods HPV and TCT. This demonstrates that PAX1 methyla-

tion gene testing also has significant diagnostic value for cervical precancerous lesions and cervical cancer.

The sensitivity, specificity, and AUC obtained in this study were lower than those in previous studies, possibly due to the specific population targeted in this research. The 79 participants in this study were all patients with incompletely visible SCJ, whereas previous studies did not restrict the type of cervical transformation zone. Patients with insufficient SCJ exposure generally exhibit a lower positivity rate compared to those with fully visible SCJ, further highlighting the difficulty of diagnosing cervical lesions and the higher rate of missed diagnoses in populations with incomplete SCJ visibility. Additionally, the small sample size in this study introduces a higher risk of bias, suggesting that further exploration with a larger sample size is warranted.

Conclusion

In populations with incomplete SCJ visibility, cervical biopsy or postoperative pathology results show distribution across various lesion grades, including inflammation, LSIL, HSIL, carcinoma in situ, and invasive cervical cancer. Compared to HPV and TCT, PAX1 demonstrated higher overall efficacy in diagnosing high-grade cervical lesions, which enhances diagnostic accuracy to some extent, making it suitable for further clinical application and promotion.

Disclosure of conflict of interest

None.

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