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HPV infection and cervical, vagina, and vulvar cancers

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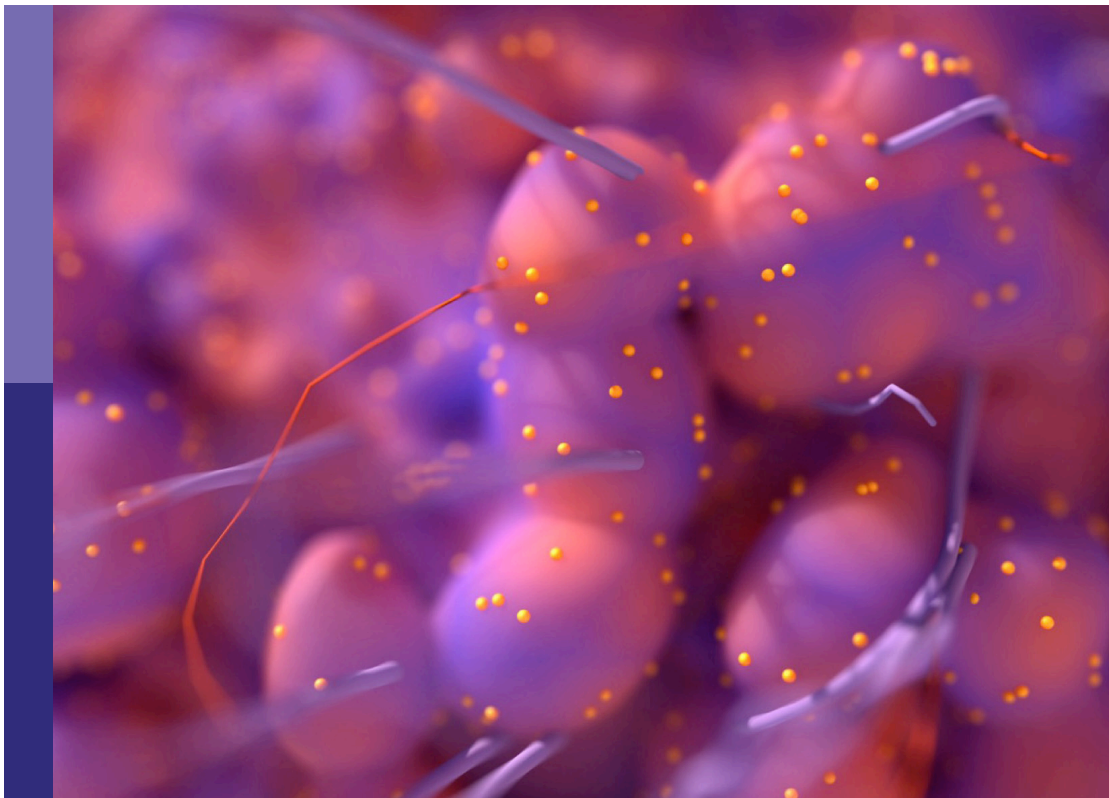

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HPV infection and cervical, vagina, and vulvar cancers

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Impact of HR-HPV infection on oncological outcomes in early cervical cancer

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Background: This study aimed to investigate the differences in long-term oncological outcomes between high-risk human papillomavirus (HR-HPV) negative and HR-HPV positive early-stage cervical cancers.

Methods: We retrospectively analysed 2061 cases of early-stage cervical cancer from the Chinese cervical cancer clinical diagnosis and treatment database. Kaplan-Meier curves were used to describe the survival outcomes of different HR-HPV infections. Cox proportional hazard regression model was used to analyze and determine independent risk factors.

Results: K-M analysis revealed no significant difference in 5-year OS between HR-HPV negative and HR-HPV positive groups (OS: 95.0% vs.95.6%, P=0.900). A significant difference was observed in 5-year DFS between the HR-HPV negative and HR-HPV positive groups (DFS: 87.2% vs.91.9%, P=0.025). Cox proportional hazard regression model indicated that HR-HPV infection (negative vs. positive) was an independent factor influencing 5-year DFS after early cervical cancer surgery (DFS: hazard ratio [HR]=1.862, P=0.022). HR-HPV infection (negative vs positive) was not an independent factor influencing 5-year OS after early cervical cancer surgery (OS: P=0.813). After 1:1 PSM pairing, there was no significant difference in 5-year OS and DFS between HR-HPV negative group and HR-HPV positive group (OS: 91.6% vs.95.0%, P=0.297; DFS: 87.2% vs.85.1%, P=0.758). Cox multivariate analysis indicated that HR-HPV infection was not an independent factor influencing 5-year OS and DFS after early cervical cancer surgery (OS: P=0.806, DFS: P=0.251).

Conclusions: The tumour results of HR-HPV negative group and HR-HPV positive group were similar, after eliminating the differences in known variables that affect the oncological outcomes of cervical cancer. The treatment plan of HR-HPV positive cervical cancer is suitable for HR-HPV negative cervical cancer.

KEYWORDS

cervical neoplasms, HR-HPV negative group, HR-HPV positive group, real-world study, oncological outcomes

Introduction

Cervical cancer is the fourth most common malignant tumor that threatens women's health worldwide. According to data from the International Agency for Research on Cancer, it is estimated that there will be approximately 604,000 new cases and 342,000 deaths due to cervical cancer globally in 2020. In low-income developing countries and regions, the number of new cases and deaths due to cervical cancer ranks second among female malignant tumor (1). Notably, etiological research on cervical cancer has seen a series of breakthroughs. In the 1980s, German virologist Harald Zurhausen proposed that high-risk human papillomavirus (HR-HPV) infection is closely associated with cervical cancer (2). Epidemiological investigations have confirmed that HR-HPV is detectable in 95–99% of cervical cancer tissues (3). With the further research on cervical cancer pathogenesis, the long-term persistent infection of HR-HPV is the decisive factor leading to the occurrence and development of cervical cancer. However, the recent study of 209 cases of cervical cancer in Sweden shows that 7% of tumor patients are still HPV negative using three different methods of genotyping and the reassessment of tumor materials by pathologists (4). In 2019, Malin et al. showed that the use of alternative methods and viral targets for extended analysis of HPV negative cervical cancer patients can reduce the HPV negative proportion from 14% to 7% (5). In clinical practice, with no matter what detection method, some patients with cervical cancer are still not found to have HR-HPV infection. However, the etiology and pathogenesis of these patients are not very clear, and the tumor outcome is rarely reported after clinical treatment. To address these gaps in the field, we compared and analyzed oncologic outcomes of open surgery in HR-HPV-negative and HR-HPV positive cases of stage IA1–IIA2 cervical cancer in real-world settings. To this end, we harnessed data on 63926 cases from databases of 37 hospitals in mainland China in order to elucidate the prognosis of patients with stage I A1–II A2 cervical cancer undergoing laparotomy.

Methods

Data sources

This study was a multicentre, retrospective, observational study, a cervical cancer specialized disease database (n=63926) that covers consecutive patients with cervical cancer in 37 hospitals in mainland China treated since January 2004. The Southern Hospital Ethics Committee of Southern Medical University reviewed the establishment of the cervical cancer database (Ethics No. NFEC-2017-135). The identifier of the clinical trial is CHiCTR180017778 (International Clinical Trials Registry Platform Search Port, http://apps.who.int/trialsearch/).

Clinical data were collected from patient files and the medical record management system in the hospitals by trained gynaecological oncology staff using standardized data collection and quality control procedures. The details of the data sources and methods were the same as those previously reported (6–8). For patients underwent surgical treatment, the collected data contained almost all the information during the treatment of cervical cancer, including demographic details, preoperative examination results, surgical information, pathological results, preoperative and postoperative adjuvant treatment details, complications, hospitalization time and expenses, and follow-up. To ensure the accuracy of the collected data, two uniformly trained staff used EpiData software (EpiData Association, Odense M, Denmark) to input and proofread the same data from each hospital.

All follow-up procedures were carried out by trained gynaecological oncology staff at each centre to keep the patients' personal data confidential and to simultaneously provide disease management guidance. Follow-up information, including the survival status, time of death, recurrence time, recurrence site, and treatment after recurrence, was gathered through the return visit system or through a telephone follow-up. Vaginal stump recurrence was usually confirmed by pathological biopsy, abdominal and pelvic recurrence is detected by computer tomography (CT) or magnetic resonance imaging (MRI), and a few patients are detected by positron emission tomography-CT. The oncological outcomes were estimated according to the recorded information, and the last day of the return visit or telephone follow-up was defined as the last follow-up. In this database, the final International Federation of Gynecology and Obstetrics (FIGO) stage was corrected by tumor size according to the FIGO 2018 staging system. Tumor size was determined by final pathological records.

Inclusion and exclusion criteria

Entry conditions and grouping were as follows (1): Chinese female, age ≥ 18 years; (2) FIGO stage included IA1 (lymphatic vascular space infiltration (LVSI)-positive) - IIA2 stage (including unknown sub-stages of IA (LVSI-positive), IB, IIA); (3) histological type was squamous cell carcinoma, adenocarcinoma, and adeno-squamous cell carcinoma; (4) no preoperative adjuvant therapy was administered; (5) surgical approach was laparotomy; (6) operation method: IA1 (LVSI-positive), IA (LVSI-positive), and IA2 patients underwent QM-B type surgery, while the remaining patients underwent QM-C type surgery; (7) survival outcomes were available; (8) Availability of HR-HPV status. The exclusion criteria were as follows: (1) accidental discovery of cervical cancer, pregnancy complicated by cervical cancer, stump cancer, and other types of malignant tumors concurrently; (2) patients who did not meet the inclusion criteria.

Definition

The staging rules for cervical cancer in FIGO 2018 are based on the combination of clinical imaging and pathological diagnosis results. The following four points should be noted for staging: 1. Two or more senior physicians should conduct a joint physical examination to clarify the clinical staging. When conditions permit, it is best to perform pelvic examination under anesthesia. 2. When there are differences in stages, the earlier stage shall prevail. 3. Allow

imaging and pathological examination results to be used for staging. 4. The diagnosis of minimally invasive carcinoma must be made by an experienced pathologist based on cervical conization specimens.

In this study, all patients were tested for HPV by in-house polymerase chain reaction (PCR). For cervical cancer patients with negative HR-HPV in the first screening, the second sampling and testing were conducted by the same method. Patients who tested negative twice were classified as HR-HPV negative patients.

The 5-year DFS was defined as the date from the operation to the date of death due to cervical cancer or recurrence of cervical cancer. OS was defined as the date from the operation to the date of death from any cause. Patients with no evidence of recurrence or death were defined by the date of the last follow-up date or the last outpatient visit.

Statistical analysis

Continuous variables are summarized by means ± standard deviation, while count variables are summarized by frequency and percentage. The comparison between the mean values of continuous variables is conducted using independent sample t-tests, and the comparison between the rates of counting data groups adopts χ 2 Test, rank variable adopts nonparametric rank sum test. The t-test and the χ 2 Test were used to analyze the clinical pathological characteristics and differences between the HR-HPV negative group and the HR-HPV positive group in early cervical cancer populations. The statistical software used was Statistical Product and Service Solutions 23.0 (SPSS, Inc., Chicago, IL, USA). The P-value <0.05 was considered statistically significant.

Kaplan-Meier curves were used to describe the survival outcomes of different HR-HPV infections. Cox proportional hazard regression model was used to analyze and determine independent risk factors, and estimate the hazard ratio (HR) and 95% confidence interval (CI) of the impact of HR-HPV infection on the 5-year OS and DFS rates. In the Cox proportional risk regression models, we included clinical variables regarded as known factors affecting the oncological outcomes of cervical cancer (age, histological type, FIGO stage, tumor diameter, depth of cervical invasion, LVSI, Parametrial invasion, vaginal margin, and postoperative adjuvant therapy).

In the propensity score matching (PSM) analysis, patients in the HR-HPV negative group were matched to patients in the HR-HPV positive group based on propensity score to reduce bias. Then, a new group of patients was constructed with different HR-HR-HPV infection but similar other clinicopathological features. The propensity score of each patient to receive HR-HPV negative patients was calculated by logistic regression model, which included clinical variables of known factors affecting the oncological outcomes of cervical cancer (age, histological type, FIGO stage, tumor diameter, depth of cervical invasion, LVSI, parametrial invasion, vaginal margin, and postoperative adjuvant therapy). This propensity score was used for one-to-one matching cases with the nearest neighbor matching with variance of 0.02.

Results

A total of 2,061 cases met the enrolment criteria. The detailed data-filtering process is presented in Figure 1.

Comparison of oncological outcomes between HR-HPV negative and HR-HPV positive surgical cases of early cervical cancer 2061 cases of cervical cancer in IA1~IIA2 stage met the initial inclusion criteria, including 153 cases in HR-HPV negative group and 1908 cases in HR-HPV negative group (Table 1).

The survival analysis revealed no significant difference in 5-year OS (OS: 96.7% vs.96.9%, P=0.900) between the HR-HPV-negative and HR-HPV positive groups, but there was a significant difference between the HR-HPV negative group and the HR-HPV positive group in the 5-year DFS (DFS: 89.5% vs.94.0%, P=0.025) (Figures 2A, B).

Cox multivariate analysis indicated that HR-HPV infection (negative vs. positive) was not an independent factor influencing 5-year postoperative death due to early cervical cancer (OS: P = 0.813) (Table 2). HR-HPV infection (negative vs. positive) is an independent influencing factor for recurrence/death of early cervical cancer 5 years after surgery (DFS: P=0.022) (Table 2). HR-HPV positive is a risk factor for DFS 5 years after surgery. The

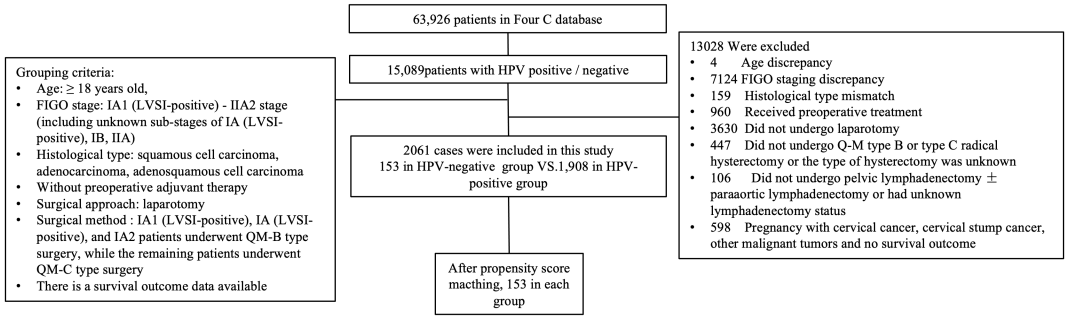

FIGURE 1
Flow diagram of recruitment and exclusions. HPV, human papillomavirus; FIGO, Federation International of Gynecology and Obstetrics; QM, Querleu-Morrow.

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TABLE 1 The clinicopathologic characteristics of patients in HPV-positive group and HPV-negative group before matching.

Characteristics	HPV- positive (n=1908)		HPV-negative (n=153)		P value
Age	47.88 ± 9.839		47.58 ± 9.397		0.716
Histological type					<0.001
Squamous cell carcinoma	1711	89.70%	121	79.10%	
Adenocarcinoma	157	8.20%	30	19.60%	
Adenosquamous carcinoma	40	2.10%	2	1.30%	
FIGO stage					0.357
IA1	39	2.00%	1	0.70%	
IA2	51	2.70%	8	5.20%	
IB1	479	25.10%	36	23.50%	
IB2	726	38.10%	49	32.00%	
IIA1	408	21.40%	37	24.20%	
IIA2	89	4.70%	11	7.20%	
IA	55	2.90%	3	2.00%	
IB	33	1.70%	4	2.60%	
IIA	20	1.00%	3	2.00%	
I	7	0.40%	1	0.70%	
II	1	0.10%	0	0.00%	
Tumor diameter					0.276
≤4cm	1731	90.70%	133	86.90%	
>4cm	89	4.70%	11	7.20%	
Unreported	88	4.60%	9	5.90%	
Depth of cervical invasion					0.869
≤1/2	894	46.90%	69	45.10%	
>1/2	835	43.80%	68	44.40%	
Unreported	179	9.40%	16	10.50%	
LVSI					0.812
Negative	1620	84.90%	131	85.60%	
Positive	288	15.10%	22	14.40%	
Parauterine infiltration					0.306
Negative	1895	99.30%	153	100.00%	
Positive	13	0.70%	0	0.00%	
Vaginal margin					0.910
Negative	1868	97.90%	150	98.04%	
Positive	40	2.10%	3	1.96%	
Postoperative adjuvant therapy					0.079
None	888	46.50%	71	46.40%	
Chemotherapy	268	14.00%	12	7.80%	

(Continued)

TABLE 1 Continued

Characteristics	HPV- positive (n=1908)		HPV-negative (n=153)		P value
Radiotherapy	286	15.00%	22	14.40%	
Radiotherapy/radiochemotherapy	466	24.40%	48	31.40%	

Values are presented as mean ± standard deviation or number (%). Bold indicates significant p-value.
LVSI, lymphovascular space invasion.

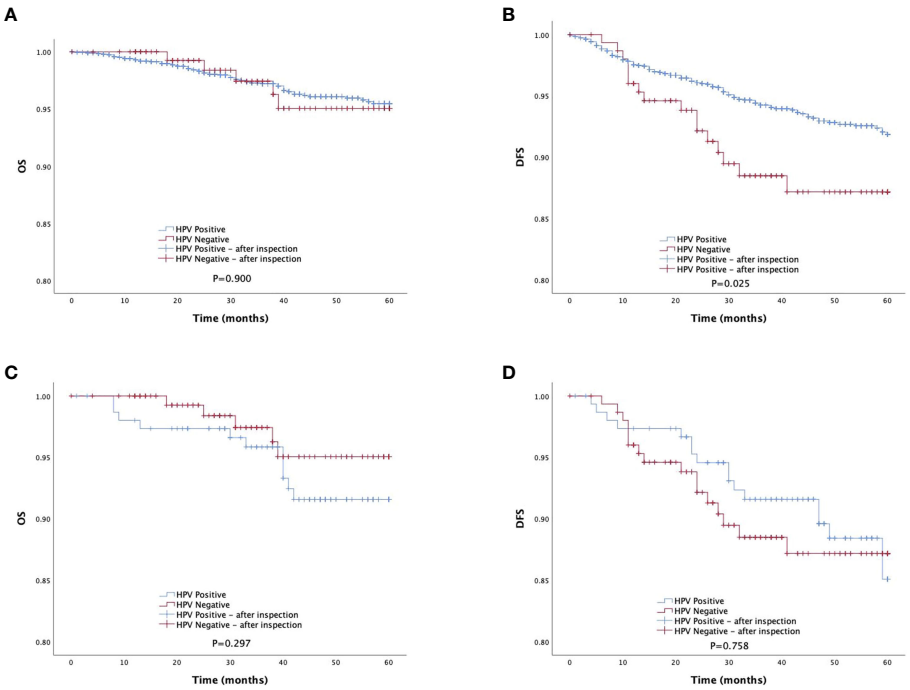

FIGURE 2
Survival outcomes between HPV-negative group and HPV-positive group in study population. DFS, disease-free survival; PSMpropensity score matching. **(A, B)** The 5-year DFS and OS of total study population. **(C, D)** The 5-year DFS and OS of total study population after PSM matching.

risk of recurrence/death in HR-HPV positive group is 1.862 times that in negative group.

Comparison of oncological outcomes between HR-HPV-negative and HR-HPV positive surgical cases of early cervical cancer after further enrolment and matching.

Meet the initial inclusion criteria and strictly follow the histological type, LVSI, postoperative adjuvant therapy 1:1 matching. The matching tolerance is 0, including 153 cases each in the HR-HPV positive and HR-HPV negative group (Table 3).

The survival analysis showed that there was no statistically significant difference between the HR-HPV negative and the HR-HPV positive group in the 5-year OS (OS: 96.7% vs.92.8%, P=0.297), and there was no statistically significant difference between the HR-HPV negative and the HR-HPV positive group in the 5-year DFS (DFS: 89.5% vs.88.9%, P=0.758) (Figures 2C, D). Cox multifactor analysis showed that HR-HPV infection (negative vs positive) was not an independent factor (OS: P=0.806) influencing 5-year mortality after surgery for early cervical cancer (Table 4), and influencing factor for recurrence/death of early cervical cancer 5 years after surgery (DFS: P=0.251) (Table 4).

Discussion

In this study, our previous study showed that HR-HPV infection (negative vs. positive) is an independent influencing factor for recurrence/death of early cervical cancer 5 years after surgery. However, after PSM matching to eliminate relevant confounders, we found that HPV infection was not an independent influencer of recurrence/death after early cancer surgery.

This study was based on the real conditions in some parts of Chinese Mainland. in order to explore the impact of HR-HPV infection on the oncological outcome of early cervical cancer after laparotomy. The subjects were patients with stage IA1~IIA1 cervical cancer treated by laparotomy. This study was a multicenter study based on the real-world study, covering a large database of 63926 cases in 37 hospitals of different regions, levels and categories in China. It can reflect the real research situation of oncological outcomes of IA1~IIA1 cervical cancer patients with different HR-HPV infection in China after laparotomy.

At present, studies have confirmed that cervical cancer is caused by HR-HPV infection. persistent infection with HR-HPV

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TABLE 2 Association of HPV infection and survival in cervical cancer by multivariable analysis.

Characteristics	OS				DFS			
	P	HR	95.0% CI		P	HR	95.0% CI	
Age	0.341	1.012	0.987	1.038	0.873	1.001	0.984	1.020
HPV	0.813	1.117	0.444	2.810	0.022	1.862	1.095	3.166
FIGO stage								
IA1	0.933				0.821			
IB1	0.895	2802.410	0.000	8.476E+54	0.868	5819.890	0.000	1.119E+48
IB2	0.889	4529.725	0.000	1.377E+55	0.862	8530.113	0.000	1.640E+48
IIA1	0.884	6278.068	0.000	2.009E+55	0.858	10687.868	0.000	2.055E+48
IIA2	0.893	3168.755	0.000	1.011E+55	0.867	6201.489	0.000	1.196E+48
IA2	1.000	1.037	0.000	9.009E+83	0.999	1.141	0.000	7.298E+70
IA	0.998	0.779	0.000	8.817E+90	0.869	5490.128	0.000	1.075E+48
IB	0.944	7435.606	0.000	1.244E+112	0.826	35659413.996	0.000	8.880E+74
IIA	0.997	0.527	0.000	1.128E+132	0.821	58772346.686	0.000	1.465E+75
I	0.999	0.866	0.000	4.375E+164	0.991	4.502	0.000	7.015E+110
II	1.000	1.073	0.000	.	0.999	0.785	0.000	.
Histological type								
Squamous cell carcinoma	0.617				0.885			
Adenocarcinoma	0.654	0.782	0.266	2.300	0.715	1.123	0.603	2.092
Adenosquamous carcinoma	0.400	1.855	0.440	7.814	0.721	1.235	0.387	3.938
Tumor diameter								
≤4cm	0.989				0.991			
>4cm	0.947	0.961	0.301	3.069	0.914	0.956	0.421	2.171
Unreported	0.900	1.067	0.387	2.944	0.929	0.966	0.450	2.073
Depth of cervical invasion								
≤1/2	0.000				0.000			
>1/2	0.000	3.700	1.923	7.116	0.000	2.550	1.667	3.901
Unreported	0.361	0.387	0.051	2.961	0.127	0.399	0.123	1.297
LVSI	0.102	1.661	0.904	3.050	0.043	1.582	1.014	2.469
Parauterine infiltration	0.266	2.387	0.515	11.055	0.132	2.534	0.756	8.491
Vaginal margin	0.560	0.553	0.075	4.059	0.586	1.326	0.481	3.656
Postoperative adjuvant therapy								
None	0.075				0.009			
Chemotherapy	0.081	0.513	0.242	1.087	0.042	0.569	0.330	0.981
Radiotherapy	0.350	0.691	0.318	1.501	0.225	0.721	0.424	1.224
Radiotherapy/radiochemotherapy	0.014	0.442	0.231	0.847	0.001	0.450	0.280	0.724

Multicollinearity test and cox proportional hazard regression models were used for analysis. Bold indicates significant p-value.
CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; LVSI, lymphovascular space invasion; OS, overall survival.

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TABLE 3 The clinicopathologic characteristics of patients in HPV-positive group and HPV-negative group after matching.

Characteristics	HPV-positive (n=153)		HPV-negative (n=153)		P value
Age	46.48 ± 9.210		47.58 ± 9.397		0.303
Histological type					1.000
Squamous cell carcinoma	121	79.10%	121	79.10%	
Adenocarcinoma	30	19.60%	30	19.60%	
Adenosquamous carcinoma	2	1.30%	2	1.30%	
FIGO stage					0.421
IA1	9	5.90%	8	5.20%	
IA2	1	0.70%	1	0.70%	
IB1	38	24.80%	36	23.50%	
IB2	65	42.50%	49	32.00%	
IIA1	30	19.60%	37	24.20%	
IIA2	4	2.60%	11	7.20%	
IA	1	0.70%	3	2.00%	
IB	2	1.30%	4	2.60%	
IIA	2	1.30%	3	2.00%	
I	0	0.00%	1	0.70%	
II	1	0.70%	0	0.00%	
Tumor diameter					0.089
≤4cm	144	94.10%	133	86.90%	
>4cm	4	2.60%	11	7.20%	
Unreported	5	3.30%	9	5.90%	
Depth of cervical invasion					0.592
≤1/2	70	45.80%	69	45.10%	
>1/2	72	47.10%	68	44.40%	
Unreported	11	7.20%	16	10.50%	
LVSI					1.000
Negative	131	85.60%	131	85.60%	
Positive	22	14.40%	22	14.40%	
Parauterine infiltration					0.082
Negative	150	98.00%	153	100.00%	
Positive	3	2.00%	0	0.00%	
Vaginal margin					0.474
Negative	148	96.70%	150	98.00%	
Positive	5	3.30%	3	2.00%	
Postoperative adjuvant therapy					1.000
None	71	46.40%	71	46.40%	
Chemotherapy	12	7.80%	12	7.80%	

(Continued)

TABLE 3 Continued

Characteristics	HPV-positive (n=153)		HPV-negative (n=153)		P value
Radiotherapy	22	14.40%	22	14.40%	
Radiotherapy/radiochemotherapy	48	31.40%	48	31.40%	

Values are presented as mean± standard deviation or number (%). Bold indicates significant p-value.
LVSI, lymphovascular space invasion.

TABLE 4 Association of HPV infection and survival in cervical cancer by multivariable analysis after PSM matching.

Characteristics	OS				DFS			
	P	HR	95.0% CI		P	HR	95.0% CI	
Age	0.114	1.046	0.989	1.107	0.294	1.021	0.982	1.061
HPV	0.806	0.869	0.282	2.672	0.251	1.529	0.741	3.156
FIGO stage								
IA1	0.997				0.987			
IB1	0.917	198.284	0.000	2.217E+45	0.858	967.793	0.000	4.408E+35
IB2	0.889	1184.989	0.000	1.296E+46	0.847	1651.144	0.000	7.505E+35
IIA1	0.887	1284.564	0.000	1.406E+46	0.849	1480.011	0.000	6.735E+35
IIA2	0.991	0.416	0.000	3.813E+63	0.987	0.400	0.000	8.542E+47
IA2	0.997	0.634	0.000	1.346E+113	0.997	0.695	0.000	9.624E+79
IA	0.985	20.476	0.000	1.384E+137	0.986	7.253	0.000	9.531E+95
IB	0.962	67647.126	0.000	8.265E+204	0.954	16614.497	0.000	9.451E+146
IIA	0.991	18.993	0.000	1.643E+215	0.946	90243.195	0.000	5.119E+147
I	0.973	33925.276	0.000	1.156E+269	0.965	18234.065	0.000	2.416E+192
II	0.996	0.393	0.000	1.725E+175	0.997	0.610	0.000	2.369E+124
Histological type								
Squamous cell carcinoma	0.563				0.389			
Adenocarcinoma	0.284	2.063	0.548	7.760	0.259	1.666	0.686	4.048
Adenosquamous carcinoma	0.954	0.002	0.000	1.619E+92	0.340	2.959	0.319	27.416
Tumor diameter								
≤4cm	0.992				0.660			
>4cm	0.980	0.000	0.000	.	0.971	0.000	0.000	3.919E+272
Unreported	0.902	1.135	0.150	8.595	0.362	1.738	0.530	5.704
Depth of cervical invasion								
≤1/2	0.294				0.241			
>1/2	0.121	3.226	0.735	14.156	0.096	2.074	0.879	4.895
Unreported	0.857	0.002	0.000	2.424E+27	0.790	0.001	0.000	1.967E+20
LVSI	0.715	0.766	0.184	3.200	0.012	9.984	1.648	60.493
Parauterine infiltration	0.009	13.453	1.891	95.707	0.015	9.239	1.550	55.079
Vaginal margin	0.580	1.964	0.180	21.406	0.782	0.722	0.071	7.294

(Continued)

TABLE 4 Continued

Characteristics	OS				DFS			
	P	HR	95.0% CI		P	HR	95.0% CI	
Postoperative adjuvant therapy								
None	0.470				0.500			
Chemotherapy	0.743	1.293	0.278	6.018	0.694	1.286	0.368	4.493
Radiotherapy	0.543	0.554	0.083	3.707	0.515	1.425	0.491	4.134
Radiotherapy/radiochemotherapy	0.211	0.409	0.101	1.661	0.348	0.624	0.233	1.672

Multicollinearity test and cox proportional hazard regression models were used for analysis. Bold indicates significant p-value.
CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; LVSI, lymphovascular space invasion; OS, overall survival; PSM, propensity score matching.

(especially type 16) can cause cancer of the cervix (9). HPV plays an important role in the pathogenesis of cervical cancer. It affects host cell apoptosis, cell cycle, cell adhesion and DNA repair mechanisms, and can also activate immune response (10, 11). In addition, the integration of HR-HPV virus is closely related to the development of cervical cancer (12). HR-HPV also affects the prognosis of cervical cancer.

However, several recent studies have shown that HR-HPV infection has a paradoxical impact on the prognosis of cervical cancer. Liana et al. believe that HPV-negative cervical cancer patients were significantly more likely to have adverse outcomes than HPV 16/18-positive patients (P=0.018; OR=3.31) (13). Ping Li et al. believed that HPV-DNA positive status was associated with good prognosis in patients with cervical cancer (OS: HR=0.610, 95% CI=0.457-0.814, P=0.001; DFS: HR=0.362, 95% CI=0.252-0.519, P < 0.001) (14). Go et al. suggested that DFS of HPV-negative cervical cancer patients was worse than that of HPV positive ones (HR=3.97; 95% CI=1.84-8.58; P=0.0005) (15). Many other publications have reported that the DFS of HPV-negative cervical cancer patients after radiotherapy or chemotherapy is low regardless of other prognostic factors (age, stage, lymph node metastasis) (16–18). In other HPV related tumor studies, Anthony et al. believed that OS and DFS of HPV positive tumor patients were improved in 3 years compared with HPV negative tumor patients in oropharyngeal squamous cell carcinoma (90% vs 65%, respectively, P=0.001; 85% vs 49%, P=0.005) (19).

There are still some reports suggesting that there is no significant correlation between HPV infection and tumor prognosis. A recent systematic study found that there was no statistically significant association between HPV16 and/or HPV18 positive and overall survival or disease-free survival of cervical cancer (20). In a study of adeno-squamous carcinoma of the head and neck, Giacomo et al. suggested that HPV positive and HPV-negative tumors had similar OS and DFS (21). These findings support the present study.

With the further study of cervical cancer, the relationship between HPV infection and prognosis has been changing. HPV infection is a decisive factor in the occurrence of cervical cancer, but in actual clinical work, a small number of cervical cancer patients have negative HPV detection. HPV negative squamous cervical carcinoma is very rare. HPV positivity of among adenosquamous cancers (ADS) may be up to 86%, the prevalence of HPV among adenocarcinoma (ADC) varies between the subtypes (Usual type 80-100%; Mucinous, Intestinal type 83-100%; Villoglandular 100%; Mucinous, signet ring cell type 100%; Endometrioid 0; Gastric Type 0; Masonephric 0; Clear cell 28%; Serous 30%) (22, 23). The pathogenesis of non HPV-associated adenocarcinoma(NHPVA) is considered irrelevant or independent of HPV (24). In fact, NHPVA is related to mutation. As for tumor inhibitor p53, the loss of its function due to the change of TP53 gene is a common event of cancer in different anatomical regions. Barreto et al. showed that there was a relationship between p53 mutation and poor prognosis (24). In Nicolás et al.'s study, 71% (15/21) HPV negative patients had p53abn (25). This mutation phenotype of NHPVA can explain that the tumor has higher relaxation and regulation ability, increased growth potential and metastasis, and worse prognosis. Other scholars' studies suggest that HR-HPV negative tumors may have become permanent and lost internal mutation control, so that somatic host mutations related to malignant growth and diffusion potential are obtained, while HR-HPV positive tumors may be better controlled by the immune system due to the expression of viral proteins, so the prognosis is relatively more positive (26).

In order to avoid the influence of different pathological tissue types, LVSI, and postoperative adjuvant therapy on the tumor outcome of cervical cancer patients as much as possible, this study strictly controls them to eliminate the influence of the above differences on the tumor outcome of cervical cancer patients. However, there are still limitations of HPV detection in clinical practice. Sampling errors may be the primary cause of false negative HPV testing. For example, low cellularity (due to cancer necrosis and/or inflammation), influence of blood or lubricants, cell fixation or cell lysis may lead to classification errors. It is reported that the use of formalin fixed and paraffin embedded samples had an impact on DNA preservation and subsequent HPV-DNA test results, leading to the high prevalence of HPV negative tumors (24). The low content of HPV DNA in some cervical cancers is considered as a possible cause of false negative test results. It is worth noting that the dedifferentiation and subsequent loss of HPV in the tumor may also change the HPV detection results (27).

In addition, many other factors may also be influencing factors that have no significant correlation between HPV infection and oncological outcome of IA1~IIA1 cervical cancer patients after abdominal surgery.

The sample size is not large enough, the definition of HPV infection status (HPV positive cases, HPV16 positive cases or other reference categories) is different, the treatment plans received by cervical cancer patients are different, the statistical definition of survival rate is different, and there are many relative confounding factors in the actual clinical treatment process.

Real-world research has garnered increasing attention in recent times, as exemplified by the "Basic Considerations for Real-World Evidence Supporting Drug Research and Development" issued by Chinese State Drug Administration in May 2019. Although treatments were not standardized, this report represents the status of cervical cancer diagnosis and treatment in China. Moreover, this study adopted PSM to eliminate baseline heterogeneity between groups. Crucially, this study more realistically reflected the treatment status and oncological outcomes of Chinese patients with HPV-negative and HPV positive IA1–IIA2 cervical cancer, providing evidence that may not be available from randomized controlled trials.

This study has several limitations that stem from the retrospective nature of data collection. Although patients were matched based on perioperative factors to minimize bias, unknown confounding factors not captured in the dataset may have created residual bias in the results. Further, this study only focused on the analysis of survival outcomes of treatment groups with different HPV conditions after laparotomy for cervical cancer, and did not analyze the impact of specific conditions on the oncological outcome in postoperative radiotherapy, chemo-therapy and follow-up treatment. We look forward to a multicenter prospective study with a larger sample and a longer follow-up time.

Conclusions

In conclusion, HPV-negative cervical precancerous lesions are not common in clinical practice, and their clinical characteristics and prognosis are not more favorable than those of HPV positive lesions. This study explored the impact of HPV infection on oncological outcomes of early cervical cancer by assessing patients with stage IA1-IIA2 cervical cancer undergoing surgery in parts of mainland China, encompassing 37 different regions, grades, and categories in China. This multicenter study based on real-world research contributes to previous gaps in the literature, as we provide novel insight into oncological outcomes after treatment for HPV-negative and HPV positive stage IA1–IIA2 cervical cancer in China. We aim to conduct further research in this area in order to provide a theoretical basis and novel ideas for individualized and differentiated treatment of different types of cervical lesions.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by The Southern Hospital Ethics Committee of Southern Medical University (Ethics No. NFEC-2017-135). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

MH: Writing – review & editing. XS: Writing – original draft, Writing – review & editing. PaL: Writing – original draft. HZ: Writing – original draft. LS: Writing – original draft. WW: Writing – original draft. SJ: Writing – original draft. HW: Writing – original draft. PiL: Writing – review & editing. CC: Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Oncological and reproductive outcomes of conization combined with pelvic node evaluation in patients with early-stage cervical cancer: a systematic review and meta-analysis

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Objective: This study aims to preliminarily assess the oncological and reproductive outcomes of fertility preservation treatment using conization combined with pelvic node evaluation in young patients with early-stage cervical cancer (ECC) through meta-analysis.

Methods: In this meta-analysis, we analyzed studies published in PubMed, Embase, Cochrane Central Register of Controlled Trials (CENTRAL), International Clinical Trials Registry Platform (ICTRP), and Clinical Trials. gov that appeared in our search from inception to 0 7/02/2023.

Results: There were 17 relevant studies with a total of 620 patients included, of which 444 patients received conization combined with pelvic node evaluation. The combined pregnancy rate was 45.4% (95% CI: 0.34–0.57), the combined live birth rate was 33.9% (95% CI: 0.26–0.42), the combined miscarriage rate was 4.8% (95% CI: 0.02–0.092), the combined preterm delivery rate was 5.1% (95% CI: 0.02–0.092), and the combined recurrence rate was 1.9% (95% CI: 0.006–0.035), which did not significantly differ from that of patients who received radical surgery (OR: 0.689, 95% CI: 0.506–0.938).

Conclusion: Cervical conization combined with pelvic lymph node evaluation for fertility preservation in young ECC patients can achieve oncological outcomes similar to radical surgery while improving pregnancy success rates and preserving postoperative fertility. In summary, fertility preservation treatment using cervical conization combined with pelvic lymph node evaluation may be considered as a viable option for young ECC patients with strong fertility preservation desire, resulting in better pregnancy and live birth outcomes.

Systematic review registration: https://www.crd.york.ac.uk/PROSPERO/#myprospero, identifier PROSPERO (CRD42023423432).

KEYWORDS

conization, pelvic node evaluation, oncological outcome, reproductive outcome, cervical cancer

1 Introduction

Cervical cancer is the fourth most common female malignancy worldwide (1). With the widespread use of HPV and cervical cancer cell screening, the detection rate of early cervical cancer has greatly increased. At the same time, the morbidity of young patients is gradually increasing due to changes in social lifestyle. It has been reported that around 35% of cervical cancer patients are under 40 years old (2) and a considerable proportion of them have not completed childbirth or still have fertility requirements. Currently, first-line treatment advised by guidelines for ECC is radical hysterectomy with bilateral pelvic lymphadenectomy and/or sentinel lymph node (SLN) biopsy with or without salpingo-oophorectomy (3), which results in loss of fertility and is not acceptable for young patients.

In ECC, many studies have shown that the incidence of parametrial involvement (PI) is low in patients with tumor size < 2 cm, negative pelvic lymph nodes, and invasion depth < 10 mm (4, 5). This supports the use of simpler fertility-preserving surgical methods for young patients with small tumor volume and limited local lesions, further improving their quality of life. For women with ECC who want to preserve fertility, both FIGO and NCCN guidelines recommend conization with lymph node evaluation for stage IA1 no lymphovascular space invasion (LVSI), radical trachelectomy or conization with lymph node evaluation for stage IA1 with LVSI and stage IA2, or radical trachelectomy with lymph node evaluation for stage IB1 and selected IB2 (6, 7).

Dargent et al. published his experience of performing RT with laparoscopic pelvic lymph node dissection for young women with ECC in 1994 (8, 9). Some studies show that RT is a safe and feasible technique with similar oncological results to cervical conization, but it has a high rate of miscarriage and preterm labor during pregnancy (10, 11), which may impair postoperative reproductive outcomes. Several studies have reported that conization has generally favorable obstetric outcomes compared with RT (12, 13). Although current guidelines recommend the application of cervical conization in ECC, the safety, feasibility, and treatment outcome of conization combined with lymph node evaluation in patients with ECC have not been fully evaluated.

We conducted a systematic review to evaluate the oncological and fertility outcomes of using cervix conization combined with pelvic lymph node evaluation surgery to treat ECC patients.

2 Materials and methods

The systematic review and meta-analysis were conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines and registered in PROSPERO (CRD42023423432).

2.1 Search strategy

We systematically examined the PubMed, EMBASE, Cochrane Library, International Clinical Trials Registry Platform (ICTRP), and Clinical Trials electronic databases to 07/02/2023, to identify relevant literature reporting the use of cervix conization combined with pelvic lymph node evaluation surgery for fertility preservation in patients with ECC. These studies reported the oncological and fertility outcomes of ECC patients. The following search terms were used to identify relevant studies on early cervical cancer: “cervical cancer” and “cervical carcinoma,” whereas the following terms were used to identify relevant studies on conization: “cone biopsy” and “conization”. The following terms were used to identify relevant studies on pelvic lymph node evaluation: “lymph node assessment,” “lymph node dissection,” “lymph node evaluation,” “lymph node excision,” “lymphadenectomy*,” and “lymphadenectomy”. The search was limited to English-language publications. We rigorously reviewed the reference lists of all the articles identified in our search based on inclusion and exclusion criteria, to identify any potentially missing studies or unpublished data. If multiple studies analyzed overlapping patient populations, we selected the most recent or comprehensive results.

2.2 Inclusion and exclusion criteria

The inclusion criteria included the following: (1) Primary cervical cancer patients who received conization combined with lymph node evaluation as initial treatment options were included. (2) The average age of patients included in the literature was less than 40 years old. (3) The clinical stage was FIGO IA1-IB1 (2018 FIGO staging). (4) Tumor diameter <2 cm. (5) No other tumors combined or history of other tumor treatments.

Exclusion criteria included the following: (1) Pathological types were cervical neuroendocrine tumors. (2) Postoperative pathology combined with endometrial cancer or other tumors. (3) Malignant

tumors of other tissue sites or metastatic cervical tumor. (4) Literature that did not analyze and statistically report pregnancy and oncological outcomes, without a clearly defined follow-up deadline or an unreasonable experimental design. (5) Fertility-damaging treatments such as radiotherapy after cone-shaped excision of the cervix combined with pelvic lymph node evaluation surgery for early cervical cancer. (6) Individual case reports or literature with repetitive data (the literature with the latest or more comprehensive results was used for repetitive data). (7) Literature with fewer than five cases.

2.3 Study selection

Two reviewers (YSW and LLZ) screened the studies initially based on titles and abstracts, removing duplicate studies and those that did not meet the review criteria, and then read the remaining articles in full to include eligible studies. Disagreements were resolved through consultation with a third reviewer (YLC). The quality of included studies was evaluated using the non-randomized studies index (MINORS) (14).

2.4 Data extraction and results calculation

Two independent reviewers (MYW and ZJQ) extracted the following data from each study: study author, publication date, study design type (prospective or retrospective), number of patients, median patient age, FIGO stage, tumor histological type, oncological and reproductive outcomes, median follow-up time, and so on.

In this study, we defined pregnancy rate as the number of women who successfully conceived divided by the total number of women who retained fertility during follow-up; live birth rate as the number of surviving infants divided by the total number of women who retained fertility during follow-up; abortion rate as the ratio of women who experienced one or more abortions to the total number of women who retained fertility during follow-up; and premature birth rate as the ratio of women who experienced one or more premature births to the total number of women who retained fertility during follow-up. Recurrence rate was defined as the number of recurrence cases divided by the total number of included patients.

2.5 Statistical analysis

The data extracted were statistically summarized and analyzed using Stata 17.0. Random-effects models were calculated using the inverse variance method, and forest plots were generated for each outcome to obtain individual study and pooled estimates with 95% CI (15). I^2 was used to assess heterogeneity of outcome data (16), and I^2 >50 was considered high heterogeneity. Sources of heterogeneity were determined through subgroup analysis and sensitivity analysis. Publication bias was assessed using Begg–Mazumdar rank correlation and funnel plots.

3 Results

3.1 Search results

A total of 518 studies were retrieved through computer databases and manual searches, which were basically in line with the query requirements. After removing 129 duplicate studies, the remaining 389 articles were screened based on titles and abstracts, and obviously ineligible articles were excluded, resulting in a total of 148 articles remaining. After reading the full texts, 17 studies that met the study criteria were eventually included in the analysis (17–33). The specific search process are detailed in Figure 1.

3.2 Included literature and characteristics of studies

A total of 17 English language studies were included in this study, consisting of 7 prospective studies and 10 retrospective studies, including 620 young patients with ECC. These studies were conducted in various countries, including the United States (n = 2), Japan (n = 1), Canada (n = 2), Germany (n = 1), China (n = 1), Italy (n = 7), United Kingdom (n = 1), Argentina (n = 1), and the Netherlands (n = 1). The average age of onset for the included patients in these studies was close (between 29 and 38 years old), and the follow-up time ranging from 16 to 79.9 months. General information of the included literature is shown in Figure 2.

3.3 Quality assessment of included studies

This article included a total of 17 English language studies. All included literature was assessed for quality using the non-randomized controlled trials methodological evaluation index: MINORS. All studies had clear objectives, but blinding was not used during the study process and the necessary sample sizes were not prospectively estimated. A total of 14 studies consecutively included patients, 14 studies collected data that was designed in the study protocol before the study began, and 13 studies had endpoints that could adequately reflect the research objectives. According to the guidelines, the follow-up time should be at least 5 years. Only 4 studies out of the 17 studies reported follow-up data for at least 5 years. One study reported a >5 follow-up loss (29). The quality assessment of all studies is shown in Figure 3.

3.4 Fertility and oncologic outcomes

3.4.1 Pregnancy rates

There were 16 studies that reported on pregnancy rates, including 569 patients. A total of 415 (72.93%) patients successfully received conization combined with pelvic node evaluation. Furthermore, 183 young women achieved at least one pregnancy, and the combined pregnancy rate was 45.4% (95% CI, 0.34–0.571) (17–22, 24–33). The heterogeneity test result for the included studies was I^2 = 81.0, P < 0.05 (Figure 4-1), indicating high

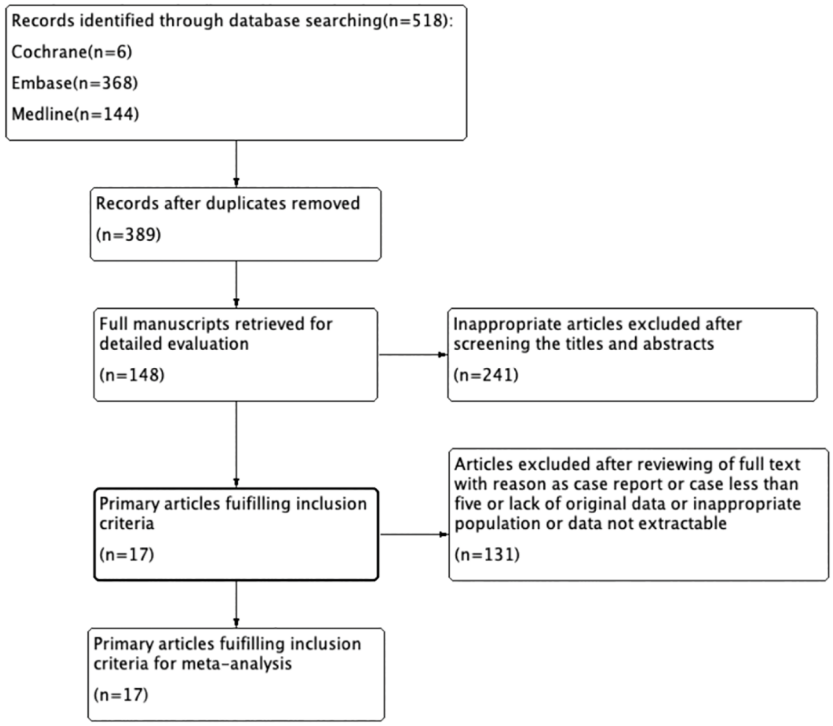

FIGURE 1
Flowchart of literature selection process.

heterogeneity among the included studies. The sensitivity analysis could not identify the source of heterogeneity by eliminating studies one by one. Subgroup analysis showed that when studies were grouped by research type, the combined pregnancy rate for prospective studies was 35.8 (95% CI, 0.227–0.442) (19, 22, 24, 26, 27, 32), $I^2 = 0$ P > 0.05 (Figure 4-2), indicating that there was no obvious heterogeneity among prospective studies. The source of heterogeneity could not be identified in other subgroups. The type

Study	Country	Study design	Median age (y)	Patients	FIGO stage, n (%)	Histology, n (%)	Women treated	Median follow-up (months)
Yamamoto 2022[17]	Japan	Retrospective	33 (28-36)	8	IA1 5(62.5%); IA2 2(25%); IB1 1 (12.5%);	SCC 7(87.5%);AC 1(12.5%);	8	60 (8-107)
Tsaousidis 2022[18]	German	Retrospective	32 (25-37)	23	IA1 6(26.1%); IA2 4(17.4%); IB1 13(56.5%);	SCC 10(43.4%);AC 13(56.6%);	16	46.8 (31.2-96)
Schmeler 2021[19]	America	Prospective	38 (23-67)	100	IA2 33(33%); IB1 67(67%);	SCC 48(48%);AC 52(52%);	42	36.3 (0-68.3)
Lucchini 2021[20]	Argentine	Retrospective	31.5 (23-41)	31	IA1 8(25.8%); IA2 11(35.5%); IB1 12(38.7%);	SCC 24(77.4%);AC 7(22.6%);	31	41.4 (2-90)
Fanfani 2013[21]	Italy	Retrospective	30 (24-43)	23	IA2 7(30.4%); IB1 16(69.6%);	SCC 11(47.8%);AC 11(47.8%);GCT 1(4.3%);	23	40 (32-125)
Ditto 2014[22]	Italy	Prospective	31 (27-40)	22	IA2 6(27.3%); IB1 16(72.7%);	SCC 10(45.5%);AC 11(55%);ASC 1(4.5%);	18	48.8 (16-81.6)
Bouchard 2014[23]	Canada	Prospective	34 (19-77)	51	IA1 30(58.8%); IA2 8(15.7%); IB	SCC 26(51%);AC 22(43.1%);ASC 3(5.9%);	29	21 (1-122)
Bogani 2019[24]	Italy	Prospective	33 (29-37)	32	IA2 9(28.1%); IB1 21(65.6%); IB	SCC 20(62.5%);AC 11(34.4%);ASC 1(3.1%);	25	75 (12-184)
ANica 2021[25]	Canada	Retrospective	31 (19-61)	44	IA1 18(40.9%); IA2 5(11.4%); IB	SCC 27(61.4%);AC 16(36.4%);ASC 1(2.3%);	34	44 (6-137)
Andikyan 2014[26]	America	Prospective	28 (18-36)	10	IA1 7(70%); IB1 3(30%);	SCC 8(80%);AC 1(10%);GCT 1(10%);	10	17 (1-83)
Martinelli 2021[27]	Italy	Prospective	33 (22-40)	39	IA1 3(7.7%); IA2 11(28.2%); IB1	SCC 22(56.4%);AC 17(43.6%);	33	51 (1-184)
Maneo 2011[28]	Italy	Retrospective	31 (24-40)	36	IB1(100%);	SCC 24(66.7%);AC 12(33.3%);	31	66 (6-168)
Lindsay 2014[29]	Britain	Retrospective	29 (22-38)	43	IA1 2(4.8%); IA2 4(9.5%); IB1 37	SCC 28(65.1%);AC 11(25.6%);ASC 4(9.3%);	35	42 (0-91)
Li 2020[30]	China	Retrospective	32 (21-41)	40	IA1 5(12.5%); IA2 21(52.5%); IB	SCC 35(87.5%);AC 3(7.5%);ASC 2(5%);	39	35 (8-74)
Fanfani 2021[31]	Italy	Retrospective	32 (19-44)	42	1BI(100%)	SCC 27(64.3%);AC 13(31%);ASC 2(4.8%);	35	54 (1-185)
Fagotti 2011[32]	Italy	Prospective	33 (30-43)	17	IA2 4(23.5%); 1B1 13(76.5%)	SCC 12(70.6%);AC 4(23.5%);GCT 1(5.9%);	13	16 (8-101)
Baalbergen 2011[33]	Netherlands	Retrospective	Unknown	59	IA1 33(55.9%) IA2 26(44.1%)	AC 59(100%);	22	79.9 (10-131)

FIGURE 2
Characteristic of the studies.

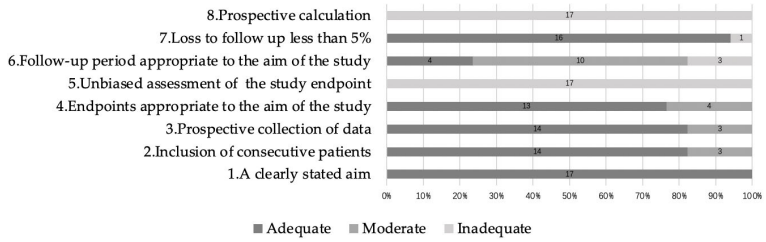

FIGURE 3
Quality assessment.

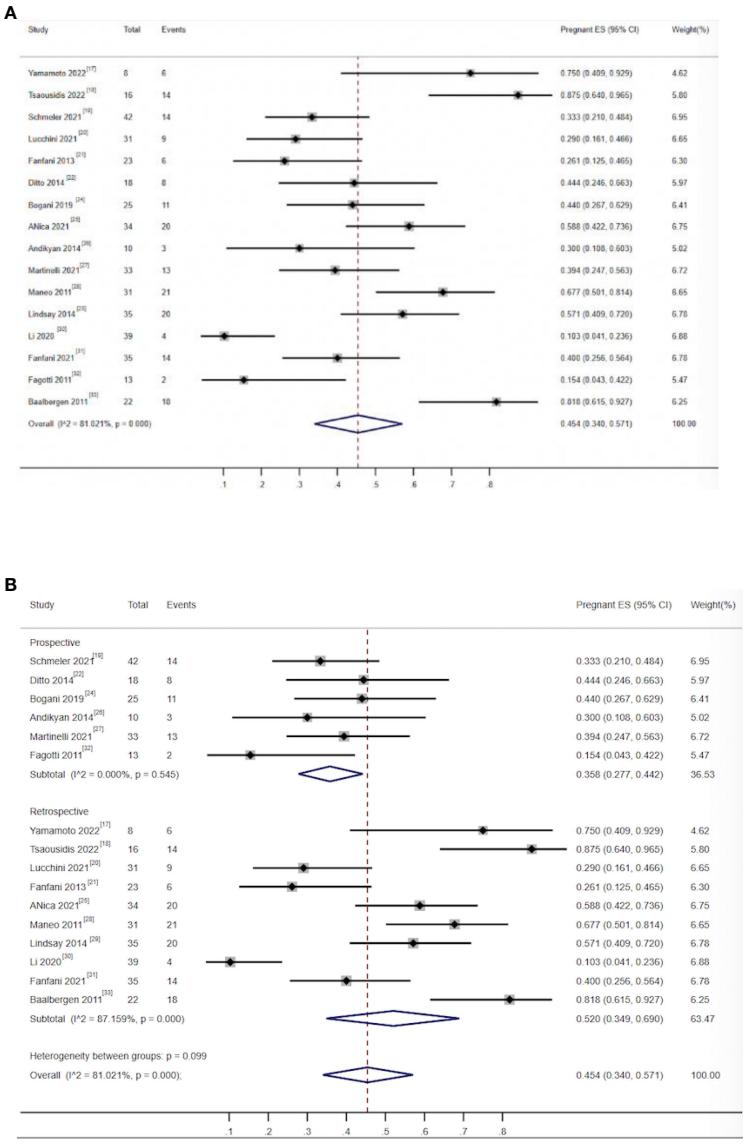

FIGURE 4
(A) Forest plot of the meta-analysis of pregnancy rate in ECC patients who underwent conization combined with pelvic node evaluation. **(B)** Forest plot of the meta-analysis of pregnancy rate in ECC patients who underwent conization combined with pelvic node evaluation using study type subgroup analysis.

of study directly affects the quality of evidence and therefore the quality of the integration result. The heterogeneity among prospective studies was significantly reduced in subgroup analysis by study type, and the overall sample pregnancy rate should be closer to the data obtained from prospective studies.

3.4.2 Live birth rate and miscarriage rate

There were 15 studies that reported on live birth rates and miscarriage rates, including 559 patients. Among them, 405 (72.45%) patients successfully received fertility-preserving treatment. Among them, 138 women gave birth to at least one healthy baby. The combined live birth rate was 33.9% (95% CI, 0.261–0.422) (17–22, 24–33), and the heterogeneity test result for the included studies was I^2 = 63.2 P < 0.05, indicating high heterogeneity among the included studies (Figure 5-1). Subgroup analysis showed no significant difference among subgroups. Sensitivity analysis identified one study as a potential source of heterogeneity (30). Excluding it greatly reduced heterogeneity (I^2 = 35.26 P > 0.05) and yielded a similar outcome as the combined live birth rate in all studies [36.6% (0.302–0.431)] (Figure 5-2). There were 25 patients who had experienced miscarriage once or more. The combined miscarriage rate was 4.8% (95% CI, 0.02–0.085), and the heterogeneity test result for the included studies was I^2 = 41.97 P = 0.044, indicating low heterogeneity among the included studies (Figure 5-3).

3.4.3 Preterm delivery rate

There were 12 studies that reported on the preterm delivery rate, including 369 patients. Among them, 310 (84%) patients successfully received fertility-preserving treatment (17, 18, 21, 22, 24, 25, 27–32). Among them, 21 women experienced at least one preterm delivery. The combined preterm delivery rate was 5.1% (95%, 0.02–0.092), and the heterogeneity test result for the included studies was I^2 = 34.03 P > 0.05, indicating low heterogeneity among the included studies (Figure 6).

3.4.4 Recurrence rate

There were 17 studies that reported on the recurrence rate, including 620 patients. Moreover, 18 patients experienced recurrence, and the combined recurrence rate was 1.9% (0.006–0.035) (17–33) (Figure 7). The heterogeneity test result for the included studies was I^2 = 0 P > 0.05, indicating no significant heterogeneity among the included studies. Three studies involving 210 patients reported recurrence rates of patients who received cervical conization combined with pelvic lymph node evaluation (1.05% 1/95) or radical surgery (2.6% 3/115) (18, 23, 33). The ratio between the two groups showed no significant difference (OR = 0.689, 0.506–0.938).

3.4.5 Publication bias

Begg–Mazumdar rank correlation test showed that the funnel plot in the meta-analysis of the main outcome indicator pregnancy rate in ECC patients undergoing cervical conization combined with pelvic lymph node evaluation is slightly asymmetric (Figure 8), indicating the possibility of publication bias in the corresponding study. This phenomenon may be due to the inadequate retrieval of negative results in literature or biased database literature inclusion criteria, which to some extent weakened the reliability of the statistical results.

4 Conclusions

Fertility preservation is becoming an increasingly important issue for young cervical cancer patients. While ensuring the outcome of oncology, the reproductive outcome should be further improved. The results of this study clarify that cervical conization combined with pelvic lymph node evaluation can achieve similar oncological outcomes as RT for ECC while also achieving more optimal obstetric outcomes.

Cervical conization combined with pelvic lymph node evaluation showed good results in terms of oncological outcomes. Nezhat et al.'s study has shown that among all fertility-sparing treatments with or without pelvic node evaluation, the overall mean cancer recurrence rate was 3.2% (34). Rob et al.'s study has shown that the recurrence rate of Dargent RT surgery is between 4.2% and 4.7% (35), and Plante summarized the recurrence rates of abdominal and laparoscopic RT as 4% and 7%, respectively (36). In our meta-analysis, the recurrence rates mentioned in the 17 articles were very low, ranging from 0% to 9.1% with a combined recurrence rate of 1.6% (95% CI, 0.005–0.03) indicating comparable recurrence results with RT. Some studies have shown that 60% of patients who underwent RT did not have residual tumor lesions in the surgical specimens, indicating that these patients can be treated with less aggressive surgery to achieve the expected oncological outcomes (37). Based on these results, we recommend that cervical conization combined with pelvic lymph node evaluation be considered as a safe alternative to RT for young women with ECC who wish to preserve fertility.

In terms of reproductive outcomes, RT is often reported to increase the risk of postoperative premature birth and miscarriage, reducing the success rate of postoperative fertility preservation in young patients. Pareja et al. summarized the pregnancy rates after RT for ECC via abdominal and vaginal routes globally to be 16.2% and 24% (38). Additionally, some studies have found high rates of miscarriage in early and middle pregnancy after RT (16%–20% and 8%–10%), with a high risk of premature birth (20%–30%) (10, 11, 39). In our meta-analysis, the combined results of cervical conization combined with pelvic lymph node evaluation seem to be more ideal for reproductive outcomes. In ECC patients undergoing cervical conization combined with pelvic node evaluation, approximately half (45.4%) of the patients can conceive, with as high as one-third (33.9%) giving birth to healthy babies and only 4.8% experiencing miscarriage and 5.1% experiencing premature birth. This may be mainly due to the relatively minor removal of para-cervical tissue and less damage to pelvic floor function during the surgery.

However, our meta-analysis has the following limitations: Significant heterogeneity was observed among studies in the analysis of pregnancy rate and live birth rate, reflecting the differences between included studies. The retrospective design and

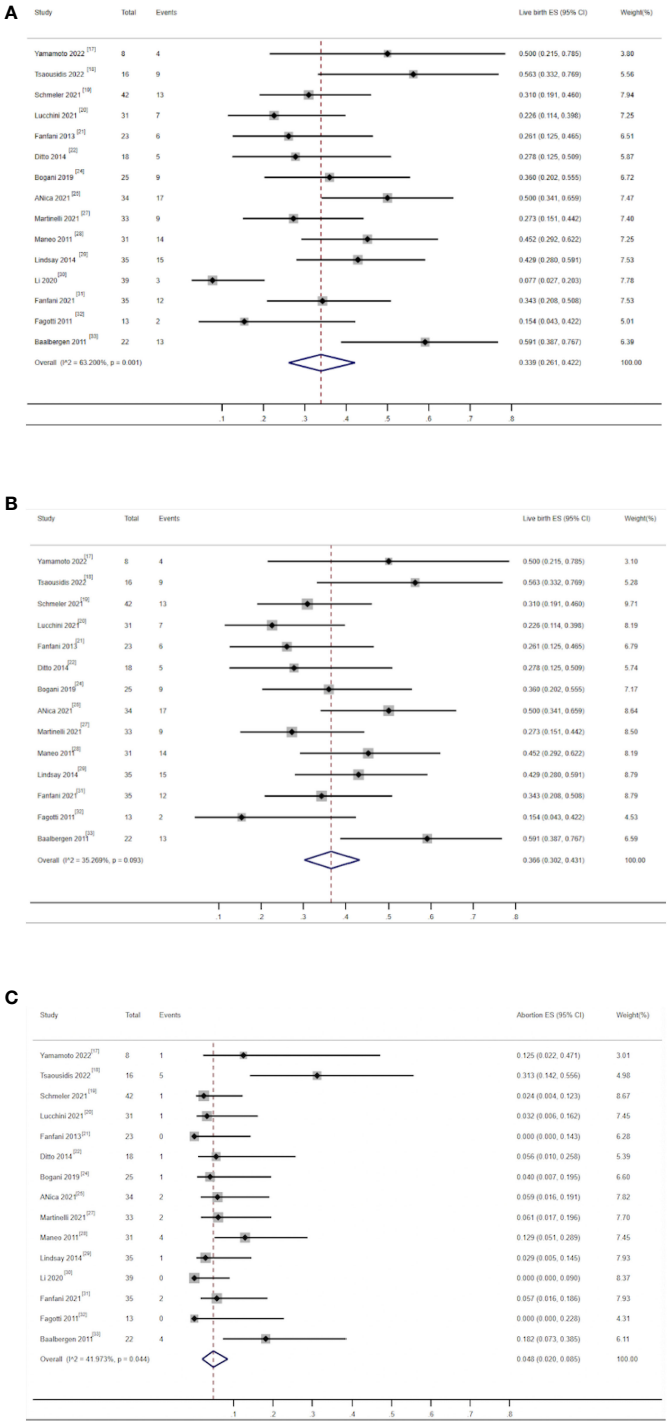

FIGURE 5
(A) Forest plot of the meta-analysis of live birth rate in ECC patients who underwent conization combined with pelvic node evaluation. **(B)** Forest plot of the meta-analysis of live birth in ECC patients who underwent conization combined with pelvic node evaluation, after removal of one study (30). **(C)** Forest plot of the meta-analysis of abortion rate in ECC patients who underwent conization combined with pelvic node evaluation.

differences in sample size of the studies may also be sources of heterogeneity. We were able to identify individual studies with significant contributions to heterogeneity, and exclusion of these studies for repeat analysis yielded similar results to the original analysis. Our study was conducted through ratio-based rather than randomized controlled trials, which may introduce many confounding effects and weaken the reliability of evidence. The inclusion of only English-language studies may also introduce biases, and the limited availability of domestic research data in this study may differ from China's genetics, environment, and

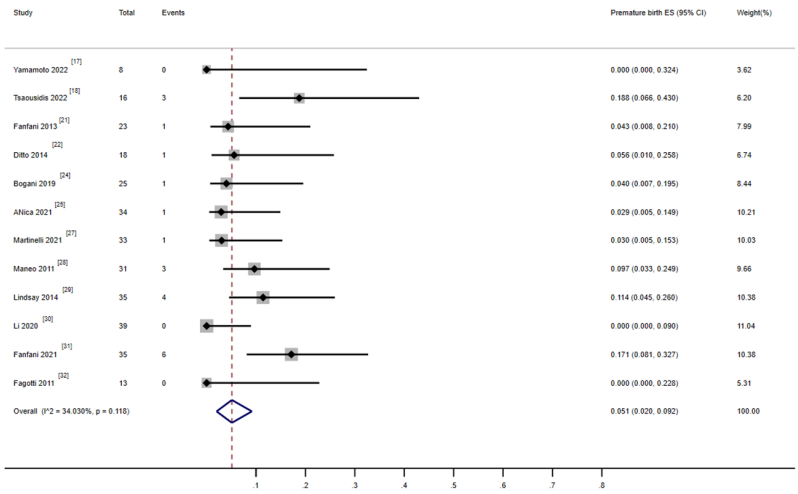

FIGURE 6
Forest plot of the meta-analysis of premature rate in ECC patients who underwent conization combined with pelvic node evaluation.

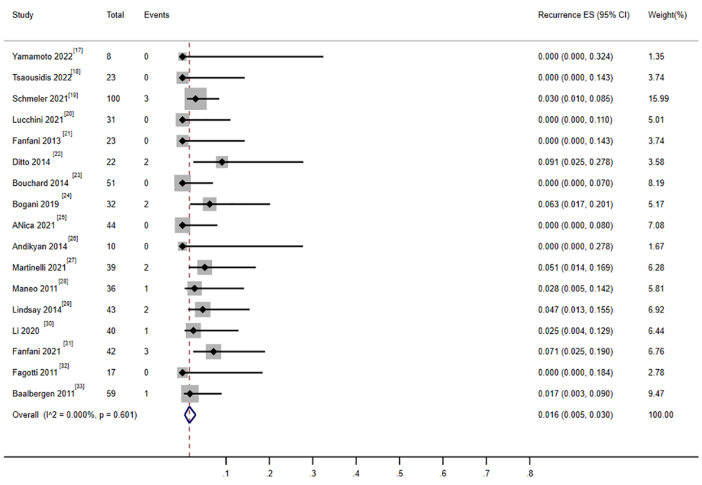

FIGURE 7
Forest plot of the meta-analysis of recurrence rate in ECC patients who underwent conization combined with pelvic node evaluation.

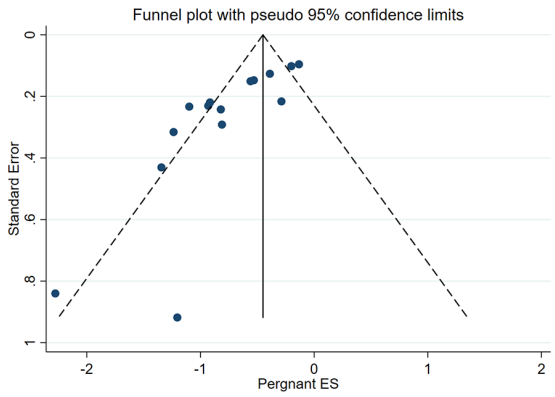

FIGURE 8
Funnel plot of the meta-analysis of pregnancy rate in ECC patients who underwent conization combined with pelvic node evaluation.

health conditions. Therefore, whether this treatment method is beneficial to domestic cervical cancer patients in preserving fertility while ensuring survival rate or specific indications still requires further verification.

Although the above limitations exist, our meta-analysis results indicate that cervical combined with pelvic lymph node evaluation has a good oncological and reproductive outcome. To our knowledge, this is the first meta-analysis to evaluate the oncological and reproductive outcomes after cervical conization combined with pelvic lymph node evaluation.

Cervical conization with pelvic lymph node evaluation seems to be an acceptable treatment for well-selected patients with low-risk, early-stage cervical cancer who wish to preserve fertility. It offers excellent oncological outcomes and good reproductive results. Further large prospective studies are warranted to prove the effectiveness of this surgery.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Author contributions

YW: conceptualization, data curation; YC: writing—original draft preparation; LZ: methodology, software, validation; MW: visualization, investigation; ZQ: methodology, formal analysis; LH: supervision: AZ: writing—review editing. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Vulvar squamous intraepithelial neoplasia epithelial thickness in hairy and non-hairy sites: a single center experience from China

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Introduction: A large-sample study focusing on VIN lesions of a more precise thickness is needed to help guide clinical treatment. This study aimed to investigate the depth of vulvar intraepithelial neoplasia (VIN) and involved skin appendages to provide evidence for laser surgery.

Methods: The study retrospectively enrolled and analyzed the clinical characteristics of VIN patients in the obstetrics and gynecology department of a university hospital between January 1, 2019 and December 30, 2021. The study further explored the thickness of epithelium and skin appendages of 285 women with low-grade VIN (VIN1) and 285 women with high-grade VIN (VIN2/3).

Results: The study included 1,139 (80%) VIN1 and 335 (20%) VIN2/3 cases. The VIN1 and VIN2/3 groups showed a significant difference in human papillomavirus infection (P<0.01) but not in cytology (P = 0.499). Most (89.90%, 1,325) cases occurred in one area of the vulva, whereas 10.11% were multifocal. VIN commonly occurred on the posterior fourchette (76.85%), labia majora (11.61%), and labia minora (9.92%). The VIN2/3 group reported a significantly higher positive rate for concurrent cervical and vaginal intraepithelial neoplasia (160 of 285) than the VIN1 group (321 of 953) (P=0.000). The involved epithelial thicknesses in VIN2/3 and VIN1 were 0.69 ± 0.44 and 0.49 ± 0.23 mm, respectively, both of which were greater than the corresponding noninvolved epithelial thickness (0.31 ± 0.19 and 0.32 ± 0.10 mm, P<0.001 and P<0.001, respectively). In cases of appendage involvement, the VIN thickness was 1.98 ± 0.64 mm.

Conclusions: VIN thickness was generally ≤1 mm for the superficial lesions in non-hairy areas. However, for lesions extending onto hairy areas, the thickness was approximately 3 mm, leading to the destruction of involved skin appendages.

KEYWORDS

vulvar intraepithelial neoplasia, vulva, HPV, squamous intraepithelial lesion, treatment, thickness

1 Introduction

In 2015, the International Society for the Study of Vulvovaginal Disease proposed a revised classification of vulvar intraepithelial neoplasia (VIN) terminology. This classification included subtypes such as low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL), alongside the VIN differentiated type (1). LSIL and HSIL correspond to the former VIN1 and VIN2/3 nomenclature, respectively. VIN2/3 is more prevalent in younger women (2) and is considered a premalignant condition. This condition is related to invasive vulvar squamous cell carcinomas (VSCC), which account for over 80% of vulvar malignancies.

The management of VIN remains challenging owing to the lack of a clear consensus regarding the best treatment modality. VIN therapy must be individualized; therefore, comparing therapies to determine the optimal treatment is often difficult. Patients with VIN1 are recommended to undergo observation without treatment owing to the high rate of spontaneous regression. For patients with visible VIN1 lesions or those whose VIN1 lesions do not improve during observation, drugs, physical therapy, and surgical procedures can be considered. Current treatment options for VIN2/3 include local surgical excision (consisting of the removal of all visible lesions using a scalpel and electrosurgery), chemotherapy (including cidofovir, photodynamic therapy, and imiquimod), photodynamic therapy, laser ablation, and vaccination (3). Local surgical excision, often in the form of vulvectomy, can be disfiguring, emotionally distressing, and cause sexual problems in many women. Additionally, the incidence of VIN among younger women has been increasing, prompting the consideration of conservative therapy as a viable option. Alternative conservative topical chemotherapy, utilizing immune-modulating agents, and antiviral therapy have varying disadvantages. These include high rates of ulceration and unclear success response rates, ranging 26–70% (4–6). Photodynamic therapy has demonstrated numerous limitations, including a high rate of treatment failure, immunosuppressive effects, and consequent increases in direct and non-direct costs (7). Laser surgery, which uses a high-energy light beam, has been proposed as a surgical intervention for various cases of VIN. This approach has yielded mixed success, with reports indicating generally favorable tolerance, satisfactory healing, and minimal sexual dysfunction (8, 9).

However, the risk of residual disease or VIN recurrence exists in different treatment methods owing to unclear identification of the lesion's macroscopic characteristics. Notably, different areas of the vulva exhibit variations in skin structure. Few studies have investigated the depth of epithelial and involved appendages and yielded consistent results. These studies suggested that depths of 1.0 mm and 2.0–2.5 mm in non-hairy and hairy sites, respectively, were appropriate for successful treatment (10, 11). However, these studies included only a small number of patients. Thus, a study with a large sample size focusing on VIN lesions with a more precise thickness is required to help guide clinical treatment. In this study, we aimed to describe the depth of involved and noninvolved vulvar epithelium and appendages in women with VIN and recommend the optimal depth for epithelial ablation during laser surgery.

2 Materials and methods

2.1 Patients

This was a single-center retrospective study conducted in a large obstetrics and gynecology hospital in China. We enrolled patients who underwent colposcopy-directed biopsy or vulvar surgical vulvectomy and subsequently diagnosed with VIN1 and VIN2/3 between January 1, 2019 and December 30, 2021. Patients with an incomplete medical history, VIN with warts (condyloma acuminata), and who were lost to follow-up were excluded. Approval was obtained from the relevant institutional review board before data extraction was commenced, and all women provided consent to participate in the study. Finally, 285 patients with VIN2/3 were enrolled in the study. Considering the large number of VIN1 cases, we randomly selected 285 VIN 1 patients who were diagnosed during the study recruitment period.

2.2 Cytology and human papillomavirus testing

Cervical or vaginal cytology tests were interpreted and reported by two pathologists based on the 2014 Bethesda System. Human papillomavirus (HPV) testing was performed using a fluorescence-based multiplex real-time HPV DNA genotyping kit (Bioperfectus, Jiangsu, China) capable of detecting both high-(16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82) and low-risk HPV types (6,11, and 81).

2.3 Histological technique and anatomicopathological features

We examined tissue specimens from all study participants Specimens were collected through biopsy or local surgical vulvectomy, subsequently fixed in buffered formalin, and embedded in paraffin. We stained 4-μm sections of paraffin-processed samples with hematoxylin and eosin. Two experienced pathologists scanned and reviewed all digital slides. All margin diagnoses were negative for intraepithelial lesions or invasive cancer. To compare the vulvar epithelium thickness before and after formalin fixation, seven radical vulvectomy samples classified as International Federation of Gynecology and Obstetrics phase I VSCC were selected. Each sample included a frozen section diagnosis of the vulvar margin, as well as its corresponding formalin-fixed and paraffin-embedded (FFPE) sample. All margin diagnoses were negative for intraepithelial lesions or invasive cancer. In total, we retrieved 21 paired sections, 3 from each case. Epithelium thickness was measured on whole-slide images, targeting similar sites on the paired sections.

Surface keratinization or surface separated from the epidermis rendered the use of surface as a reference point inaccurate. Consequently, we initiated vertical measurements at the stratum

corneum-granulosum junction and extended them to the basal layer. We measured multiple foci and recorded the maximum values. We also obtained the thickness of involved and noninvolved epithelium or appendages in the same section. All available data were recorded, including patient cytology records, history of HPV and CIN/VaIN disease (excluding other diseases, such as diabetes, hypertension, and autoimmune diseases), and age.

2.4 Statistical analysis

Statistical analyses were performed using SPSS Statistics for Windows, version 16.0 (SPSS Inc., Chicago, IL, USA). We utilized independent samples t-tests and chi-squared tests to assess differences between groups. Statistical significance was set at $P<0.05$.

3 Results

A total of 1,474 women who underwent vulvar biopsy or local surgical vulvectomy between January 1, 2019 and December 30, 2021 were diagnosed with VIN (Table 1), with an average of 42.72 ± 14.31 years. Of them, 1,139 (80%) and 335 (20%) were diagnosed with VIN1 and VIN2/3, respectively. In addition, women in the VIN2/3 group were significantly older than those in the VIN1 group ($P<0.01$). We also found a significant difference in HPV infection rate ($P<0.01$) but not in cytology ($P = 0.499$) between the VIN1 and VIN2/3 groups. For the VIN lesions, 90% (1,325 of 1,474) were unifocal, and 10% (149) were multifocal. In our study, VIN was commonly found on the posterior fourchette (76.85%), labia majora (11.61%), and labia minora (9.92%). We recorded 1,374 of 1,678 (77.83%) VIN lesions in non-hairy areas and 372 (22.17%) in hairy areas. Cervical squamous intraepithelial neoplasia (CIN) and/or vaginal squamous intraepithelial neoplasia (VaIN) were observed in 38.85% (481 of 1,238) of VIN cases without a history of CIN/VaIN/any other disease. We noted a significantly higher positive rate for concurrent CIN and VaIN in the VIN2/3 group (56.14%, 160 of 285) compared with that in the VIN1 group (33.68%, 321 of 953) ($P=0.000$).

We randomly selected 285 VIN1 patients who were diagnosed during the same period as VIN-2/3 patients, all of whom had no history of CIN/VaIN. The clinical characteristics of the women with VIN1 and VIN2/3 are shown in Supplementary 1. In the VIN1 group, six cases had two lesion sites, specifically the posterior fourchette and labia majora. In the VIN2/3 group, 20 cases had 2 lesion sites, and 3 cases had 3 lesion sites, specifically the posterior fourchette, labia majora, and perianal areas. In the VIN1 group, CIN/VaIN 1 and CIN/VaIN 2/3 were detected in 35 (12.28%) and 8 (2.81%) cases, respectively. Two patients (0.70%) also had squamous cell carcinoma of the cervix (SCC). In the VIN2/3 group, CIN/VaIN 1, CIN/VaIN 2/3, SCC, and vaginal squamous carcinoma (VaSCC) were detected in 90 (31.6%), 59 (20.7%), 8 (2.8%), and 3 (1.1%) patients, respectively.

Table 2, Figures 1, 2 show the epithelial thickness of VINs in different sites. We examined 291 and 309 sections of tissue from 285 patients with VIN1 and 285 patients with VIN 2/3, respectively. Of the 600 tissue sections, VIN was detected on the posterior fourchette (45.33%), labia majora (18.83%), and labia minora (14.83%). The maximum depths of epithelial lesions were 1.6 mm and 2.75 mm in the VIN1 and VIN 2/3 groups, respectively. In the VIN2/3 group, significant differences in the thickness of involved and noninvolved epithelia were detected across all vulvar sites ($P<0.05$). Moreover, in the VIN1 group, the thickness of involved epithelia was greater than that of the noninvolved epithelia, except in the clitoris, urethral opening, and navicular fossa. The thickness of involved epithelium were 0.69 ± 0.44 mm and 0.49 ± 0.23 mm in the VIN2/3 and VIN1 groups, respectively ($P=0.000$). However, the depth of noninvolved epithelia was projected to be consistent across all VIN grades. We found that 32.81% (187 of 570) of VINs were involved in hairy areas. The rates of involvement in hairy areas were 28.07% (80 of 285) and 37.54% (107 of 285) in the VIN1 and VIN2/3 groups, respectively. The most common lesion site in non-hairy areas was the posterior fourchette in both groups. Conversely, the labia majora was the most common lesion site in hairy areas. We noted significant differences in the epithelial thickness between VINs in non-hairy and hairy areas (0.52 ± 0.30 mm vs. 0.78 ± 0.45 mm, $P<0.001$).

Table 3 shows the depth of involved epithelial and skin appendages in VIN and noninvolved tissue. Compared with nondysplastic samples, we found no significant difference in the depth of stratum corneum between the VIN groups. The thickness of involved skin appendages in VIN ranged 0.91–5.44 mm (mean depth, 1.98 ± 0.64 mm), whereas that of noninvolved skin appendages ranged 0.26–4.38 mm (mean depth, 1.66 ± 0.85 mm). VIN appeared to affect hair follicles in only one patient, with the depth reaching 5.44 mm. The thickness of epithelium of the involved skin appendages in VIN was consistently greater than that of the involved epithelium at the same section (1.98 ± 0.61 mm vs. 1.01 ± 0.52 mm, $P<0.001$). Hair follicles represented the most commonly involved appendage, followed by sebaceous glands. The involvement of sweat glands was not detected in any VINs (Figure 3).

As shown in Supplementary 2, the thickness of involved epithelia in all VIN grades was consistently greater than that of the noninvolved epithelia across all age groups. We observed a significant decrease of thickness with age in both noninvolved and involved epithelia in all VIN grades ($P<0.001$ for all comparisons). In comparisons between the VIN2/3 and VIN1 groups, the differences in thickness of the involved epithelia were statistically significant across the different age groups, including pre- and postmenopausal women ($P<0.001$ for all comparisons). Comparisons of the thickness of noninvolved epithelia showed no significant differences between the VIN2/3 and VIN1 groups in subgroup-level (age, premenopausal and postmenopausal group; $P>0.05$ for all comparisons).

To compare the vulvar epithelium thickness before and after FFPE treatment, we analyzed 21 pairs of frozen and corresponding FFPE-treated sections. The epithelial thickness was 0.32 ± 0.18 mm and 0.31 ± 0.11 mm for the frozen and FFPE sections, respectively, indicating no significant difference in size changes due to tissue fixation ($P=0.56$).

TABLE 1 Clinical Characteristics of the 1474 Women with Vulvar Intraepithelial Neoplasia.

	Total	VIN2/3		VIN1		P
	n=1474	n=335		n=1139		
Age(y)	42.72±14.31	44.69±14.75		41.28±13.82		0.007
Cytology						0.499
≤LSIL	1279	287	85.67%	992	87.09%	
≥HSIL	195	48	14.33%	147	12.91%	
HPV infection						0.008
Yes	1357	320	95.52%	1037	91.04%	
NO	117	15	4.48%	102	8.96%	
Number of lesion site						<0.001
1	1325	301	89.85%	1024	89.90%	
≥2	149	34	10.15%	115	10.10%	
Lesion site						<0.001
non-hairy						
Posterior forchette	1036	182	46.79%	854	66.25%	
labia minora	182	58	14.91%	124	9.62%	
Navicular fossa	52	14	3.60%	38	2.95%	
Urethral opening	29	13	3.34%	16	1.24%	
clitoris	7	4	1.03%	3	0.23%	
hairy						
labia majora	240	80	20.57%	160	12.41%	
interlabial grooves	68	9	2.31%	59	4.58%	
perianal areas	64	29	7.46%	35	2.72%	
Accompanied with cervical/vaginal SIL*						0.002
Yes	481	160	56.14%	321	33.68%	
NO	757	125	43.86%	632	66.32%	
History with CIN/VaIN/any other disease						0.538
Yes	236	50	14.93%	186	16.33%	
NO	1238	285	85.07%	953	83.67%	

HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; ≤LSIL, atypical squamous cells of undetermined significance, no intraepithelial or malignant lesions, or low-grade squamous intraepithelial lesion; SIL, squamous intraepithelial lesion; *VIN cases were accompanied with cervical/vaginal SIL and they had no history of any other disease at the same time.

4 Discussion

This study aimed to investigate the depth of involved and noninvolved vulvar epithelium and appendages in women with VIN to provide evidence for laser surgery. Our study showed that the value of cytology results was limited in identifying the severity of VIN lesions as no differences was observed between cytology results of the VIN1 and VIN2/3 groups. However, when the cytology results are positive for HSIL, care should be taken to avoid missing serious lesions during colposcopy. In our study, most VINs were associated with HPV infection, consistent with other studies that reported HPV positivity rates >80% (12–15), confirming the cause-and-effect relation.

VINs tend to be multifocal and multicentric, with approximately 18–56% VIN patients simultaneously having cervix, vaginal, and anal lesions (2, 16–19). In our study, 31.58% of VIN2/3 cases were concurrent with LSIL, 20.27% with HSIL, 2.81% with SCC, and 1.05% with VaSCC; VIN2/3 cases were more likely to be accompanied by cervical and vaginal lesions. Our data supported the concept that HPV-related disease can manifest as multicentric lesions in the lower female genital tract rather than being confined to one particular organ. Thus, detecting VIN on clinical examination

TABLE 2 Involved and noninvolved vulvar epithelial thickness in patients of different sites.

Site		VIN2/3						VIN1					
	total	No. patients	Involved	(range)	Noninvolved	(range)	P	No. patients	Involved	(range)	Noninvolved	(range)	P
Non-hariy	413	202	0.59±0.36	0.15-2.37	0.30±0.20	0.08-1.20	<0.001	211	0.46±0.31	0.15-1.40	0.31±0.10	0.18-0.64	<0.001
Posterior forchette	272	153	0.62±0.39	0.15-2.37	0.30±0.21	0.08-1.2	<0.001	119	0.50±0.24	0.29-1.40	0.33±0.11	0.22-0.64	<0.001
labia minora	89	26	0.47±0.22	0.25-1.19	0.30±0.20	0.10-0.70	0.006	63	0.42±0.16	0.15-0.80	0.26±0.05	0.18-0.37	<0.001
clitoris	14	5	0.59±0.05	0.51-0.65	0.31±0.09	0.23-0.47	<0.001	9	0.43±0.14	0.28-0.65	0.36±0.10	0.26-0.51	0.224
Navicular fossa	19	10	0.54±0.31	0.36-1.40	0.27±0.07	0.11-0.47	0.023	9	0.30±0.10	0.15-0.43	0.23±0.03	0.19-0.28	0.059
Urethral opening	19	8	0.52±0.13	0.31-0.7	0.27±0.13	0.11-0.50	0.002	11	0.48±0.19	0.28-0.82	0.36±0.10	0.26-0.51	0.072
Hariy	187	107	0.92±0.51	0.1-2.75	0.33±0.18	0.12-1.18	<0.001	80	0.58±0.27	0.24-1.60	0.36±0.11	0.21-0.78	<0.001
interlabial grooves	35	10	0.66±0.25	0.40-1.10	0.30±0.11	0.15-0.47	0.001	25	0.52±0.21	0.30-1.00	0.31±0.07	0.21-0.45	<0.001
labia majora	113	70	0.95±0.52	0.25-2.75	0.30±0.19	0.12-1.18	<0.001	43	0.63±0.29	0.32-1.6	0.37±0.11	0.27-0.64	<0.001
perianal areas	39	27	0.93±0.52	0.10-1.82	0.40±0.16	0.18-0.91	<0.001	12	0.57±0.27	0.24-1.15	0.41±0.14	0.28-0.78	0.048
Total	600	309	0.69±0.44	0.10-2.75	0.31±0.19	0.08-1.2	<0.001	291	0.49±0.23	0.15-1.60	0.32±0.10	0.18-0.78	<0.001

VIN, Vulvar Intraepithelial Neoplasia.

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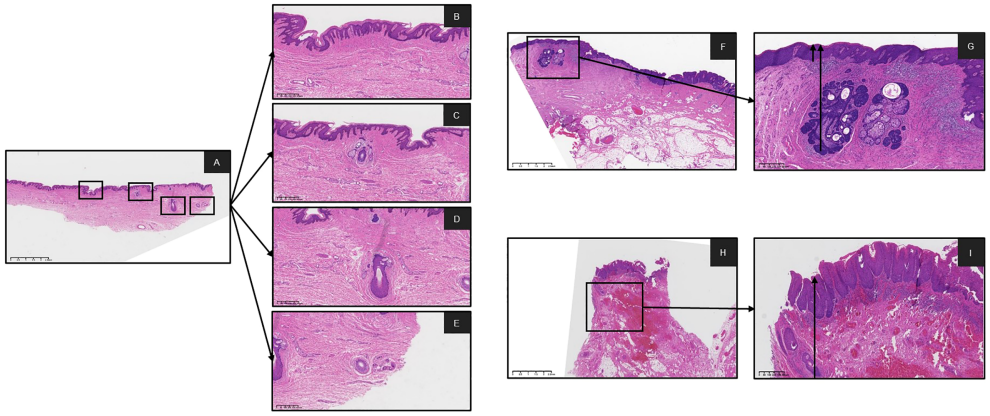

FIGURE 1
Digital pathology slides were scanned by K-scanner (KF-BIO-120, digital pathology slides scanner, KFBIO) and reviewed on K-viewer software. **(A)** was showing normal issue of epithelial and skin appendages on the same slide HE X 1. **(B-E)** were showing the epithelial, Hair Follicles, Sebaceous Gland and Sweat Gland, on the A slide HE X 4, respectively Measurement of depth from the basal layer to the surface of the squamous epithelium was obtained as the arrow was pulled at the locus of normal tissue. **(F, G)** with the involved epithelium and Sebaceous Gland on the slide HE X 1, HE X 4, respectively. **(H, I)** with the involved epithelium and Hair Follicles on the slide HE X 1, HE X 4, respectively. (HE, hematoxylin-eosin staining).

should prompt a thorough examination from the cervix to the perianal area (20). Meanwhile, owing to the lack of effective screening methods for VIN2/3, a comprehensive and careful examination of the vulvar and perianal areas remains the main method for the early detection of VIN2/3.

The thickness of involved epithelium was ≤1 mm in 87.67% (526 of 600) of VIN cases. However, 61 (of 301, 20.37%) VIN2/3 and 13 (of 291, 4.47%) VIN1 cases showed an epithelial thickness >1 mm. Further, the number of VIN2/3 lesion sites with depths >1 mm in hairy sites (including the labia majora and perianal areas) was higher (at 49 of 97 cases). Meanwhile, 53.85% (7 of 13) VIN1 cases occurred in hairy sites (including the labia majora and perianal areas). Therefore, a treatment depth of 1 mm may be sufficient for most VIN cases, although patients with lesions in hairy areas should be monitored. According to our results, more than half of the patients with lesions in hairy sites had lesion depths of 1–3 mm.

In our results, lesions in hairy sites were thicker than those in non-hairy areas. Histologically, non-hairy sites include the clitoris, labia minora, and posterior fourchette, which are characterized by the absence of hair follicles and sweat glands. Hair-bearing skin of the inter-labial grooves, labia majora, lateral perineal, and perianal areas consist of skin appendages, including hair follicles, sebaceous glands, and apocrine and exocrine sweat glands. The upper parts of hair root sheath and lining of the sebaceous gland duct are susceptible to the extension of epithelial lesions owing to their contiguity with the surface epithelium and the presence of similar cell types. Exocrine and apocrine gland ducts are lined by their own independent epithelium, which are not contiguous with the surface epithelium. Involvement of hair root sheaths to depths of 0.8–2.5 mm has been documented (21). Involvement of the sebaceous duct occurs less often, secondary to that of the sheath.

Baggish and Dorsey suggested a uniform depth of 3 mm for laser vaporization for all areas of the vulva (22). Buckley et al. conducted a study involving 28 patients with involved skin appendages and reported that CO2 laser eradication of the skin to a depth of 5 mm can eliminate all atypical epithelium in skin appendages. However, it is unclear whether tissues within the 5–10 mm depth should also be destroyed to ensure that no appendages remain, considering that the appendages may penetrate deeper than 5 mm (21). Based on our results, the depth of lesions extending into the appendages was much deeper than that of the involved epithelium in the same section. We

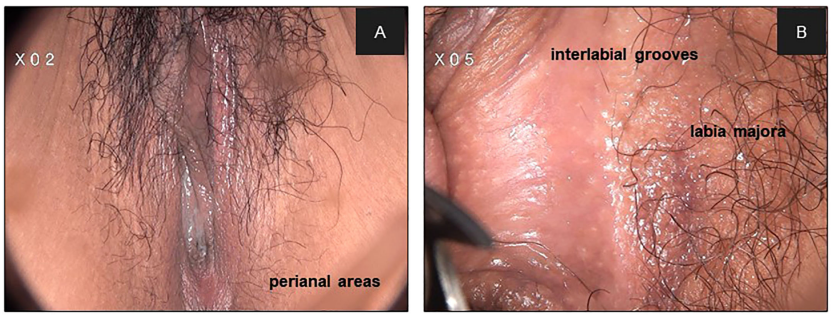

FIGURE 2
The hairy parts of the vulva were showed under colposcopy. **(A)** 2X; **(B)** 5X.

TABLE 3 Depth of epithelial and skin appendages in vulvar intraepithelial neoplasia and noninvolved tissue.

	VIN			Non-involved			P
Diagnosis	No. patients	Mean±SD(mm)	Range(mm)	No. patients	Mean±SD(mm)	Range(mm)	
Stratum Corneum	57	0.10±0.06	0.04-0.25	47	0.08±0.08	0.01-0.52	0.331
Epidermis	57	1.01±0.52	0.25-2.75	57	0.28±0.16	0.12-1.08	<0.001
Appendage	57	1.98±0.61	0.91-5.44	186	1.66±0.85	0.26-4.38	/
Hair Follicles	45	2.05±0.66	0.91-5.44	56	1.86±0.99	0.31-4.38	0.271
Sebaceous Gland	12	1.71±0.29	0.98-2.1	63	1.77±0.60	0.79-3.33	0.715
Sweat Gland	/	/	/	67	1.40±0.87	0.26-4.12	/

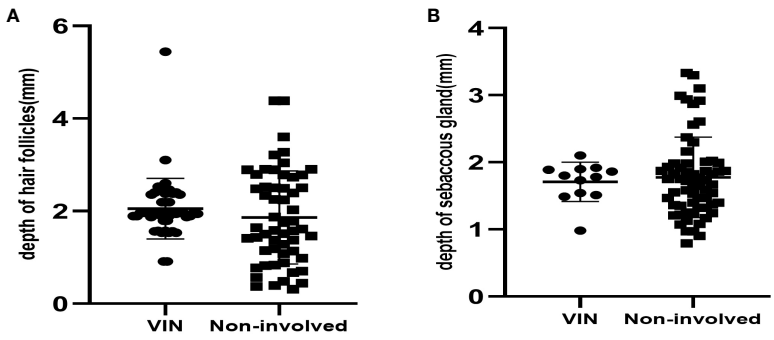

FIGURE 3
(A) The depth of hair follicles in 45 VINs and 56 non-involved cases. **(B)** The depth of sebaceous gland in 12VINs and 63 non-involved cases.

found that superficial lesions in non-hairy areas were vaporized by the laser to a ≤1 mm depth; however, lesions extending onto hairy areas were vaporized to 3 mm to destroy involved skin appendages. Our results were similar to previous findings of a study involving only 29 patients, of which only 5 cases had involved skin appendages (23). However, in our study, we detected only one case with involved appendages with a lesion depth >5 mm. Therefore, we were unable to provide evidence supporting laser surgery with a >5 mm depth for VIN patients.

VIN treatment aims to completely destroy the lesion, improve symptoms, exclude invasion, preserve normal vulvar anatomy and function, and avoid recurrence (3). VIN has a recurrence rate of 20–36.7% despite treatment (24, 25), with 2–15% of cases progressing to vulvar cancer (26, 27). The risk factors for recurrence and progression of VIN remain poorly understood. To eliminate VIN and avoid recurrence, health-care professionals must understand the structure of the skin and recognize the involvement of appendages.

In summary, the epithelium of VIN2/3 lesions was thicker than that of VIN1 lesions, especially in hairy areas. The depth of involvement of appendages was greater than the thickness of epithelial lesion in the same section. The lesion depth in hairy areas was 1–3 mm, with or without appendage involvement. This was deeper than the lesion depth in non-hairy areas, which was approximately 1 mm. Hence, the removal of involved epithelium and appendages would be advisable for laser surgery.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by Fudan University Obstetrics and Gynecology Hospital Ethical committee approval. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

JX: Writing – original draft, Writing – review & editing, Formal Analysis, Investigation, Methodology. ZC: Methodology, Writing – original draft. YX: Formal Analysis, Writing – original draft. LS: Conceptualization, Formal Analysis, Methodology, Writing – original draft, Writing – review & editing, Investigation. CW: Conceptualization, Formal Analysis, Writing – original draft, Writing – review & editing. QC: Conceptualization, Formal

Analysis, Methodology, Writing – original draft, Writing – review & editing, Data curation, Investigation.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1254820/full#supplementary-material

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Associations of multi-human papillomavirus infections with expression of p16 in a cohort of women who underwent colposcopy: a retrospective study of 5165 patients

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Objective: Investigate HPV types in cervical specimens, their correlation with p16 expression in lesions, and diagnostic value for cervical lesions. Enhance clinical diagnosis reliability.

Methods: Retrospective cross-sectional study at Fujian Maternity and Child Health Hospital's Cervical Disease Center (Jun 2019-Dec 2021). Patients with abnormal cervical screening underwent colposcopy and conization. Pathological diagnosis based on colposcopy, cervical biopsy, ECC, and conization. Analyzed HPV genotyping (18 HR-HPV, 5 LR-HPV) and p16 expression correlation. Statistical analysis used R software.

Results: he expression of p16 is significantly associated with the infection of high-risk HPV types, such as 16, 33, 52, and 58, with an increased risk of 1.4 times or higher (OR=1.91, 3.14, 1.40, and 1.78, respectively). The risk of p16 expression increased 4-fold for multiple high-risk HPV types [adjusted OR (95% CI) = 4 (2.92~5.5), P-value <0.001]. Compared to the p16(-) group, the p16(+) group had a higher association with cervical lesions worse than HSIL (High-grade Squamous Intraepithelial Lesions).In the group with multiple Human Papillomavirus Infections with types 16, 33, 52, and 58, the risk of cervical lesions worse than HSIL increased by up to 660-fold compared to the negative group (adjusted OR=660.62, 95% CI: 91.39~4775.53, P<0.001), indicating that this combination of HPV types posed the greatest risk for cervical lesions above HSIL.

Conclusions: p16 plays a crucial role in cervical lesion progression, linked to high-risk HPV. Combining p16 with HPV screening improves cervical cancer detection. Studying multiple HPV infections will enhance prevention and management.

KEYWORDS

HPV, cervical cancer, p16, cervical lesions, retrospective study

1 Background

Cervical cancer is one of the most common gynecological malignancies worldwide, with the highest incidence among malignant neoplasms of the female reproductive system, only second to breast cancer (1). At present, cervical cancer causes up to 30,000 deaths of women in China every year, which poses a huge threat to women's health in the country (2).

The development of cervical cancer is a long-term and continuous process of tumor progression, which includes cytological abnormalities, low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), and finally, carcinogenesis. This process requires the involvement of multiple pathogenic factors, multiple oncogenes, and occurs through a series of steps (3, 4). One crucial factor in the development of cervical cancer is persistent infection with human papillomavirus (HPV). The World Health Organization (WHO) has listed cervical cancer as the first most common cancer caused by HPV infection (5).

Currently, HPV-DNA detection is the primary screening method for cervical cancer in China. However, it has its limitations, including high sensitivity and low specificity due to the influence of various factors in both the host and the virus (6). Especially for precancerous lesions, HPV-DNA testing is only a qualitative test, which cannot classify the severity of lesions nor distinguish between transient and persistent infections. As a result, it cannot guarantee the accuracy of cancer diagnoses (6, 7). There was also research revealed the importance of the HPV mRNA test to define how severe is a cervical lesion, more research is needed to prove (8).

To improve the accuracy of cervical cancer detection and prognosis, researchers have been investigating the role of p16, a tumor suppressor gene involved in the progression of uterine cervical lesions (9). The p16 protein, produced by this gene, has been found to inhibit the cell cycle, thereby negatively regulating cell growth, and controlling cell hyperproliferation. Dysfunctional pathways resulting from aberrant p16 protein expression may induce cervical intraepithelial neoplasia (CIN) and influence the occurrence and development of cervical cancer (10–12).

However, while the significance of p16 in cervical cancer progression has been studied, there is still a lack of research on its interaction with different HPV infection genotypes (13). As a result, the relationship between p16 expression and cervical lesions remains unclear, and the potential value of combining HPV detection with p16 testing in differentiating cervical lesions needs further exploration.

In this study, we conducted a retrospective analysis of patients with cervical lesions using histopathology as the standard for diagnoses (14). All patients underwent HPV typing and p16 expression testing. The main objective was to evaluate the diagnostic significance of HPV typing and p16 detection alone or in combination for cervical lesions, aiming to provide a more reliable clinical diagnosis method. This approach would help avoid overtreatment and reduce the rate of misdiagnosis in patients with mild lesions confirmed by postoperative pathology (14).

2 Materials and methods

2.1 Study population

This cross-sectional study included patients who underwent colposcopy and conization due to abnormal cervical cancer screening results at the cervical disease center of Fujian Maternity and Child Health Hospital from June 2019 to December 2021. Cervical cancer screening involved ThinPrep Cytology Test (TCT) and/or HPV genotyping. Abnormal cytology results were defined as Atypical Squamous Cells of Undetermined Significance (ASC-US), Low-grade Squamous Intraepithelial Lesion (LSIL), High-grade Squamous Intraepithelial Lesion (HSIL), Atypical Glandular Cells (AGC), Endocervical Adenocarcinoma *in situ* (AIS), Squamous Cell Carcinoma (SCC), and Adenocarcinoma. The interval between cervical cancer screening and histological examination was less than 3 months. Clinical information, including age, gravidity, parity, HPV genotypes, and cervical pathology, was extracted from the department's medical records (Figure 1).

The study was conducted in compliance with the Declaration of Helsinki (as revised in 2013) and was approved by the Ethics Committee of Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University (2023KY038). Due to the retrospective nature of the study, informed consent was exempted.

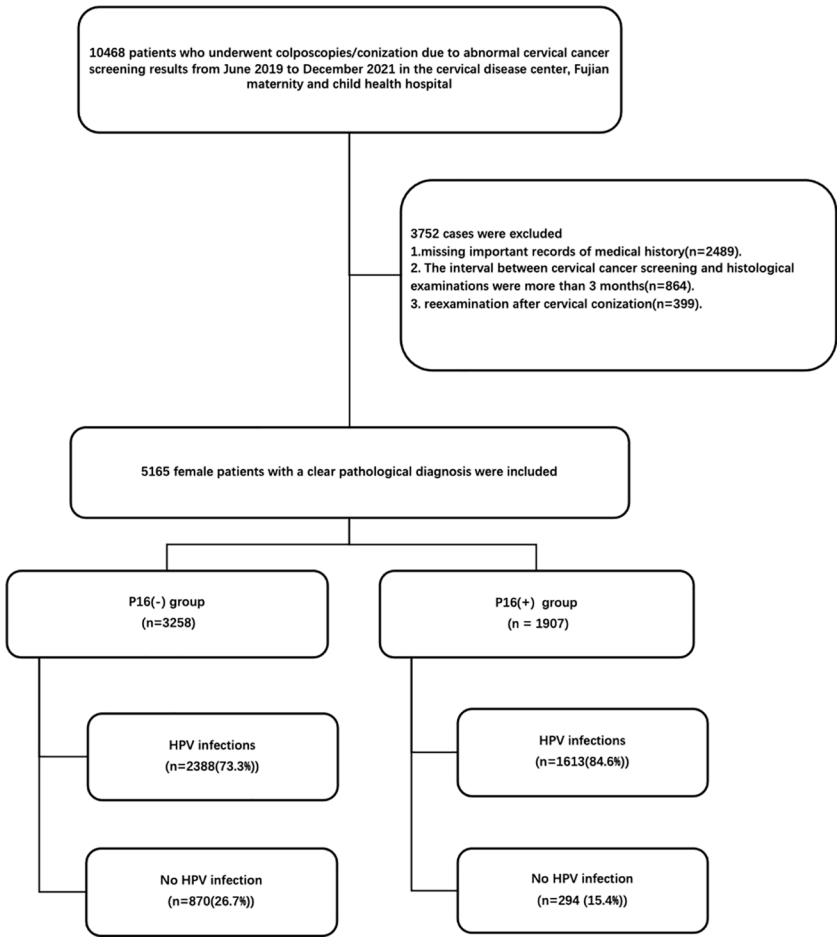

FIGURE 1
Study design. A total of 5165 female patients with clear pathological diagnoses were included. HPV, human papillomavirus.

2.2 HPV Genotyping

PCR-RDB HPV genotyping (Yaneng Biotech) was performed to identify 18 genotypes of high-risk HPV (HR-HPV): HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, and 83, as well as 5 types of low-risk HPV (LR-HPV): HPV-6, 11, 42, 43, and 81.

2.3 Pathological diagnosis

Colposcopy referrals were based on the ASCCP guidelines (10). All patients underwent colposcopy and cervical biopsy. Additionally, patients with HPV-16 and 18 infections, AGC/AIS/HSIL cytology, and type 3 cervical transformation zone underwent endocervical curettage (ECC). Cervical cone resection was performed in cases with liquid-based cytology results indicating HSIL, AGC-FN (atypical glandular cell, favor neoplastic), AIS, or cervical pathological biopsy and ECC results indicating CIN2-3 (cervical intraepithelial neoplasia 2-3). Two blinded senior pathologists independently performed the pathological evaluation of cervical biopsies, ECC, and conization tissues. Standard haematoxylin-eosin stain was used in this study, standard H&E protocol allows visualization of tissue morphology by imparting blue-stained nuclei and pink-stained cytoplasm/connective tissue. It is the routine stain for histopathology, providing an overview of tissue architecture and cytology.

The final pathological diagnosis was determined using the most severe result among evaluations of cervical biopsies, ECC, and conization tissues. The histologic endpoints were defined according to the 2014 WHO classification of tumors of the female reproductive organs (4th Edition) (11) and Lower Anogenital Squamous Terminology (LAST) recommendations as follows (12): Normal cervix; LSIL, which includes CIN1 and p16 negative CIN2; HSIL, including p16 positive CIN2 and CIN3; AIS; invasive cervical cancer. Furthermore, HSIL, AIS, and invasive cervical cancer were classified as HSIL+.

2.4 Procedure for colposcopic examination and immunocytochemical staining

The Leisegang D-10625, Model1DS Ur Nr 55764, Colposcope from Berlin, Germany, was used for cervix examination. After exposing the cervix using an appropriately sized Cusco's

speculum, the vulva, vagina, and cervix were examined before the application of 3% acetic acid solution for each patient. Colposcopic abnormalities were classified as normal, abnormal, or unsatisfactory. Biopsies were taken from abnormal areas using punch cervical biopsy tissue forceps. The cervical specimens were processed in the histopathology laboratory, and a histopathologist blinded to the HPV status of the participants performed the diagnosis. Immunocytochemical staining was performed using the P16/Ki67 double staining kit on each cervical specimens. Experimental operations were strictly in accordance with the kit instructions and the technical instructions for double staining of cervical cells, and two experienced pathologists conducted and interpreted the double staining of cervical epithelial cell.

2.5 Statistical analysis

Categorical variables were presented as frequencies (percentages), and statistical analyses were performed using R software and its packages (Open Access, Version 4.0.2). Descriptive statistics showed mean ± standard deviation for continuous variables, while frequency and percentage were used for categorical variables. The statistical differences among p16 status for clinical characteristics were tested with t-tests for continuous variables and Chi-square tests for categorical variables. Univariate and multivariate logistic regression analyses, adjusting for age, gravidity, parity, and pregnancy, were used to determine the association between multiple HPV infections and cervical lesions. Two-tailed P-values less than 0.05 were considered statistically significant.

3 Results

3.1 Characteristics of patients

The analysis included a total of 5165 female patients with definitive pathological diagnoses. Among them, there were 3258 cases with p16(-) and 1907 cases with p16(+). The mean age of patients with p16(+) was significantly older than that of patients with p16(-) (42.5 ± 11.1 vs. 39.4 ± 10.9, p<0.001). The prevalence of HPV infection was 73.3% (n=2388) in patients with p16(-), whereas it was 84.6% (n=1613) in patients with p16(+) (P<0.001). P16(+) was associated with the infection of high-risk HPV types 16, 33, 52, 56, 58, and low-risk HPV type 81 (P<0.05) (Table 1). Figure 2 shows

TABLE 1 Characteristics of the study patients.

Variables	Total (n = 5165)	p16- (n = 3258)	p16+ (n = 1907)	p
Ages, Mean ± SD	41.3 ± 11.1	39.4 ± 10.9	42.5 ± 11.1	< 0.001
gestation, n (%)				0.022
0	406 (7.9)	230 (7.1)	176 (9.2)	
1	797 (15.4)	493 (15.1)	304 (15.9)	
2	1357 (26.3)	851 (26.1)	506 (26.5)	
3	1165 (22.6)	765 (23.5)	400 (21)	
≥4	1440 (27.9)	919 (28.2)	521 (27.3)	
parity, n (%)				0.001
0	730 (14.1)	417 (12.8)	313 (16.4)	
1	1862 (36.1)	1220 (37.4)	642 (33.7)	
2	1929 (37.3)	1211 (37.2)	718 (37.7)	
≥3	644 (12.5)	410 (12.6)	234 (12.3)	
hpv16, n (%)				< 0.001
0	3507 (67.9)	2398 (73.6)	1109 (58.2)	
1	1658 (32.1)	860 (26.4)	798 (41.8)	
hpv18, n (%)				0.179
0	4047 (78.4)	2572 (78.9)	1475 (77.3)	
1	1118 (21.6)	686 (21.1)	432 (22.7)	
hpv31, n (%)				0.916
0	5050 (97.8)	3186 (97.8)	1864 (97.7)	
1	115 (2.2)	72 (2.2)	43 (2.3)	

(Continued)

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TABLE 1 Continued

Variables	Total (n = 5165)	p16- (n = 3258)	p16+ (n = 1907)	p
hpv33, n (%)				< 0.001
0	5036 (97.5)	3210 (98.5)	1826 (95.8)	
1	129 (2.5)	48 (1.5)	81 (4.2)	
hpv35, n (%)				0.526
0	5096 (98.7)	3217 (98.7)	1879 (98.5)	
1	69 (1.3)	41 (1.3)	28 (1.5)	
hpv39, n (%)				0.789
0	5020 (97.2)	3165 (97.1)	1855 (97.3)	
1	145 (2.8)	93 (2.9)	52 (2.7)	
hpv45, n (%)				0.617
0	5103 (98.8)	3217 (98.7)	1886 (98.9)	
1	62 (1.2)	41 (1.3)	21 (1.1)	
hpv51, n (%)				0.414
0	4916 (95.2)	3107 (95.4)	1809 (94.9)	
1	249 (4.8)	151 (4.6)	98 (5.1)	
hpv52, n (%)				< 0.001
0	4456 (86.3)	2857 (87.7)	1599 (83.8)	
1	709 (13.7)	401 (12.3)	308 (16.2)	
hpv53, n (%)				0.242
0	4894 (94.8)	3078 (94.5)	1816 (95.2)	
1	271 (5.2)	180 (5.5)	91 (4.8)	
hpv56, n (%)				0.017
0	5029 (97.4)	3159 (97)	1870 (98.1)	
1	136 (2.6)	99 (3)	37 (1.9)	
hpv58, n (%)				< 0.001
0	4835 (93.6)	3091 (94.9)	1744 (91.5)	
1	330 (6.4)	167 (5.1)	163 (8.5)	
hpv59, n (%)				0.24
0	5031 (97.4)	3167 (97.2)	1864 (97.7)	
1	134 (2.6)	91 (2.8)	43 (2.3)	
hpv66, n (%)				0.264
0	5053 (97.8)	3193 (98)	1860 (97.5)	
1	112 (2.2)	65 (2)	47 (2.5)	
hpv68, n (%)				0.838
0	5021 (97.2)	3166 (97.2)	1855 (97.3)	
1	144 (2.8)	92 (2.8)	52 (2.7)	
hpv73, n (%)				0.281
0	5142 (99.6)	3241 (99.5)	1901 (99.7)	

(Continued)

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TABLE 1 Continued

Variables	Total (n = 5165)	p16- (n = 3258)	p16+ (n = 1907)	p
1	23 (0.4)	17 (0.5)	6 (0.3)	
hpv82, n (%)				0.383
0	5131 (99.3)	3239 (99.4)	1892 (99.2)	
1	34 (0.7)	19 (0.6)	15 (0.8)	
hpv42, n (%)				0.066
0	5029 (97.4)	3162 (97.1)	1867 (97.9)	
1	136 (2.6)	96 (2.9)	40 (2.1)	
hpv43, n (%)				0.403
0	5082 (98.4)	3202 (98.3)	1880 (98.6)	
1	83 (1.6)	56 (1.7)	27 (1.4)	
hpv44, n (%)				0.518
0	5142 (99.6)	3242 (99.5)	1900 (99.6)	
1	23 (0.4)	16 (0.5)	7 (0.4)	
hpv81, n (%)				< 0.001
0	5021 (97.2)	3141 (96.4)	1880 (98.6)	
1	144 (2.8)	117 (3.6)	27 (1.4)	
hpv83, n (%)				0.39
0	5083 (98.4)	3210 (98.5)	1873 (98.2)	
1	82 (1.6)	48 (1.5)	34 (1.8)	
hpv, n (%)				< 0.001
0	1164 (22.5)	870 (26.7)	294 (15.4)	
1	4001 (77.5)	2388 (73.3)	1613 (84.6)	

the intersections of the HPV genotype. HPV genotypes 16, 18, 52, 51 and 33 had the most frequent infections, and there was coinfection (Figure 2).

Each row corresponds to a set of infection genotype(s), and the bar chart on the left demonstrates the size of each set. Each column corresponds to a possible intersection: the filled-in cells show which set is a part of an intersection.

3.2 Association between different HPV genotype and p16

Figure 3 depicts the relationship between different HPV genotypes and p16 expression. In the crude models, high-risk HPV types 16, 33, 52, 56, 58, and 81 showed a significant correlation with p16 expression, whereas a negative relationship was observed for HPV type 18. After adjusting for confounding factors, the results remained consistent with the univariate analysis. Infection with high-risk HPV types increased the risk of p16(+) by approximately 1.4 times or higher (OR=1.91, 3.14, 1.40, and 1.78, respectively).

Models adjusted for age, gravidity, parity, and cervical histology.

3.3 Association between multiple HPV infections and p16

Table 2 presents the results of univariate and multivariate logistic regression analyses of multiple HPV infections and p16 expression. The highest incidence of p16(+) was identified in individuals infected with HPV33+ and multiple high-risk HPV infections (MH-HPV+). Moreover, there was an increased risk of p16(+) for HPV genotypes 16, 33, 52, and 58 alone, as well as for multiple high-risk HPV infections.

Specifically, the risk of p16(+) increased 4.38-fold when infected with HPV33 alone [adjusted OR (95% CI) = 4.38 (2.617.36), P < 0.001], and 4-fold when infected with multiple high-risk HPV genotypes [adjusted OR (95% CI) = 4 (2.925.5), P < 0.001].

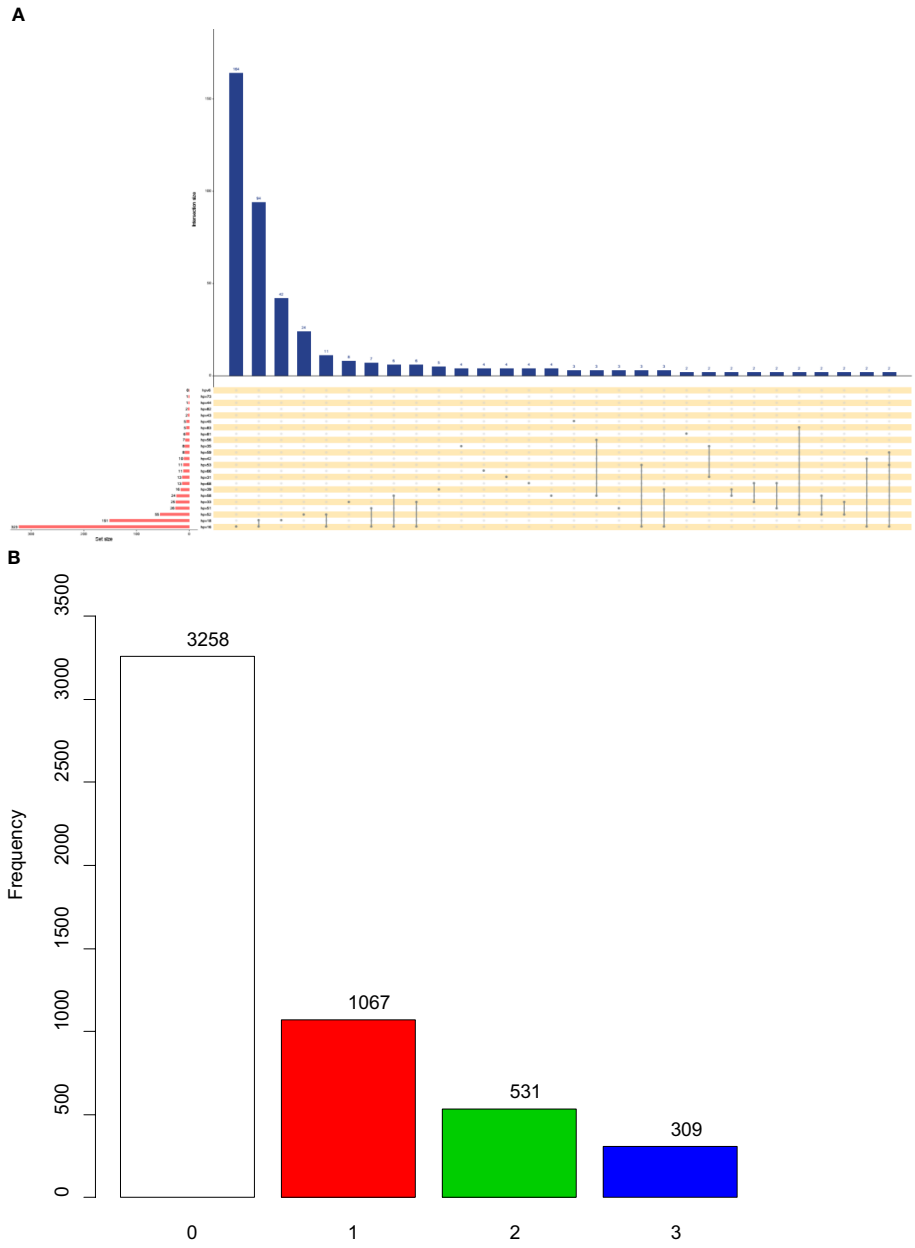

FIGURE 2
Upset plots of the intersections of HPV genotype **(A)** and different expression level of p16 **(B)**. Each row corresponds to a set of infection genotype(s), and the bar chart on the left demonstrates the size of each set. Each column corresponds to a possible intersection: the filled-in cells show which set is a part of an intersection.

3.4 Association between multiple HPV infections and cervical lesions above HSIL

Table 3 presents the comparison of lesions more severe than HSIL between the p16(-) and p16(+) groups. Across all groups, our study found that compared to the p16(-) group, the p16(+) group had a higher association with cervical lesions worse than HSIL.

In the negative+ group, the risk of cervical lesions above HSIL was 43.06-fold higher than that of the negative group [adjusted OR=43.06, 95% CI: 28.8264.33, P<0.001]. In the SH+ group, the risk of cervical lesions above HSIL was 93.58-fold higher than that of the SH group [adjusted OR=93.58, 95% CI: 64.47135.85, P<0.001]. The MH+ group demonstrated the highest risk increase, with p16(+) patients having a 660-fold higher risk of cervical lesions above HSIL compared to the negative group [adjusted OR=660.62, 95% CI: 91.394775.53, P<0.001]. This group represented the most significant risk for cervical lesions above HSIL. In the other+ group, the risk of cervical lesions above HSIL was 41.54-fold higher than that of the other group [adjusted OR=41.54, 95% CI: 28.9659.59, P<0.001]. Similarly, in the MO+ group, the risk of lesions above HSIL was 80.91-fold higher than that of the other group [adjusted OR=80.91, 95% CI: 53.28~122.87, P<0.001].

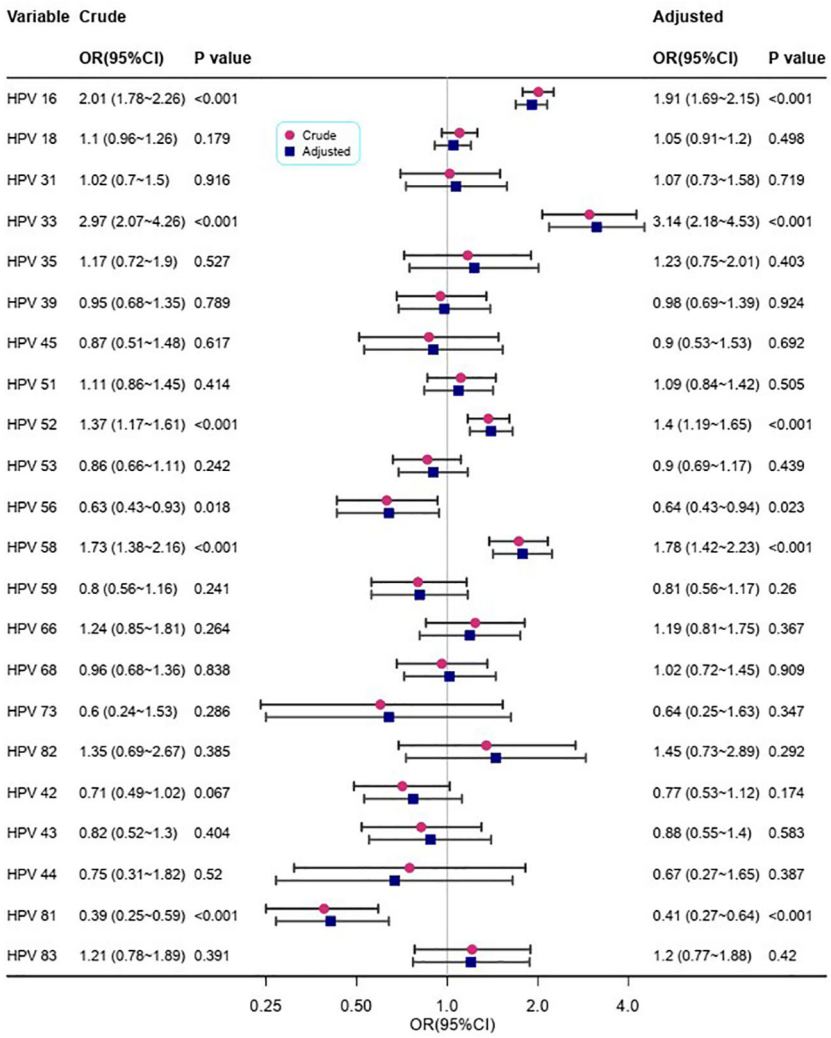

FIGURE 3
The relationships between different HPV genotypes and p16.

Discussions

Cervical cancer is unique as it is the only type of cancer with a clear etiology and complete tertiary prevention measures. The two most common ways of screening for cervical cancer are cervical cytology and HPV detection (13). Cytology is based on microscopic morphology and has limitations, such as complex grading, subjectivity, and variable diagnostic repeatability, leading to insufficient sensitivity. On the other hand, HPV tests have high sensitivity but lower specificity due to potential transient infections being missed, and they cannot reflect the extent or severity of HPV-induced lesions.

Countries with established cervical cancer screening programs are increasingly adopting HPV primary screening as the preferred method (14, 15). Early detection through improved screening methods can significantly improve survival rates for cervical cancer patients. Abnormal expression of p16 is closely related to HPV-16 and HPV-18 infections, and its expression increases with the progression of CIN and cervical cancer (16–18). Patients with p16-negative HPV-associated cervical cancer tend to have worse prognoses (19). Combining TCT with dual staining of p16/Ki67 has shown high sensitivity and specificity in detecting HSIL, making it an effective screening method (20).

Multiple infections are common in healthy women (15.8%) but less prevalent in cervical cancer patients (3%-4%), and the relationship between multiple infection and pathogenicity requires further study (18).

In our study, 5165 female patients with definite pathological diagnoses were included, with 1907 exhibiting positive p16 expression and 3258 showing negative p16 expression. P16 expression correlated positively with high-risk HPV types, including HPV-16, HPV-33, HPV-52, and HPV-58, with an increased risk compared to p16(-) cases (9).

The p16 gene, located on chromosome 9, encodes the p16 protein, which inhibits cell proliferation by preventing cells from entering the S phase (21, 22). Variations in the p16 gene and inactivation of its proteins are common in various malignant tumors, including cervical cancer (23).

TABLE 2 Univariate and multivariate logistic regression analyses of HPV infection patterns and p16.

Variables	Total	Event (%)	Crude		Adjusted	
			OR (95%CI)	P value	OR (95%CI)	P value
negative	1164	294 (25.3)	1(Ref)		1(Ref)	
HPV16+	820	399 (48.7)	2.8 (2.32~3.39)	<0.001	2.65 (2.19~3.22)	<0.001
HPV33+	65	39 (60)	4.44 (2.66~7.42)	<0.001	4.38 (2.61~7.36)	<0.001
HPV52+	390	163 (41.8)	2.12 (1.67~2.7)	<0.001	2.08 (1.63~2.66)	<0.001
HPV58+	160	75 (46.9)	2.61 (1.86~3.66)	<0.001	2.62 (1.86~3.68)	<0.001
MH-HPV+	195	112 (57.4)	3.99 (2.92~5.46)	<0.001	4 (2.92~5.5)	<0.001
OtherHPV+	1375	379 (27.6)	1.13 (0.94~1.34)	0.19	1.11 (0.93~1.33)	0.249
MultiOtherHPV+	996	446 (44.8)	2.4 (2~2.88)	<0.001	2.31 (1.92~2.77)	<0.001

Models adjusted for age, gravidity, parity, and cervical histology.
MH-HPV+, Multiple HPV16/33/52/58 infection. OtherHPV+, Single HPV infection with genotype other than HPV16, 33, 52, and 58. MultiOtherHPV+, Multiple HPV infection with genotype other than HPV16, 33, 52, and 58.

Persistent infection with high-risk HPV is associated with cervical intraepithelial neoplasia and cervical cancer (24, 25). HPV can exist in free or integrated form, and persistent infection may lead to gene instability and lesion escalation (26, 27). The E7 gene of HPV inactivates the pRb protein, promoting cell cycle progression and potentially leading to feedback overexpression of p16 (22). Thus, the overexpression of p16 in tumor cells is linked to HPV infection (28).

Over 80% of patients with HPV infections experience transient infections, while 4% to 10% develop persistent HPV infections, leading to cervical lesions and potentially cancer (29). Among the 200 identified HPV types, HPV-16 and HPV-18 are the most common and pathogenic types (30). Multiple HPV infections are more common in LSIL and HSIL patients, with longer durations of infection increasing the risk of cervical lesions (30).

Positive p16 protein expression is correlated with increasing cervical lesion levels, making it a predictor of cervical lesion escalation (31, 32). Combining HPV with p16 testing can enhance cervical cancer detection and risk assessment (33). P16 expression has been proposed as a new indicator for cervical cancer screening (19).

The current study's limitations include its retrospective cross-sectional design, which may introduce selection bias, and the potential impact of residual confounding factors. Multicenter prospective cohort studies are needed to validate the findings. Another potential limitation of this study is that heavy methylation of the p16 gene promoter region can lead to silencing and decreased expression of p16, resulting in false negative results by immunohistochemistry. In the latter study, it will be important to understand the potential confounding effects of high p16 methylation

TABLE 3 Associations of HPV infection patterns and cervical lesion grades with p16.

Variables	Total	Event (%)	Crude		Adjusted	
			OR (95%CI)	P value	OR (95%CI)	P value
negative	870	125 (14.4)	1(Ref)		1(Ref)	
negative+	294	259 (88.1)	44.1 (29.55~65.84)	<0.001	43.06 (28.82~64.33)	<0.001
SH	759	99 (13)	0.89 (0.67~1.19)	0.439	0.88 (0.66~1.17)	0.371
SH+	676	636 (94.1)	94.76 (65.36~137.39)	<0.001	93.58 (64.47~135.85)	<0.001
MH	83	12 (14.5)	1.01 (0.53~1.91)	0.982	1 (0.53~1.9)	0.999
MH+	112	111 (99.1)	661.56 (91.54~4781.28)	<0.001	660.62 (91.39~4775.53)	<0.001
MO	550	75 (13.6)	0.94 (0.69~1.28)	0.7	0.93 (0.69~1.27)	0.67
MO+	446	416 (93.3)	82.65 (54.51~125.3)	<0.001	80.91 (53.28~122.87)	<0.001
other	996	148 (14.9)	1.04 (0.8~1.35)	0.764	1.03 (0.8~1.34)	0.794
other+	379	332 (87.6)	42.1 (29.39~60.31)	<0.001	41.54 (28.96~59.59)	<0.001

Models adjusted for age, gravidity, parity and cervical histology.
negative, HPV negative group; negative+, HPV negative group with p16(+); SH, Single HPV-16, 33, 52, or 58 infection; SH+, Single HPV-16, 33, 52, or 58 infection with p16(+); MH, multiple HPV-16, 33, 52, and 58 infection; MH+, multiple HPV-16; 33, 52, and 58 infection with p16(+).
MO, Multiple HPV infection with genotype other than HPV16, 33, 52, and 58. MO+, Multiple HPV infection with genotype other than HPV16, 33, 52, and 58 with p16(+). other, Single HPV infection with genotype other than HPV16, 33, 52, and 58. other+, Single HPV infection with genotype other than HPV16, 33, 52, and 58 with p16(+).

when interpreting p16 immunohistochemistry results in cervical specimens.

In conclusion, p16 expression is crucial in cervical lesion progression and is associated with high-risk HPV genotypes (HPV-16, 33, 52, and 58). Incorporating p16 testing into HPV screening can enhance cervical cancer detection. Further research on multiple HPV infections' role in cervical lesion development will improve cervical cancer prevention and management.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The paper was approved by the Ethics Committee of Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University (2022YJ002). The studies were conducted in accordance with the local legislation and institutional requirements. Informed consent was waived due to the retrospective nature of the study.

Author contributions

YZ: Writing – original draft, Writing – review & editing. HBL: Writing – original draft, Writing – review & editing. XL: Data curation, Methodology, Writing – original draft, Writing – review & editing. ZL: Data curation, Writing – original draft, Writing – review & editing. QY: Data curation, Investigation, Writing – original draft. HWL: Data curation, Investigation, Writing – review & editing. ZZ: Data curation, Investigation, Writing – original draft. HY: Writing – review & editing. YS: Writing – review & editing. XZ: Writing – review & editing. YC: Writing – review & editing. JC: Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Co-creation of human papillomavirus self-sampling delivery strategies for cervical cancer screening in rural Zimbabwe: nominal group technique

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Background: Human papillomavirus (HPV) self-sampling is recommended for cervical cancer screening, particularly among women who do not participate in or have access to current screening methods offered in Zimbabwe. Key stakeholder involvement is critical in co-creating acceptable delivery strategies for implementing HPV self-sampling to ensure demand and facilitate uptake by the target population. The main objective of this study was to engage key stakeholders in co-creating acceptable HPV self-sampling delivery strategies for cervical cancer screening in rural Zimbabwe.

Methods: We invited key stakeholders and employed a nominal group technique (NGT) for data collection. We employed the NGT to (1) identify barriers to access and utilisation of available cervical cancer screening services and (2) co-create delivery strategies for HPV self-sampling. The workshop included 8 participants (women $n = 4$, health workers $n = 2$ and policymakers $n = 2$). Quantitative data was gathered by ranking ideas and qualitative data were collected from participant group discussions and analysed thematically. The results of the ranking exercise were fed back to the participants for comments.

Results: The most significant barriers to accessing and utilising current cervical cancer screening services by women were: Inadequate information and education on cervical cancer, lack of resources and funding for cervical cancer programmes, long distances to nearest health facilities, and low perceived personal risk of cervical cancer. Key stakeholders recommended enhanced education and awareness, results notification, linkage to care, community-based self-sampling, and the choice of sampling devices as potential HPV self-sampling delivery strategies.

Conclusion: Our study demonstrated the utility of the NGT for reaching a consensus. Using the NGT, we established priority delivery strategies for HPV self-sampling cervical cancer screening. Adequate education and awareness, early results notification, choice of sampling device and community-based self-sampling were crucial to HPV self-sampling screening in rural Zimbabwe. The proposed delivery strategies can guide the development of guidelines for designing and implementing an HPV self-sampling intervention. We recommend a study to determine women's most preferred HPV self-sampling delivery strategies before implementing the intervention.

KEYWORDS

cervical cancer, HPV self-sampling, co-creation, delivery strategies, nominal group technique, Zimbabwe

Background

Despite being preventable through HPV vaccination, cervical cancer screening, and treatment of cervical precancer, cancer of the cervix is a significant public health challenge in the world. It is the fourth leading cause of cancer deaths among women globally. In 2020 an estimated 604, 000 women were diagnosed with cervical cancer and 342, 000 women died from the disease (1). Compared to high-income countries, low-middle-income countries (LMICs) are disproportionately affected. According to the World Health Organisation (WHO), 19 of the top 20 countries with the highest cervical cancer incidence are in Africa (2). Zimbabwe has one of the highest global mortality rates for cervical cancer with an estimated age-standardised mortality rate of (43.0/100000) which is remarkably higher than the global average of 13.3/100,000 (3). An estimated 3043 women were diagnosed with cervical cancer, and 1976 lost their lives to it in 2020 alone (3). The burden of cervical cancer is compounded by the high prevalence of HIV in the country. According to the last national survey conducted in 2020, the prevalence of HIV among women aged 15 years and older was 15.3% (4). HIV infection is known to increase the risk of developing cervical cancer by up to six-fold (5), making HIV and cervical cancer important public health problems for Zimbabwe.

The marked difference in incidence and mortality between developing countries such as Zimbabwe and high-income countries is largely due to the lack of organised cervical screening using cytology. Similar to other LMICs, Zimbabwe's cervical cancer screening programme using cytology failed to reduce the incidence of cervical cancer due to lack of funding, infrastructure, trained personnel and financial resources (6, 7). Currently, visual inspection with acetic acid and cervicography (VIAC) forms the basis for the majority of cervical cancer screening in Zimbabwe and it is available at 14% of all government health facilities (8). Although available at some of the health facilities, the country's screening coverage remains low with the majority of women never screened and presenting with advanced disease (9, 10). An estimated 20% of all eligible women are ever screened in their lifetime for cervical cancer in Zimbabwe (8). In addition to limited access and unavailability of screening services, other barriers are responsible for preventing women from accessing and utilising available screening services.

Several factors at the individual, interpersonal, community and health system level have been established as barriers to access and utilisation of services. Nyamambi and colleagues identified barriers at the intrapersonal, sociocultural, and health system levels and the lack of education was credited as the most significant individual barrier to the uptake of cervical cancer screening by women (11). Another study conducted in Zimbabwe by Mapanga et al. further reinforced the role of individual factors as significant barriers to the uptake of screening services with the lack of knowledge and awareness of cervical cancer being the most common barrier (12). The same study found that economically disadvantaged women were less likely to seek screening services, which disproportionately affects rural women in Zimbabwe (12).

Besides the primary prevention of vaccinating girls who have never had sex, the WHO recommends the secondary prevention of cervical cancer by HPV testing in LMICs where there are enough resources. The WHO aims to achieve a screening coverage of 70% using HPV testing by 2030 by screening women twice at age 35 and again by age 45 (13). HPV testing has superior sensitivity compared to cytology and VIAC and allows for longer screening intervals after a negative test (14). Additionally, women can collect cervicovaginal specimens for testing in a process called HPV self-sampling. The use of self-collected specimens for HPV testing in screening cervical cancer among women is in line with WHO recommendations for the use of self-care interventions to promote a people-centered approach to health and well-being including for sexual and reproductive health and non-communicable diseases to achieve universal health coverage (15).

HPV testing has been used on clinician and self-collected specimens with comparable clinical accuracy (16). HPV self-sampling can potentially overcome some of the barriers that prevent women from accessing screening services (17). Evidence points to the acceptability of HPV self-sampling due to its ease of use, privacy and convenience (17). Studies conducted in limited resource settings such as Cameroon (18), Ethiopia (19), Tanzania (20) and Malawi (21) have demonstrated the acceptability of HPV self-sampling for cervical cancer screening. There is still limited HPV testing for cervical cancer screening

in Zimbabwe, with the majority of work undertaken so far being led by developmental partners such as the Clinton Health Access Initiative. According to a WHO 2023 report, only 60 sites provide HPV testing services in Zimbabwe (8). The government of Zimbabwe is integrating HPV testing, including the use of HPV self-sampling, to increase screening coverage by reaching under-screened women. It is highly probable that the country will enhance screening coverage by incorporating HPV testing alongside other existing screening methods. However, since HPV testing is still a relatively new screening tool in the country, there is a shortage of evidence regarding effective delivery strategies to implement an HPV self-sampling screening programme. In order to ensure that HPV self-sampling is widely accepted and adopted by the end-users, it is crucial to develop effective delivery strategies. It is recommended that stakeholders from relevant disciplines in cervical cancer prevention and control participate in the development of these strategies.

The main aim of this study was to come up with acceptable HPV self-sampling delivery strategies using the NGT for a cervical cancer screening programme. This would aid in increasing the uptake of cervical cancer screening in rural Zimbabwe. In the past, researchers have successfully used the NGT to find the most effective delivery methods for implementing HIV self-testing programmes (22), co-creating health education programmes (23), and determining acceptable hypertension intervention packages to promote hypertension adherence (24). The findings of this study are expected to be useful to policymakers within the Ministry of Health and Child Care in Zimbabwe and concerned development partners for the design and implementation of HPV testing using self-collected specimens.

Materials and methods

Study setting

The study was conducted in a village called Chidamoyo in Hurungwe rural district in Mashonaland West Province in Zimbabwe (Figure 1), with the study area defined to be Ward 13/15 which is the approximate catchment area of Chidamoyo Christian Hospital. The estimated population served by Chidamoyo Mission Hospital is 32,000 people, with ∼3200 eligible women i.e., those 18 years and older (22). The researcher chose Chidamoyo village in Hurungwe as it is a rural area and traditionally rural areas have been associated with low screening coverage, poor access to or unavailability of health services (23). Additionally, it was convenient for the researcher because of a previous working relationship with the hospital administration.

Study design

We invited key stakeholders involved in cervical cancer control and prevention in Zimbabwe. This study was part of a multiphase sequential exploratory mixed methods study to develop acceptable HPV self-sampling approaches for cervical cancer screening in Zimbabwe. The mixed methods study is underpinned by the socio-ecological model which emphasises that the interplay between individual, interpersonal, community, and societal factors influence behaviour and health outcomes (24). The results of the scoping review we conducted revealed the acceptability of HPV self-sampling and highlighted the need for more qualitative work involving stakeholders and further research on the impact of self-sampling devices (17). The systematic review highlighted the interplay of intrapersonal, interpersonal, community and health system level factors on women, health workers and policymaker's experiences and perspectives on HPV self-sampling in SSA. We combined these findings and sought to co-create acceptable delivery strategies for HPV self-sampling using the NGT.

The NGT is a highly structured face-to-face group interaction that allows participants to contribute equally and have their opinions heard by other group members. The NGT ensures that there is no domination of ideas by a single individual (25, 26). The NGT process consists of four main phases (i) silent generation-where participants generate ideas independently and write them down on a sheet of paper or sticky notes. (ii) Round robin sharing-participants take turns to share their responses without discussion or critique and these are listed on a flipchart visible to all. This process continues until all participants have shared their responses. (iii) Discussion phase-where group members discuss and ask questions in order to clarify items on the list and elaborate on their responses. During this phase, items with similar meanings are combined and duplicate items can be removed; (iv) Voting phase, here each participant is asked to prioritise the listed items by assigning ranks to them. The ranking results are then collated to produce a single list of priorities for the wider group (25).

Study participants

The researcher invited 8 key stakeholders involved in cervical cancer screening programmes to collaborate in a co-creation workshop. The participants included four women from the target population who resided in Hurungwe, two registered nurses (one male and one female) involved in cervical cancer screening and care working at Chidamoyo Hospital, and two policymakers (one Gynaecologist in the Ministry of Health and an Epidemiologist from a development partner) and the principal investigator (as facilitator and convener) and one research assistant. Detailed characteristics of the NGT participants are presented in the next section.

Target women

A community health worker who works closely with the women in the community used purposive sampling to identify women and recommended them to the researcher. Interest in the study was discussed between the researcher and the prospective participants taking into consideration their age and other demographic

information. Four women were considered for the nominal group workshop and were informed of the workshop date and venue.

Other key stakeholders

The researcher used a purposeful sampling strategy to invite key stakeholders to collaborate in the co-creation workshop. In our study, the term "key stakeholder" was used to refer to subject matter experts (SMEs). We defined SMEs as individuals who have expert knowledge of barriers that prevent women from accessing cervical cancer screening services and an interest in developing acceptable HPV self-sampling delivery strategies. The researcher invited these SMEs via email, printed letters and telephone calls, where an invited individual was unable to take part but suggested another person, snowball sampling was used to invite the suggested individual. Four key stakeholders were considered for the workshop and notified of the date and venue.

Sampling strategy

A sample size of eight participants was chosen based on the researcher's assessment that the team possessed the necessary expertise and represented diverse perspectives relevant to the research question, Additionally due to the limitation in resources and the added challenge of bringing together all stakeholders at the same time, it was convenient to have 8 participants for the NGT workshop. Based on previous research, our decision for the number of participants for a nominal group workshop is influenced by the recommendations of other authors. According to Harvey and Holmes, a group consisting of 6 to 12 participants would be appropriate to gather the necessary information from each participant (25). Similarly, an NGT study conducted in Australia, which aimed to achieve consensus on graduate attributes for nurses pursuing postgraduate certification in neonatal intensive care, used a sample size of 8, similar to our study (27).

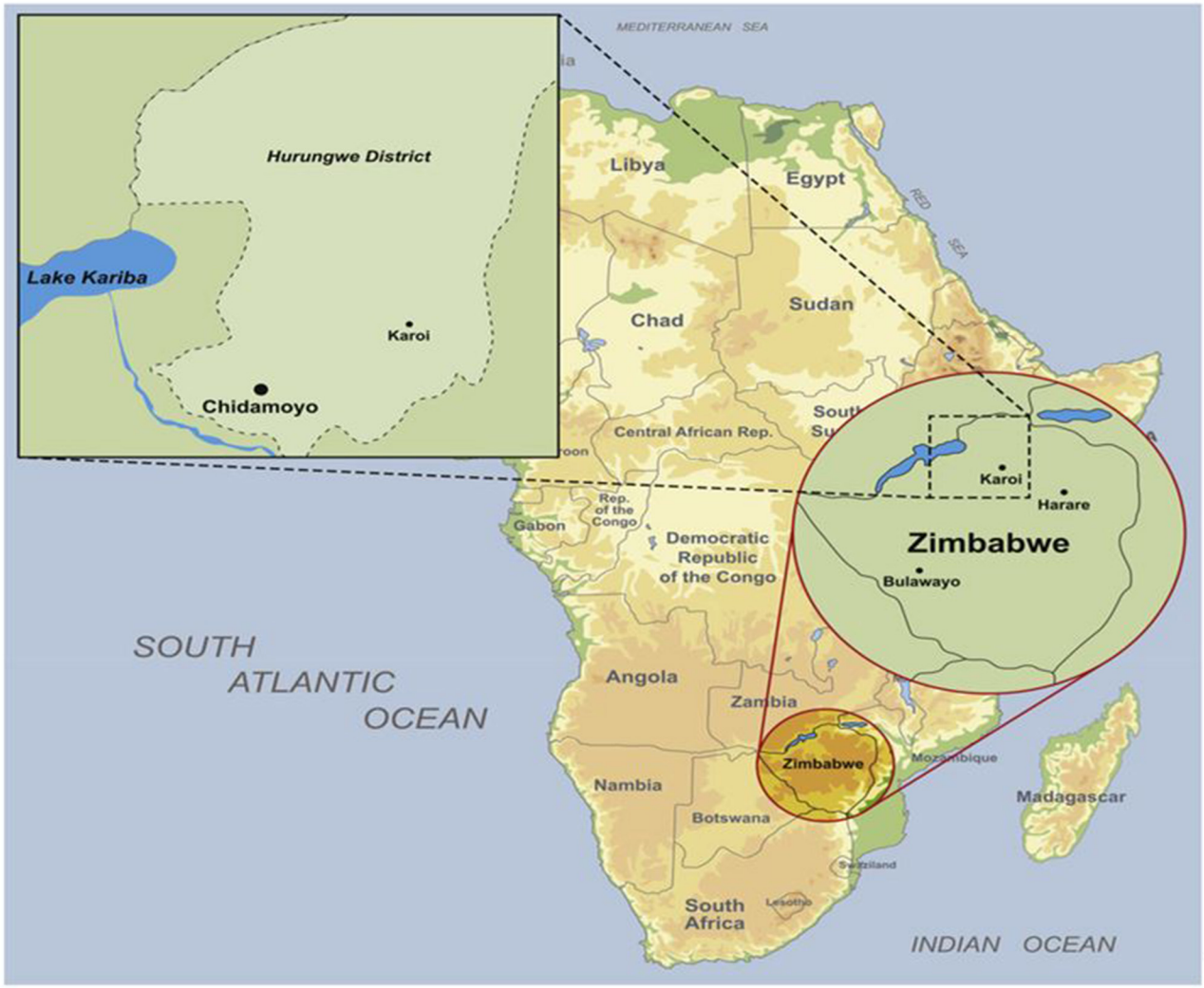

FIGURE 1
Map of Chidamoyo village in Hurungwe district (25).

Inclusion criteria

The study included people who fulfilled the following criteria:

- Women aged between 18 and 60 years from Chidamoyo Village, Zimbabwe
- Health workers involved in cervical cancer screening
- Programme managers/policymakers working for the Ministry of Health or development partners

Exclusion criteria

The study excluded individuals based on the following criteria:

- Women who were non-Chidamoyo residents
- Individuals who did not give consent to participate in the study

Nominal group process

The invited stakeholders gathered on the 4th of April 2023 at Chidamoyo Mission Hospital and we employed the NGT for data collection (25, 26). The workshop was conducted in two phases in a structured group discussion to achieve consensus on the priorities in response to the research questions (Figure 2): In phase 1, the stakeholders identified barriers that prevent women from accessing and utilising cervical cancer screening services. In the second phase, key stakeholders collaborated to determine acceptable HPV self-sampling delivery strategies for cervical cancer screening. The nominal group discussion was conducted in the local language. The convener of the discussion was the researcher (MD) and was assisted by a research assistant (RV). The participants were divided into two subgroups of four, with equal representation of 2 women, 1 health worker, and a policymaker in each subgroup. The questions asked to the participants at each phase were as follows:

Phase 1

To start the workshop, the researcher (MD) posed the following question to participants: What are the barriers to access and utilisation of current cervical cancer screening services? The following steps were followed to answer this question:

Silent brainstorming

Participants were allocated 10 minutes to write down responses on sticky notes provided with one idea on a separate note silently without discussing it with other participants. The participants were allowed to raise their hands to get the attention of the convener if they needed clarity or stationery.

Round robin session

A total of 10 minutes was allocated to allow each group participant to present their ideas in a round-robin fashion. The ideas from the participants were grouped into similar themes and the sticky notes were put on a flipchart for presentation and discussion in the next stage of the workshop.

Discussion and clarification of ideas

Each sub-group selected one representative to present their ideas according to the themes they had agreed upon. During this session, the audience was allowed to seek clarification and probe the presenters. The researcher with the help of the assistant collated all the ideas and highlighted similar themes. The ideas presented by each group representative were captured verbatim. The collated results were presented to the wider group as priority areas to be ranked during the ranking session.

Ranking of ideas

The ranking process followed the approach of assigning a value to an idea according to its priority as emphasised by Delbecq et al. (28). Participants were given a short break and refreshments were provided. During this time the researcher and the research assistant printed a ranking questionnaire for each participant. Other researchers have used tools such as Google forms for the ranking stage of the NGT (29). The questionnaire was made up of barriers to access and utilisation of cervical cancer screening services as presented by the two sub-groups. The questionnaire was handed to each participant for ranking ideas using a Likert scale of 1–5 scores with 1 representing very low priority and 5 representing highest priority. The ranking process was conducted independently and without discussion. The results were collated and analysed using an Excel spreadsheet as explained in the data analysis section below.

Phase 2

The researcher (MD) posed the following question to participants: "Which HPV self-sampling delivery strategies can help to improve women's uptake of cervical cancer screening" The steps in phase 1 were repeated in phase 2 of the workshop until the last stage of ranking the priority HPV self-sampling delivery strategies.

Data management

During the nominal group discussions, we collected two types of data: qualitative and quantitative. We managed the two data types separately using different tools, and combined the outcomes to answer our research questions. The study assistant (RV) recorded all the qualitative data in a notebook for later

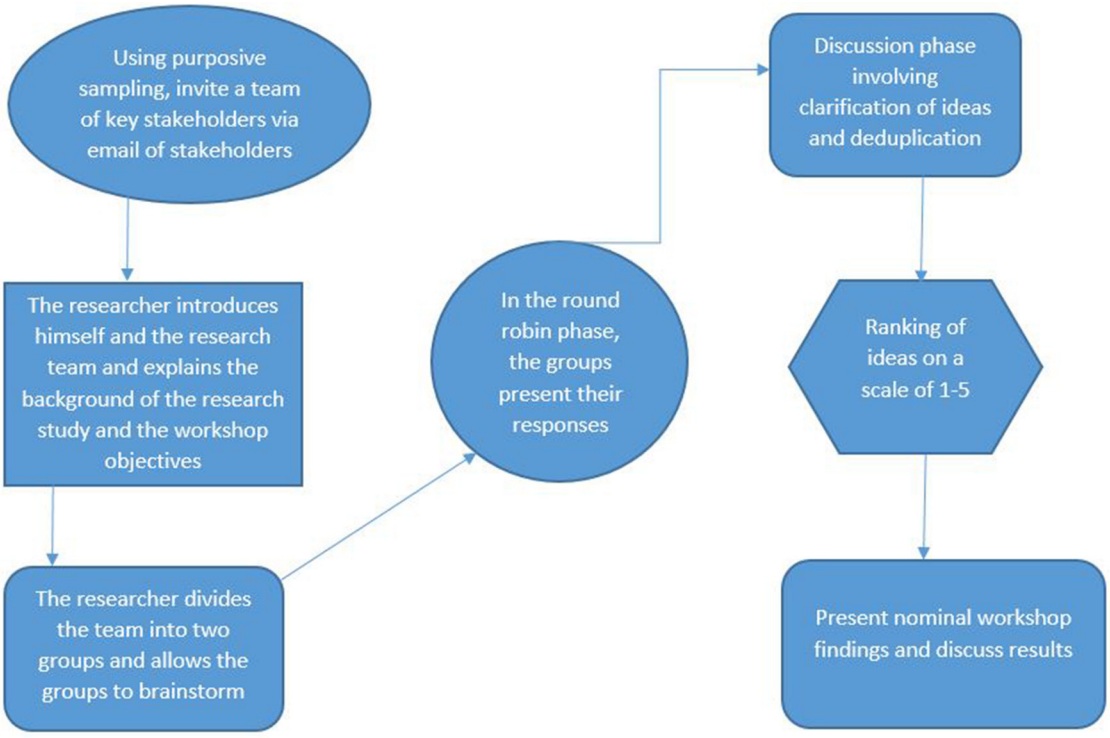

FIGURE 2
Nominal group technique (NGT) workshop workflow.

analysis. For quantitative data, we entered the information into Microsoft Excel spreadsheets for further analysis. In addition to this, we received extra qualitative data from key stakeholders who provided comments on the workshop report that was sent to them immediately after the workshop.

Data analysis

During phase 1 of the NGT, quantitative data was gathered to rank the barriers that prevent women from accessing cervical cancer screening services. Each participant provided individual scores, which were then added up to calculate a total importance score for each barrier. In phase 2, each HPV self-sampling delivery strategy was assigned a total importance score based on its effectiveness in addressing the identified barriers in phase 1. The ranking scores were on a scale of 1 to 5, with 1 being the least severe and 5 being the most severe barrier.

Qualitative data

We conducted qualitative analysis of the top 5 ranked themes. Qualitative data from the nominal group workshop was translated into English by the researcher (MD) and the assistant (RV) who are both native Shona speakers. The transcribed text was repeatedly read to familiarise with the data. We employed the thematic analysis approach by inductively generating codes from the data presented during the discussion (30). This approach has been shown to limit researcher bias due to preconceived ideas or other theoretical perspectives (31). The first and second authors performed the data analysis.

Ethics statement

This study was ethically reviewed and approved by two institutional review boards: University of Pretoria Faculty of Health Sciences Research Ethics Committee (approval number 548/2022) and the Medical Research Council of Zimbabwe (approval number MRCZ/A/2993). Additional written permission was sought and granted by the Ministry of Health and Childcare, Medical Directorate of Mashonaland West Province, and Chidamoyo Mission Hospital. Before participating in the study, all participants were fully informed about the study's background, objectives, and procedures and the researcher responded to questions regarding the study. Study participants also signed an informed consent forms to indicate their willingness to take part in the workshop. During the nominal group workshop, the participants were divided into groups with equal representation to ensure power balance and free participation. The researcher and the assistant maintained an enabling atmosphere to encourage the active participation of all stakeholders. The identities and personal information of all participants will be kept confidential and all the information shared during the discussion was anonymised to protect privacy.

Results

All 8 invited stakeholders accepted the invitation and took part in the NGT, making it an acceptance of 100%. The stakeholders were aged between 33 and 58 years, of these, 62% were female. Fifty per cent were formally employed, three were self-employed vendors and one was unemployed. Table 1 describes the characteristics of the workshop participants.

Quantitative findings

Phase 1

Stakeholders reported 10 factors as barriers to access and utilisation of current cervical cancer screening services (Figure 3). The voting scores revealed that *inadequate information and education on cervical cancer* (with a score of 40) was the leading barrier followed by *inadequate funding for cervical cancer screening programmes* (33), *long distance to a screening health facility* (32), *fear of a positive diagnosis* (31), *low perceived risk of cervical cancer* (31), *fear of speculum examination* (30), *embarrassment of getting screened by male a health worker (30)* and *lack of treatment options after a positive result* (26). The *attitude of health workers* (22) and the *need for seeking male partner permission* were the least ranked barriers (22).

After considering the voting scores, participants identified five priority barriers to access and utilisation of available cervical cancer screening services in Chidamoyo village (Table 2). *Inadequate information and education on cervical cancer and screening methods was the highest priority barrier* (100%), followed by *inadequate funding for cervical cancer screening programmes* (82.5%), *long distance to screening health facility* (80%), *fear of a positive diagnosis* (77.5%) and the *low perceived risk of cervical cancer* (77.5%).

Phase 2

Stakeholders reported 9 HPV self-sampling delivery strategies for a cervical cancer screening programme (Figure 4). The voting results showed that the highest-ranked strategy for the delivery of an HPV self-sampling intervention was *education and awareness* (39). This was followed by *early results notification* (37), *community-based self-sampling* (36), *choice of sampling device* (36), *local language for instructions* (35), *linkage to care after a positive result* (35), *facility-based self-sampling* (33), *and privacy* (31) and *male partner involvement* (28) *in HPV self-sampling* were voted as the least prominent delivery strategies for the implementation of HPV self-sampling.

According to participant voting scores, the 5 HPV self-sampling delivery strategies of high priority were *Adequate education and awareness on HPV self-sampling* (97.5%), *early results notification* (92.5%), *choice of sampling device* (90%), *community-based self-sampling* (90%) and *linkage to care after positive result* (87.5%) (Table 3).

Qualitative findings

Thematic analysis of the top 5 HPV self-sampling delivery strategies

The top 5 ranked priority HPV self-sampling delivery strategies as voted by the key stakeholders were (1) adequate education and awareness on HPV self-sampling (2) early results notification (3) choice of sampling device (4) community based self-sampling and (5) linkage to care. Each theme is presented below with supporting quotes.

Adequate education and awareness on HPV self-sampling

According to the stakeholders who participated in the workshop, the most effective delivery strategy for an HPV self-sampling screening programme was education. They suggested that the focus of education should be on providing information about cervical cancer, including its causes, prevention methods, and advantages of HPV testing using self-collected specimens over VIAC and provider-collected HPV testing. This would equip women with the necessary knowledge to make informed decisions and educate others in their communities. The women stressed that education on HPV self-sampling should be offered in their native language, such as Shona, to ensure a full understanding of the instructions and the procedure. They also requested education for their male partners to encourage their support and understanding.

TABLE 1 Characteristics of workshop participants.

I. D	Gender	Marital status	Age	Highest qualification	Designation	Work experience
P1	Female	Divorced	37	Diploma	Registered general nurse	8
P2	Female	Married	35	Ordinary level	Vendor	10
P3	Female	Married	46	Junior secondary school	Vendor	10
P4	Female	Married	52	Junior secondary school	Vendor	20
P5	Female	Married	58	Ordinary level	Unemployed	*
P6	Male	Single	33	Tertiary/masters	Epidemiologist	8
P7	Male	Married	40	Tertiary/masters	Obstetrician and Gynaecologist	13
P8	Male	Married	39	Diploma	Registered general nurse	5

*Not applicable.

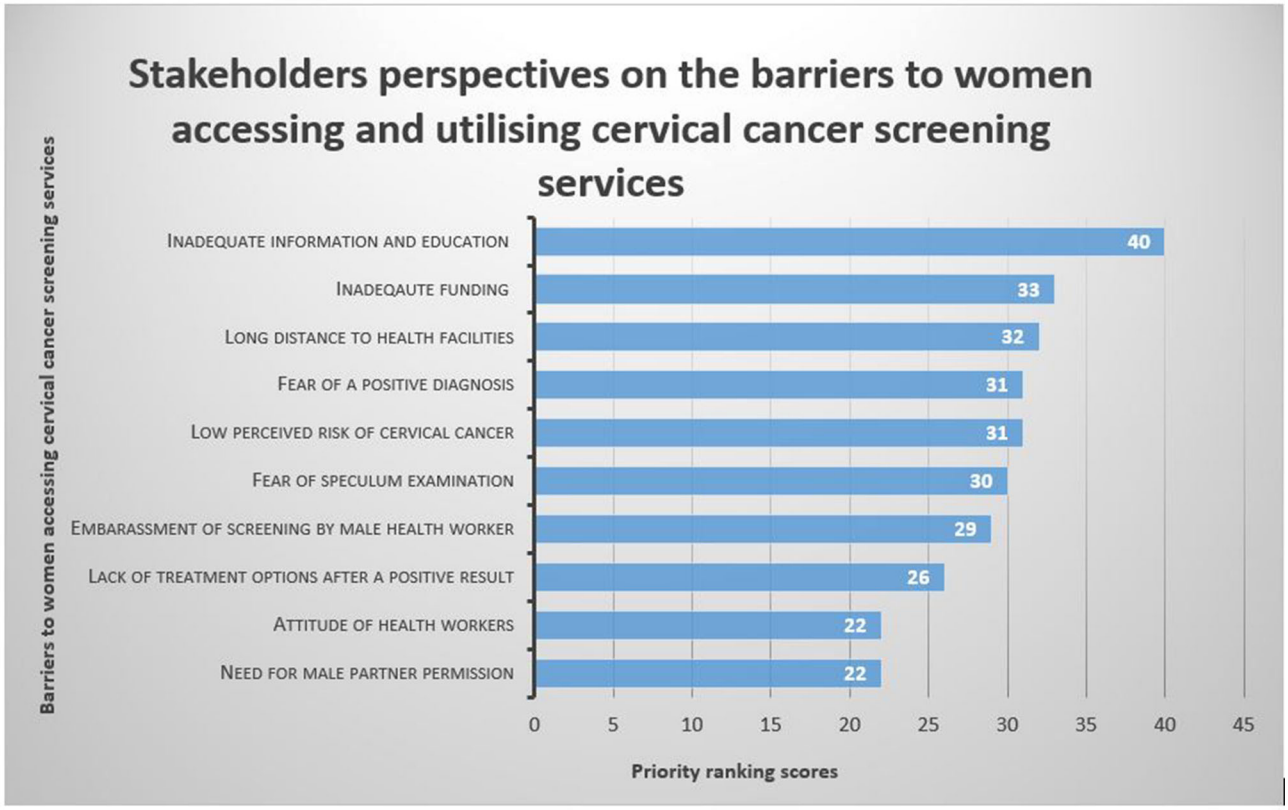

FIGURE 3
Key stakeholders' voting scores for barriers to access and utilisation of cervical cancer screening services.

TABLE 2 Priority barriers total voting scores and percentages.

Priority barriers to accessing cervical cancer screening	Summing by votes 1 = low priority 5 = highly priority					Total number of voting scores (number of votes × ranking score)
	1	2	3	4	5	40
Need for male partner permission		2	6			22
Attitude of health workers	3		2	2	1	22
Lack of treatment options after a positive result	1	2	1	2	2	26
Embarrassment of screening by male health worker	1		3	1	3	29
Fear of speculum examination		1	2	3	2	30
Low perceived risk of cervical cancer		1	1	4	2	**31**
Fear of a positive diagnosis			3	3	2	**31**
Long distance to screening health facility		1	2	1	4	**32**
Inadequate funding		1	1	2	4	**33**
Inadequate information and education					8	**40**

The bold values represent the top rankled barriers and delivery strategies respectively.

Community health workers were identified as key players in this initiative, as they are close to the women and wellgrounded within the communities. Education was also identified as a means to dispel misinformation and fight the stigma surrounding cervical cancer.

"Before we can do this self-sampling, may we get adequate information on how it is done so that we are able to do it correctly. It is also important for all the education and instructions on self-sampling to be conducted in (Shona) a language that we understand"

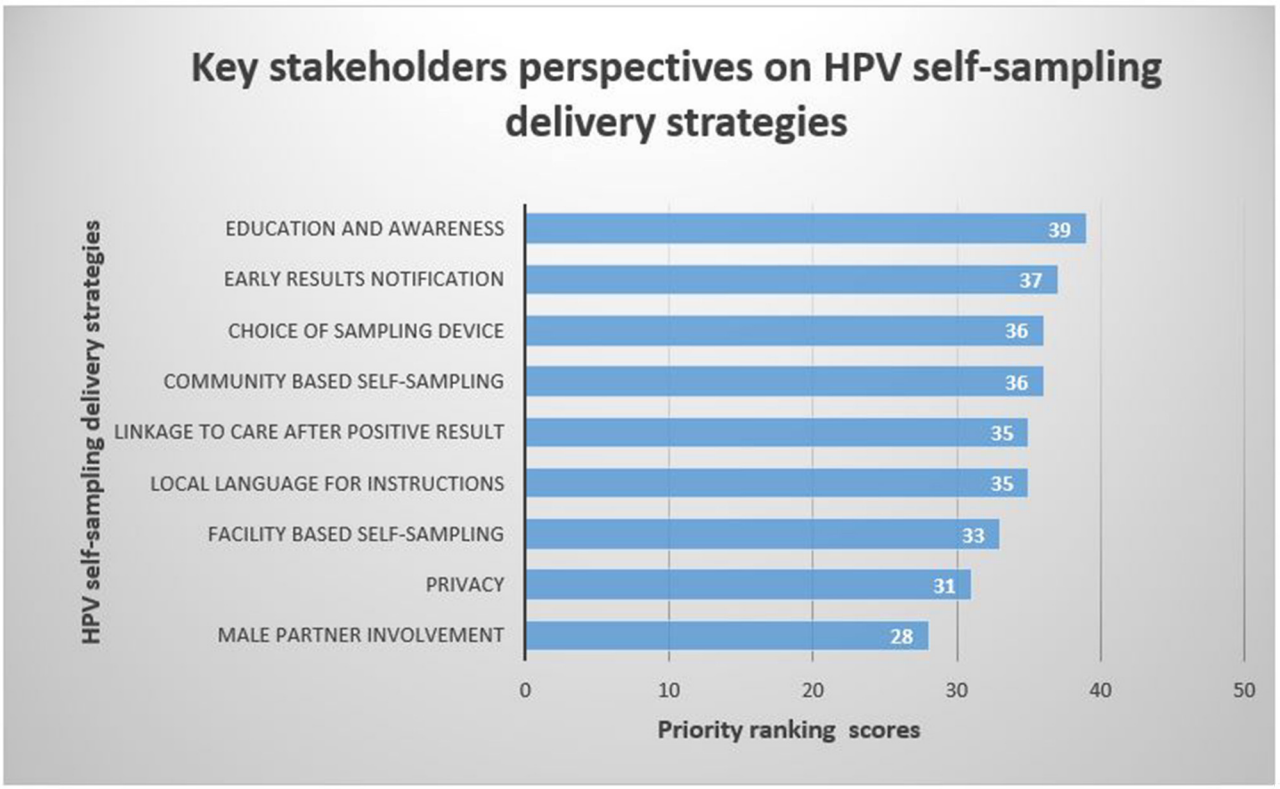

FIGURE 4
Key stakeholders' voting scores for HPV self-sampling delivery strategies for cervical cancer screening.

TABLE 3 **Priority HPV self-sampling delivery strategies for cervical cancer screening.**

Priority HPV self-sampling delivery strategies for cervical cancer screening	Summing by votes 1 = low priority 5 = highly priority					Total number of voting scores (number of votes × ranking score)
	1	2	3	4	5	40
Male partner involvement			5	2	1	28
Privacy		1	2	2	3	31
Facility based self-sampling			2	3	3	33
Local language for instructions			1	3	4	35
Linkage to care after positive result			1	3	4	**35**
Community based self-sampling			1	2	5	**36**
Choice of sampling device			1	2	5	**36**
Early results notification		1			7	**37**
Adequate education and awareness on cervical cancer				1	7	**39**

The bold values represent the top rankled barriers and delivery strategies respectively.

> "For the majority of women, there is some satisfaction in receiving a service through a healthcare worker. Self-sampling removes this contact with the healthcare worker especially when deployed within the community. There is need to educate women that self-sampling is equally good so as to encourage uptake"

Early results notification

Stakeholders have emphasised that early notification of results is crucial for a successful HPV self-sampling screening programme. Women experience anxiety and may discontinue the screening process if results are delayed. Compared to VIAC, results may take

longer to issue to clients due to the time required to transport specimens to the laboratory. Therefore, it is essential to educate women about the advantages of HPV testing over VIAC and to make them aware that the wait is worthwhile. Stakeholders also agreed that point-of-care testing technologies with quicker turnaround times should be used. This would encourage the release of results to clients earlier. They also suggested that text-based messaging could be used to notify clients of their results. This method would be convenient since most clients have mobile phones.

> "If we collect our own samples for cervical cancer screening, are we going to get our results early because we once collected samples for a screening programme and some of us have not received the results so I need to know if I was okay or not. Self-sampling is good but it should give us results quickly just like we get from VIAC "
>
> "After being screened there is anxiety about the outcome. The shorter the anxiety period the better so as to encourage uptake of services minimise loss to follow-up"
>
> "From a practical point of view, VIAC will always have the fastest turnaround time so as we transition to HPV testing, we need to equip all the women with adequate knowledge on the benefits of HPV screening compared to VIAC so that they don't say, VIAC is better because we get results faster, Also we can also invest in point of care and near point of care technologies with quicker turnaround times compared to the big molecular platforms in central laboratories in big towns and cities"

Choice of the sampling device

Women who participated in the workshop reported a general dislike for the metal speculum that health providers have traditionally used due to the discomfort and pain associated with its use. To make HPV self-sampling more acceptable to women, it is important to ensure that the devices used are visually appealing, easy to use, and cause little discomfort. The stakeholders emphasised that, since HPV self-sampling is a relatively new intervention, it is crucial that women have positive experiences with it, which will encourage them to share the information with their peers in the community.

> "We hope that the thing that will be used for self-collection is not uncomfortable or painful, because I once collected my own sample and I had to stop the moment I felt some pain, now I do not know if I collected the sample properly. So, if we can have a very soft collection device which is comfortable it will be easy to perform the procedure"
>
> "Devices causing minimal discomfort and which are visually appealing in terms of size and shape are more likely to encourage high uptake"
>
> "I just want to know if the thing that I will use to collect is soft because the metal they put inside us is very uncomfortable, I don't like it, I am sure some of the women in here can agree with me, am I lying about the metal ladies? *No, No…they all agree with the lady* so, if a soft and painless thing is provided we are going to welcome this new method and we will tell others about it so that they also get screened"

Community-based self-sampling

During discussions with community-based HPV self-sampling was suggested as a crucial a delivery strategy to promote the uptake of cervical cancer screening using self-collected samples, They emphasised that accessibility was a current challenge which disproportionately affects women in rural areas, therefore, if women are afforded the opportunity to perform self-sampling screening in their communities it would be convenient for most of them. The role of community health workers in spearheading community programmes such as cervical cancer screening was highlighted and their role in educating women, raising awareness and mobilising women is key to achieving high screening coverage in communities. Stakeholders also suggested that if women would perform self-collection in the communities it was important to ensure that there would be privacy during collection. Some of the participants had this to say:

> "The coverage of current screening methods is still low to achieve the coverage required to eliminate cervical cancer as a public health problem by 2030. This is because these methods cannot be scaled up to achieve that coverage due to cost, human resources, infrastructure and other challenges. Community-based self-sampling presents an opportunity for mass screening beyond the limitations of a health facility to reach underserved communities"
>
> "This removes barriers associated with long distances to healthcare facilities and associated costs of travel, hence it is very important"
>
> "Because some women face challenges in coming to the health facility, which may be a distance from where they stay. It would be helpful if programmes such as self-sampling can be brought to the community where the women live for convenience"

Linkage to care after positive results

Participants were of the view that for any cervical cancer screening programme to succeed there should be follow-up on women who screen positive so that they can be triaged by another method such as VIAC. It was also highlighted that the availability of care after the screening was an important enabler for cervical cancer screening as some women reported that they were unwilling to get screened when they were unsure of getting treatment after an abnormal test.

> "I don't think we will have any problems using these things to collect samples. As for me I think I want it this way instead of having that metal put inside me, however we want to know if I will be treated when found with some problems done there, because when you are HIV negative sometimes you are asked to pay for treatment but people living with HIV are treated for

> free. So if it stay like that I will be afraid of getting screened so I hope things change with this method of self-collection"
>
> "HPV testing when deployed as a primary screening method requires a visual triage step for a positive result. This step will determine the treatment to be offered based on defined criteria. The goal of screening is to detect precancerous lesions and treat them. Without treatment that goal will not be met"
>
> "When women know that something can be done for them after an abnormal result is obtained, it will encourage better uptake than if there is no plan or strategy to take care of them after a positive screen"

Discussion

This study presents findings from a stakeholder's workshop to co-create acceptable HPV self-sampling delivery strategies for cervical cancer screening in rural Zimbabwe. Our study findings indicate that barriers at the individual, interpersonal, community and health system levels prevented women from accessing and utilising screening services. The following 5 priority barriers were identified: (1) inadequate information on cervical cancer, (2) inadequate funding, (3) long distances to health facilities, (4) fear of a positive diagnosis, and (5) low perceived risk of cervical cancer. In response, the stakeholders proposed delivery strategies to overcome some of the identified barriers, for instance, education and awareness was identified as the highest-ranked strategy to overcome the lack of knowledge, low perceived risk of cervical cancer and fear of a positive diagnosis while community-based self-sampling was proposed as a strategy to overcome long distances to health facilities. Our findings on barriers to access and utilisation of screening services are corroborated by studies that were conducted in Zimbabwe (11, 16, 23, 32) and other countries in SSA (33, 34). The lack of education and information on cervical cancer and screening methods was considered a significant barrier by the stakeholders in the current study. Women in this study reported that compared to other diseases such as HIV and TB, there were no widespread campaigns and awareness on cervical cancer and this likely contributed to the lack of knowledge on the disease. Similar findings were reported in previous studies (35, 36). A qualitative study to explore community knowledge, facilitators and barriers to cervical cancer screening in rural Uganda revealed a belief among women that screening should be accessed at the onset of symptoms (35). Additionally, a Swedish study revealed that women lacking education had positive perceptions towards screening but prioritised other things in their lives, particularly when asymptomatic (36). This underscores the need for extensive education of women on cervical cancer prevention and the importance of seeking screening services early before the onset of symptoms when the cancer would probably have advanced. According to stakeholders, women were fearful of the pain and discomfort associated with the use of a metal speculum during pelvic exams. This is further emphasised by a qualitative study conducted in rural Kenya where women expressed a preference for self-sampling over pelvic exams due to the invasive and painful nature of the latter (37). We identified the lack of funding for cervical cancer control and prevention programmes and inaccessible health facilities as health system-level barriers. Petersen and colleagues reported the significant impact of factors such as (low budgetary support), infrastructure, and health workers on the accessibility and utilisation of cervical cancer screening services by women in limited resource settings (38).

HPV self-sampling delivery strategies

The highest-ranked strategies in our study included: education and awareness on HPV self-sampling, early results notification, community-based HPV self-sampling, choice of the sampling device, and linkage to care. Education and awareness was the highest-ranked delivery strategy which is important in overcoming barriers such as the lack of education, fear of positive diagnosis and the perceived low risk of cervical cancer. A systematic review conducted in Uganda (39) identified the fear of screening procedures as the major barrier to the uptake of screening services, but the authors revealed that this was due to misconceptions and myths which could all be dispelled by proper education of women. Therefore the role of education in overcoming many of the barriers at the individual level cannot be ignored. Delivery of education through peer educators has also been shown to increase the acceptability and uptake of cervical cancer screening (40, 41). According to findings from a systematic review by Makadzange et al. the use of peer educators and culturally sensitive and tailored material significantly impacted the delivery of educational messages for cervical cancer prevention to the target population in Africa (40). Similarly, a study conducted in India also supports using culturally appropriate educational material and interventions to reach communities and promote the uptake of cervical cancer screening especially among rural communities where the lack of education is the major hurdle to increased screening uptake among women (41).

The early delivery or notification of results was suggested as an important strategy for an HPV self-sampling screening programme. Stakeholders emphasised the need for early notification to avoid the anxiety associated with one not knowing their result, further contributions on this strategy highlighted the importance of early results notification to minimise the loss to follow-up and to encourage women participation in cervical cancer screening in the future. Not much is known on how the wait for HPV results affect women particularly in low income countries where VIA has been the main screening modality that ensures same day results. Considering the extensive mobile network coverage in Zimbabwe and that almost every person owns a mobile phone, text based messaging will be the most ideal notification method in Zimbabwe. A study in rural Tanzania that employed text messaging for results notification revealed the method to be acceptable and it encouraged women to attend a follow-up appointment after receiving HPV results (42). Additionally, leveraging on point-of-care diagnostic technologies which were widespread throughout Zimbabwe's districts during the COVID-19 pandemic may encourage faster turnaround times leading to early results notification for women in rural areas as compared to referring specimens for laboratory testing in towns and cities (43).

Stakeholders agreed that for HPV self-sampling to be an appealing screening method, women who screen positive for HPV must be easily linked to care. Current screening methods using VIAC in Zimbabwe encourage same-day treatment after an abnormal test and therefore it is critical to ensure that women get treatment services near them if they are eligible so that they do not lose trust in the HPV self-sampling screening method. A study by McRae et al. in which women were transitioning from cytology to HPV testing reported women's frustration with the lack of adequate treatment services after an HPV test because they were used to getting treatment and care without delay when undergoing cytology screening (44). There is a need for adequate education of women on the procedures relating to HPV testing such as the triaging of women who screen positive to determine their eligibility for treatment so that they appreciate the delay in getting care is necessary. It is worth noting that the success of any screening method ultimately rest on the identification of those at risk and their treatment, it is therefore crucial that such services are easily accessible to women in need.

The choice of sampling device was identified as a priority by stakeholders. Studies conducted in some countries in SSA highlighted the ease of use and comfort women experienced during self-sampling, making it easy for them to prefer future screening using self-collected devices (45, 46). Stakeholders in this study emphasised the need for visually appealing devices that cause minimal discomfort and less pain to ensure that women have a positive experience after screening. This has been shown to encourage the willingness to participate in future screening and to spread positive messages to peers and family members which in turn increases screening coverage (47). Megersa et al. (48) revealed that the choice of sampling device was a very important aspect of an HPV self-sampling intervention as the fear of the Evalyn brush in their study affected the quality of the sample collected and participants were less willing to use the brush next time for self-sampling. In the Zimbabwean context where resources are limited, there is likely going to be a single type of device for self-sampling. It is, therefore, vital to decide on the most preferred device before implementing the intervention. Adequate education in the local language including the use of pictures and videos can improve understanding of the self-sampling process ultimately increasing self-efficacy in performing self-sampling. Stakeholders in our study strongly recommended community-based HPV self-sampling approaches. Studies conducted in Zimbabwe (22) and Malawi (21) have all demonstrated the utility of bringing HPV testing closer to people. Likewise, in Cameroon, campaigns for HPV self-sampling in the community have proven feasible and cost-effective in increasing screening coverage, as demonstrated by same-day screening and treatment initiatives (18). In Zimbabwe, we recommend that the Ministry of Health and Child Care and development partners take advantage of traditional gatherings for women, such as "China cheMadzimai", a gathering of religious women on Thursdays to reach women and promote HPV self-sampling cervical cancer screening.

The collaboration of different stakeholders enabled different delivery strategies for implementing HPV self-sampling to be heard. The proposed approaches have the potential to promote demand and increase the acceptability of HPV self-sampling in Zimbabwe and other similarly resource-limited areas. To determine the most preferred delivery strategies for HPO self-sampling among women, we suggest employing the discrete choice experiment with a large sample size from the same study setting.

Strengths and limitations of the study

The collaboration of different stakeholders to co-create delivery strategies for an HPV self-sampling screening intervention is a notable strength of this study. Another strength of this study was the involvement of rural women as stakeholders. Rural women are disproportionately affected by cervical cancer due to their low socioeconomic status and lack of access to healthcare facilities and capturing their perspectives is vital in tailoring interventions to improve cervical cancer screening uptake not only in Zimbabwe but also in similarly low-resource rural settings globally. The collection of both quantitative and qualitative data allowed the ranking of strategies and allowed the researchers to obtain qualitative data. The themes were not selected a priori but rather actively constructed by the group. Future researchers may replicate the methods for co-creation purposes. A limitation of our study was the absence of other important stakeholders such as community and traditional leaders, community health workers and laboratory personnel during the workshop to offer their perspectives on barriers and potential delivery strategies. It would have been beneficial to involve these individuals to ensure that no crucial insights were overlooked. Although our sample size was small, we managed to capture diverse perspectives from the present stakeholders. However, it is possible that the women felt intimidated and were unable to participate freely due to the presence of familiar faces from their local hospital who were also health workers, even though the researcher ensured that participation was voluntary.

Conclusion

The purpose of the study was to develop effective HPV self-sampling delivery strategies for cervical cancer screening in rural Zimbabwe through co-creation with different stakeholders. Our research has demonstrated the effectiveness of the NGT for reaching a consensus on the barriers to access and utilisation of available screening services and identifying potential delivery strategies for HPV self-sampling to overcome identified barriers. The stakeholders identified and ranked them according to their priority in the following order: (1) education and awareness, (2) early results notification (3) choice of sampling device (4) community-based self-sampling and (5) and linkage to care. We anticipate that these proposed delivery strategies will be used by the Ministry of Health and Child Care, development partners and other relevant stakeholders to design an effective HPV testing screening programme using self-collected specimens in rural Zimbabwe.

Recommendations

After the success of NGT in identifying delivery strategies for HPV self-sampling and ranking them according to their priority, we recommend more stakeholder involvement in designing and implementing a national programme for HPV testing using self-collected specimens. To achieve this, we suggest involving the government, community health workers, traditional and community leaders, youth advocates, laboratory personnel, and supply chain experts. To ensure better access and utilisation of cervical cancer screening services, we also recommend an education programme targeting rural women, male partners, and community leaders on HPV-based cervical cancer screening using self-collected specimens. Lack of education was identified as the main barrier to accessing these services. The education programme can be championed by community health workers who work closely with women and the wider community on a day-to-day basis. To improve HPV testing turnaround times, we suggest leveraging point-of-care technologies used for COVID-19 testing and using text messaging for result notifications. Further research is needed in Zimbabwe to evaluate the impact of waiting for results on women's willingness to participate in cervical cancer screening with HPV testing. Also, before implementing HPV self-sampling screening, there is need to conduct a follow-up study to determine rural women's preferences for delivery strategies using a larger sample size with a discrete choice experiment survey.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by University of Pretoria Faculty of Health Sciences Research Ethics Committee and the Medical Research Council of Zimbabwe. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

MD: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Visualization, Writing—original draft. TD: Conceptualization, Investigation, Supervision, Writing—review & editing, Methodology. GM: Writing—review & editing. TC: Writing—review & editing. RM: Writing—review & editing. MF: Writing—review & editing. TM-T: Conceptualization, Investigation, Methodology, Supervision, Writing—review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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AI-assisted system improves the work efficiency of cytologists via excluding cytology-negative slides and accelerating the slide interpretation

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Given the shortage of cytologists, women in low-resource regions had inequitable access to cervical cytology which plays an pivotal role in cervical cancer screening. Emerging studies indicated the potential of AI-assisted system in promoting the implementation of cytology in resource-limited settings. However, there is a deficiency in evaluating the aid of AI in the improvement of cytologists' work efficiency. This study aimed to evaluate the feasibility of AI in excluding cytology-negative slides and improve the efficiency of slide interpretation. Well-annotated slides were included to develop the classification model that was applied to classify slides in the validation group. Nearly 70% of validation slides were reported as negative by the AI system, and none of these slides were diagnosed as high-grade lesions by expert cytologists. With the aid of AI system, the average of interpretation time for each slide decreased from 3 minutes to 30 seconds. These findings suggested the potential of AI-assisted system in accelerating slide interpretation in the large-scale cervical cancer screening.

KEYWORDS

HPV, cervical cancer screening, artificial intelligence, slide interpretation, low-resource areas

Introduction

Cervical cancer is threatening women's health and caused 342,000 deaths worldwide in 2020 (1). Screening plays an important role in eliminating cervical cancer, such as diagnosing precancerous cervical intraepithelial neoplasia (CIN) that can be surgically eliminated to prevent the incidence of cervical cancer (2, 3). However, there are disparities

of cervical cancer screening globally (4, 5). Self-sampling has dramatically ameliorated inequity of human papillomavirus (HPV) testing especially in resource-limited settings (6–9). In contrast, cervical cytology remains an issue in low-resource regions due to the shortage of cytologists (10, 11).

Emerging studies indicated the potential of artificial intelligence (AI) system for cervical cytology (12–16). For instance, Cheng et al. applied a recurrent neural network-based whole slide image (WSI) classification model to achieve high specificity and sensitivity for slide classification (14). Nevertheless, most of prior reports assessed the potential of AI system in classifying cervical lesions (12–15). Besides to the diagnosis of cervical lesions, it is important to lessen the dependence of cervical cytology on professionals in resource-limited settings. Given the indispensable role of cytologists in cervical cancer screening, it is imperative to reduce the number of cytologist-interpreted slides and to shorten the interpretation time for each slide.

Our study aimed to evaluate how AI-assisted system improved the work efficiency of cytologist-based cytology. To fulfill this goal, well-annotated cervical slides were applied to develop the model of slide classification. Then we assessed the feasibility of AI system in excluding NILM(Negative for Intraepithelial Lesion or Malignancy) slides. Subsequently, the efficiency of slide interpretation was evaluated for cytologists with and without the aid of AI system. These findings should have the potential to promote the implementation of cervical cytology in China.

Materials and methods

Automated staining and microscopic imaging of cervical exfoliated cells

We applied a liquid-based sedimentation cytology approach RQLCT1000 (Ruiqian co. ltd, Jiangsu) to achieve stained slides. Then, the automated slide scanner RQ1000 (Ruiqian co. ltd, Jiangsu) using continuous array scanning technology was applied to rapidly generate multi-depth images. The scanning process included two stages: 10X and 20X microscope scanning and multi-depth scanning as well as seamless layer fusion via Z-stack technology (17).

Image annotation

Three experimental clinicians and two expert pathologists from tertiary hospitals annotated scanned slides, adhering to TBS-2014 guidelines (18). In detail, digital images were divided into three non-repetitive sets for annotation by distinct medical professionals. Annotation cells, which results were agreed by two experts, were integrated into a standardized database for robust data management.

Cell detection and classification

A 10× image acquisition system was used for Pap test AI detection to identify single cells and cell clusters. Cell clusters were characterized by closely packed cells without easily distinguishable cytoplasm (Supplementary Figure 1). Meanwhile, each cell cluster should include more than three cells.

We then classified cells via two modules: cell primary screening and cell classification module. The cell primary screening module applied Yolov5 as the basic framework, and the cell classification module utilized ResNet as the basic framework (19–21). Among them, the cell primary screening module was used for detecting all suspicious lesioned cells in the image to ensure a high recall rate of detected diseased cells. The cell classification module further screened and classified diseased cells on the basis of the cell primary screening module to improve the accuracy of detecting diseased cells.

The dataset of the cell primary screening module was composed of annotated cells, that were classified into NILM(Negative for Intraepithelial Lesion or Malignancy), HSIL(High-Grade Squamous Intraepithelial Lesion), LSIL (Low-Grade Squamous Intraepithelial Lesion), and ASU(Atypical Squamous Cells of Undetermined Significance) types.

The dataset of the cell classification module was composed of the detected positive cell images of the cell primary screening module, wherein the 'false' diseased cells detected by the cell primary screening module were regarded as NILM, and the detected 'true' diseased cells were regarded as lesion-positive.

Slide classification

To determine if the slide was lesioned, we first ranked all single cells and cell clusters based on lesion probability. Afterwards, we calculated the average probability of top 10 single cells as well as the average probability of top 5 cell clusters. Then scatter plotting was conducted for all slides to determine the cutoff of probability, using the average of top 10 single cells as x-axis and the average of top 5 cell clusters as y-axis. And SVM classifier was applied to determine the degree of lesion, such as ASU and HSIL (22).

Statistic analysis

To assess the feasibility of AI system in excluding NILM slides, we calculated the percentage of AI-reported NILM slides in the validation group. Then we compared it to cytologist-interpreted results.

For slides in the validation group, the interpretation time of cytologists was recorded for two types of slide interpretation: with and without the aid of AI system. To compare the

differences of consumed time in slide interpretation, the average of the interpretation time was calculated by the formula: the total of consumed time/slide number. In addition, cytologist-reported results were also compared between two interpretation models.

Results

Automatic AI system to perform cell staining and imaging

We devised an integrated approach for the automated diagnosis of cervical lesions, utilizing the cytology of cervical exfoliated cells (Figure 1). All experiments were seamlessly integrated into an automated and cohesive process, encompassing the staining of exfoliated cells and multi-depth scanning fusion imaging.

Within this automatic system, we totally scanned 43,057 cervical slides in two hospitals within 74 days (10 running hours/day), resulting in an average production rate of nearly 30 high-quality images per hour. Then we selected 5,000 high-quality slides for developing cell classification model and the remaining 31,753 high-quality slides were applied to assess the performance of developed models.

Performance of AI-system in classifying cervical cells

Based on 5,000 high-confidence slides, 98,212 high-quality 10× images of individual cells were chosen, being annotated as NILM (n=50,100), ASU (n=16,602), LSIL (n=13,968), and HSIL (n=17,542) (Table 1). Additionally, we selected 64,087 10× high-quality images for cell clusters, which were annotated as NILM (n=34,775), ASU (n=9,062), LSIL (n=9,552), and HSIL (n=10,698) (Table 1). The confusion matrix showed that the accuracy of NILM prediction from single cell and cell cluster was 81.40% and 88.85%, respectively. The highest accuracy were found for HSIL prediction (90.0% and 85.0% for single cell and cell cluster), compare to the ASU (42.54% and 37.17% for single cell and cell cluster) and LSIL (68.23% and 65.75% for single cell and cell cluster) (Table 2).

The same methods were applied in 20× microscope classification, but combination of cell clusters and single cells were used to train the classification model. As the accuracy of NILM remained at 81.49%, there was a reduction in misclassification ratio from ASU, LSIL and HSIL to NILM by 10.61%, 2.71% and 4.45% (Table 3).

To achieve the higher accuracy of NILM prediction, we calculated both the average probability of the top 10 single cells

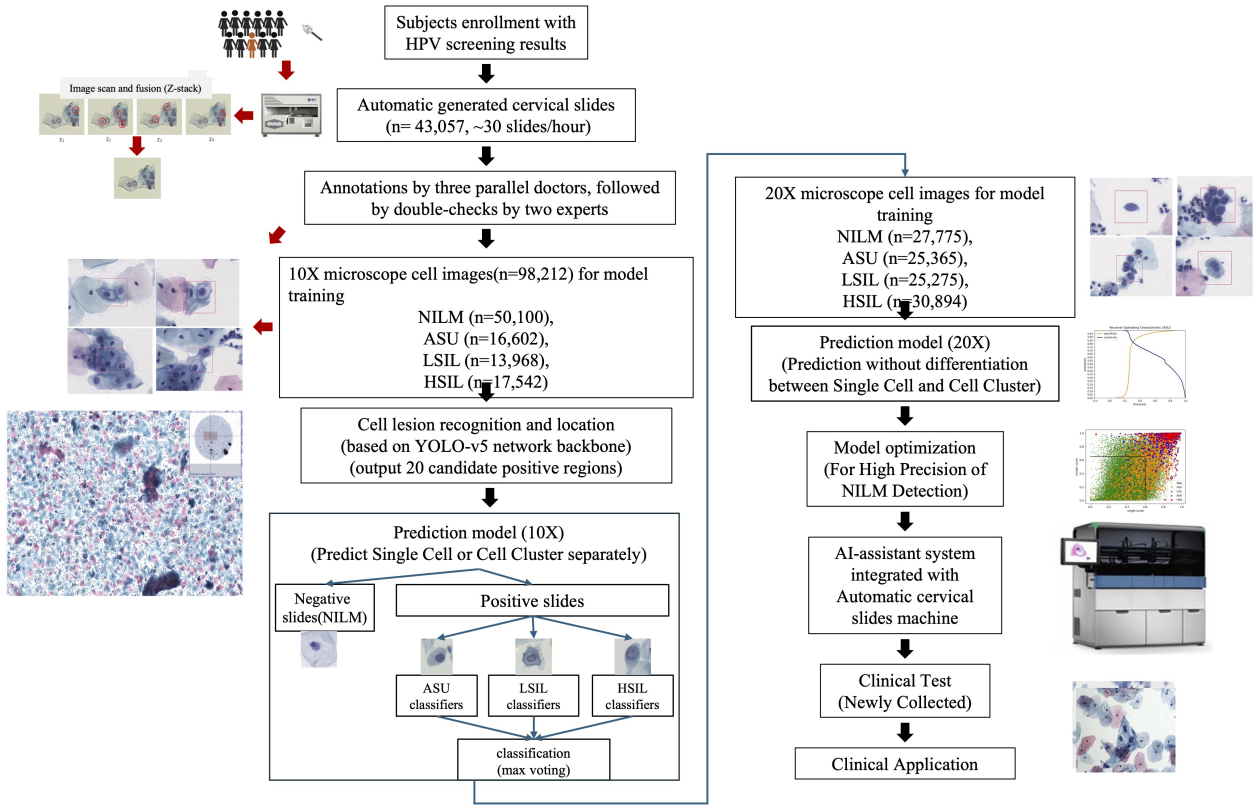

FIGURE 1
The workflow of automatic AI-assisted cervical cytology.

TABLE 1 The types and distribution of 10× single cell and cell cluster.

Types		Total	Train set	Validation set	Test set
Single Cell	**NILM**	50,100	35,070	10,020	5,010
	ASU	16,602	11,622	3,320	1,660
	LSIL	13,968	9,778	2,793	1,397
	HSIL	17,542	12,280	3,508	1,754
Cell Cluster	**NILM**	34,775	24,343	6,955	3,477
	ASU	9,062	6,344	1,812	906
	LSIL	9,552	6,687	1,910	955
	HSIL	10,698	7,489	2,139	1,070

and the average probability of the top 5 cell clusters. By applying a cutoff of 0.6 for single cell scores and 0.65 for cell cluster scores (Figure 2), we achieved the highest accuracy in NILM prediction.

Performance of AI system in excluding cytology-negative slides and improving the efficiency of the slide interpretation

We further applied our AI-system on 31,753 slides in the validation groups. AI system reported 29,625 slides with NILM among which 98.27% (29,113/29,625) were also diagnosed as NILM by two expert cytologists who reviewed slides together and provided a consensus result. For remaining 512 AI-reported NILM slides, 1.67% (496/29,625) and 0.05% (16/29,625) were diagnosed as ASU and LSIL by cytologists. None of AI-reported NILM slides was diagnosed as HSIL.

Besides to accurately exclude cytology-negative slides, our AI system shortened the interpretation time for cytologists. Based on AI-assisted system, cytologists only need to interpret the top 20 cells which were ranked by the lesion probability. Therefore, the average interpretation time for each slide can decrease from 3 minutes to 30 seconds. And we observed no differences of interpretation results for 979 AI-reported lesion-positive slides between whole slide image and AI-provided top 20 cells, including 129 HSIL, 137 ASH and 713 LSIL slides.

Discussion

Cervical cancer screening play an important role in eliminating cervical cancer (2, 3). Despite the potential of AI-assisted system in facilitating cervical cytology, professionals play essential roles in near future. Nevertheless, there is a deficiency in assessing the improved work efficiency of cytologists and the lessened dependence on professionals based on AI-assisted system. Our study evaluated the exclusion of cytology-negative slides and shortened interpretation time for cytologists with the aid of AI system.

Our large-scale cervical cancer screening projects indicated that nearly 80% of cervical slides were cytology-negative (23–26). Therefore, it can dramatically decrease the workload of cytologists if the AI-system can exclude these cytology-negative slides with high accuracy. In this study, we identified the potential of AI-assisted system in excluding cytology-negative slides, thus decreasing the number of slides to be interpreted by cytologists.

TABLE 2 The confusion matrix of 10× single cell and cell cluster.

Prediction Labels		True Labels			
		NILM	ASU	LSIL	HSIL
Single Cell	NILM	81.40%	31.98%	6.74%	4.68%
	ASU	8.0%	42.54%	15.05%	3.93%
	LSIL	2.25%	11.42%	68.23%	1.39%
	HSIL	8.34%	14.06%	9.98%	90.0%
Cell Cluster	NILM	88.85%	23.32%	6.13%	8.96%
	ASU	1.58%	37.17%	20.82%	2.68%
	LSIL	1.98%	32.72%	65.75%	3.37%
	HSIL	7.59%	6.78%	7.31%	85.0%

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TABLE 3 The distribution and confusion matrix of 20× cervical cell image.

Types	Total	Train set	Validation set	Test set	True Labels			
					NILM	ASU	LSIL	HSIL
NILM	27,775	19,443	5,555	2,777	81.49%	10.61%	2.71%	4.45%
ASU	25,365	17,756	5,073	2,536	9.79%	63.04%	18.24%	3.96%
LSIL	25,275	17,693	5,055	2,527	6.33%	18.81%	72.88%	2.31%
HSIL	30,894	21,626	6,179	3,089	2.39%	7.54%	6.17%	89.27%

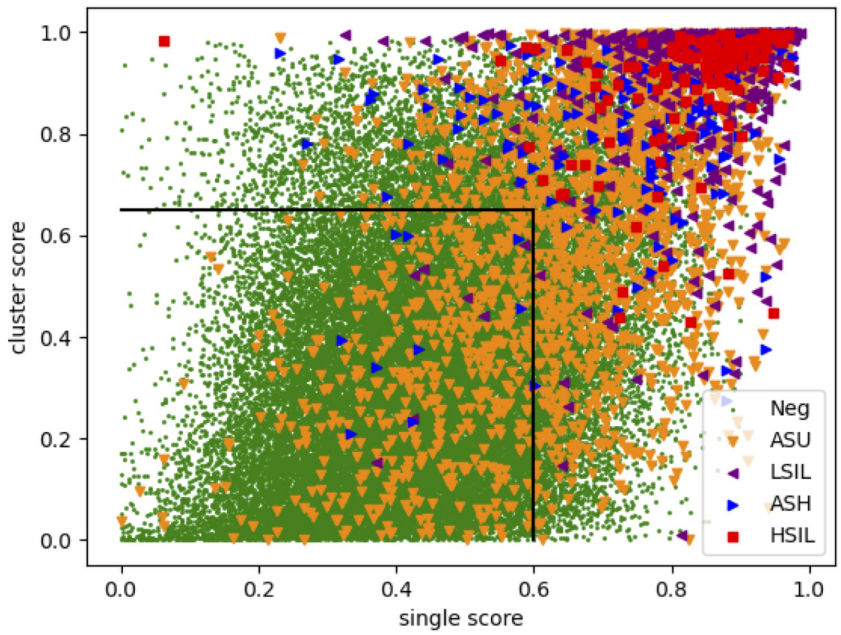

FIGURE 2
Scatter plot for slide assessment based on single cell and cell cluster scores. Each specific represents a slide and the colors represent expert-conducted diagnosis of the slide. X-axis and y-axis represent the average of probability for top 10 single cells and top 5 cell clusters, respectively.

Nearly 70% of analyzed slides were reported as NILM by AI-assisted system. Among 29,625 AI-reported NILM slides, 98.27% were diagnosed as NILM. The remaining 1.72% were diagnosed as ASU as well as LSIL by cytologists. Thus, the risk of missing HSIL slides is low when applying the AI system to exclude cytology-negative slides.

Besides to excluding about 70% cytology-negative slides, AI system can shorten the interpretation time of AI-reported positive slides (27). In prior, it took about 3 minutes for to interpret a slide. With the aid of the AI-assisted system, cytologists only need to interpret the top 20 cells which were ranked by the lesion probability. Therefore, the average interpretation time for each slide can decrease from 3 minutes to 30 seconds.

The limitations of our study included the retrospective design. And it was not tested in real resource-limited settings. In addition, it was completed with two steps: preparing slides then conducting slide scanning and AI-assisted cytology. Thus, it would bring additional burden and decrease work efficiency in resource-limited settings. However, our team and other researchers are testing one-stop machine, which can automatically perform high-throughput slide preparation, staining, imaging and AI-assisted cytology. Then it can further improve the feasibility and efficacy of cervical cytology in large-scale cervical cancer screening projects. And this should ensure the equity of cervical cancer screening and precancer/cancer treatment for underserved communities in China.

In summary, AI-assisted system can improve the work efficiency of cytologists, such as decreasing the number of slides to be interpreted and shortened the time of slide interpretation. Additionally, automatic sample processing and AI-assisted cytology can lessen the dependence of cytology on medical resources. And this should increase the coverage of cervical cancer screening in low-resource regions.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

This study was approved by the Ethics Committee of Peking University Shenzhen Hospital (registration number: 2023-116).

Author contributions

HD: Conceptualization, Funding acquisition, Resources, Writing – original draft. WD: Methodology, Visualization, Writing – original draft. QZ: Methodology, Writing – original draft. CL: Methodology, Software, Writing – original draft. SL: Methodology, Writing – original draft. CW: Data curation, Resources, Validation, Writing – original draft. JT: Data curation, Validation, Writing – original draft. XW: Conceptualization, Supervision, Writing – review & editing. RW: Conceptualization, Resources, Supervision, Writing – review & editing.

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Conflict of interest

Authors XW and CL is employed by Suzhou Ruiqian Technology Company Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1290112/full#supplementary-material

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Relationship between vaginal microecology and human papillomavirus infection as well as cervical intraepithelial neoplasia in 2,147 women from Wenzhou, the southeast of China

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Objective: The female reproductive tract is a significant microecological region, and its micro-environment can directly affect women's cervical health. This research aimed to investigate the effect of vaginal microecology on human papillomavirus (HPV) infection and cervical intraepithelial neoplasia(CIN).

Methods: A retrospective cohort study enrolling 2,147 women who underwent a colposcopic examination between August 2021 and August 2022 was conducted. The relationship between vaginal microecology and HPV infection as well as cervical lesions were assessed using the chi-square test, univariate and multivariate logistic regression analyses, and Cochran-Armitage trend test.

Results: HPV infection was linked to the imbalance of vaginal microecology [odds ratio (OR)=3.00, 95% confidence interval (CI)=1.66–5.43; P<0.001]. Clue cell (OR=1.59, 95% CI=0.99–2.54; P=0.054) and sialidase (OR=1.54, 95% CI=1.01–2.35; P<0.046) were considered as significant risk factors for HPV infection. Further analysis showed that vaginal microecological disorder was more likely to be detected in patients infected with HPV 16/18 subtypes (OR=9.86, 95% CI=2.37–41.80; P=0.002). Although there was no significant correlation between the incidence of vaginal microecological disorder and the severity of cervical lesions (P > 0.05), the proportions of abnormal PH value (OR=2.6, 95% CI=1.63–10.42; P=0.001) and abnormal vaginal cleanliness (OR=2.6, 95% CI=1.36–4.0; P= 0.004) increased as the histological stage progressed.

Conclusion: Vaginal microecology associates with HPV infection and the progression of cervical lesions. Detection of vaginal secretion may contribute to the development of targets for micro-environmental modulation with probiotics and the reduction of the incidence of cervical cancer.

KEYWORDS

vaginal microecology, human papillomavirus, cervical intraepithelial neoplasia, cervical cancer, probiotics

1 Introduction

Cervical cancer (CC) is the second most common malignant tumor in females worldwide, after breast cancer (1). Human papillomavirus (HPV) infection, especially persistent high-risk human papillomavirus (HR-HPV) infection, plays a crucial role in the occurrence of pre-invasive precursors and even cervical cancer. According to epidemiological researches, the lifetime risk of acquiring human HPV infection exceeds 70% in sexually active women, and the infection in a considerable proportion of them regresses within 2 years after spontaneous clearing by innate immune responses (2). Long-term retention of HPV may not be easily eliminated due to various factors such as the menopausal status, immune deficiency, multiple sexual partners, vaginal microecological abnormality, etc., leading to cervical dysplasia (3–5). Early detection and timely treatment of sustained HPV infection and cervical lesions timely may be one of the principal means to prevent further carcinogenesis.

In recent years, increasing attention has been paid to identifying risk factors for HPV infection and CIN. The vagina and cervix are the first lines of physical and immunological defense against foreign microorganisms, such as viruses and bacteria. Vaginal microecology is relevant to its anatomical structure, local vaginal flora and its metabolites, immunological factors, and even endocrine condition. Under normal circumstances, the microecological flora in the vagina is dominated by lactobacillus, which can produce lactic acid, bacteriocins and reactive oxygen species (ROS) (5). Once the vaginal microbiota disorders and the local immunity gets weakened, exogenous microorganisms invade the female reproductive tract, causing infectious and inflammatory processes and increasing the risk of genital tract diseases and even cancer. Clinically, we usually evaluate the vaginal microecology by testing the morphology and function of vaginal secretion, including pathogens (trichomonas, fungus and clue cell), vagina cleanness, vaginal PH, H_2O_2, leukocyte esterase and sialidase respectively. It has been proved previously that HPV infection and CIN may relate to a changed flora structure and environmental dysregulation (3). Aiming at exploring the relationship between vaginal microecology and HPV infection as well as CIN, this study provides a basis for regulating the vaginal microecological environment, preventing HPV infection, and hampering the development of cervical lesions.

2 Materials and methods

2.1 Study population

This retrospective study included 2,147 women who were transferred for the colposcopic examination as per ASCCP guidelines at the First Affiliated Hospital of Wenzhou Medical University between August 2021 and August 2022 (6). The inclusion criteria and exclusion criteria in this study were as follows. Inclusion criteria: (1) Women with a history of sexual life. (2) Women underwent a colposcopic examination. (3) No sexual activity within 3 days before sampling. (4) No vaginal irrigation or administration within 7 days before sampling. Exclusion criteria: (1) Pregnant or in the lactating period. (2) Women with a history of HPV preventive vaccination. (3) Women received treatment of HPV infection or cervical lesions in the past. The designing and reporting of this study followed STROBE guidelines. This study was approved by the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University, and all participants obtained informed consent.

2.2 Vaginal microecology testing

All participants were advised to restrain from sex for at least 3 days and required not to use vaginal medication or take vaginal treatment for 7 days before the examination. The secretion was collected from the vaginal wall by using a gynecological brush (Dirui Medical Technology Co., Ltd). The standardized specimen was observed under a manual microscope and tested by using a vaginal secretions analysis strip (Dirui Medical Technology Co., Ltd). According to the National Clinical Laboratory Operating Guidelines, pathogens(trichomonas, fungus and clue cell)-positive results, vaginal cleanness grade III~IV, vaginal PH>4.5, H_2O_2>2 μmol, sialidase>7 u/mL and leucocyte esterase>7 u/mL were indicators of abnormal results. As for repeated sampling, the worst detection result before medication was considered the final outcome. Diagnosis of vaginal microecology disorder was made when any item mentioned above was abnormal.

2.3 HPV genotyping

Specimens for HPV genotyping were gathered from the endocervix by using a disposable sterile cervical brush and inoculated into a nucleic acid genotyping kit (Jiangsu Jianyou Medical Technology Co., Ltd.). The sample was subject to laboratory measurement including flow cytometry testing and fluorescence *in-situ* hybridization. Generally, there are 18 HR-HPV genotypes(i.e., HPV16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82) and 8 low-risk HPV (LR-HPV) genotypes (i.e., HPV6, 11, 40, 42, 44, 55, 61, and 83).

2.4 Cervical cytology

Specimens for cervical cytology were also collected from the endocervix by using disposable cervical sampler and stored in PreservCyt solution (Ningbo HLS Medical Products Co., Ltd.). Pathological slides were automatically made by a ThinPrep 2000 system (Beijing Hologic Technology Co. Ltd) and then reviewed by two experienced pathologists. The cytological diagnosis was established based on Bethesda criteria 2001. Specifically, the diagnostic results were classified into negative for no intra-epithelial lesion cells and malignancy (NILM), atypical squamous cells of undetermined significance (ASCUS), atypical squamous cells, exclude high-grade squamous epithelial lesion (ASC-H), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), atypical glandular cells (AGC), and carcinoma (CC), which included squamous cell carcinoma (SCC) and adenocarcinoma (AC) (7).

2.5 Colposcopy and histology

Indications and standards for colposcopy from the ASCCP guidelines were adopted in this study (8). The biopsy was performed after specimens were stained with pigment, and endocervical canal curettage (ECC) was an alternative if necessary. According to the 2014 WHO classification for cervical precancerous lesions, cervical tissues were pathologically divided into the following categories: with normal limits (WNL), LSIL, HSIL (CIN II/CIN III), and cervical cancer (9). Two independent histological experts in cervical histopathology were involved in the classification of the lesions. The highest grade of the cervical lesion was regarded as the final diagnosis if the results were different in the multipoint biopsy.

2.6 Statistical analysis

All statistical computations were performed using SPSS Version 20.0 (IBM Corp., Armonk, NY, USA). Continuous variables were presented with mean and standard deviation (SD), and categorical variables were indicated by numbers and percentages. Chi-square test, univariate and multivariate logistic regression analyses, and Cochran-Armitage trend test were performed to explore the correlation of vaginal microecology with HPV infection and CIN, being expressed with 95% confidence intervals (CI) and relative risk [odds ratio (OR)]. P-values (from two-sided tests) < 0.05 were considered to indicate statistical significance.

3 Results

3.1 Patient characteristics

Clinical characteristics of patients are described in Table 1. Almost all patients (n = 2097, 97.7%) had vaginal microecological disorders. Among them, 887 (41.3%) women tested abnormal for vaginal PH, 1817 (84.6%) women positive for H_2O_2, 231(10.8%) women positive for sialidase, 1950 (90.8%) women positive for leukocyte esterase, and 711 (33.1%) women grade III~IV for vagina cleanness. Moreover, trichomonas, fungus and clue cell were detected in 20 (0.9%), 50 (2.3%) and 191 (8.9%) women, respectively (Figure 1). Among 1774 (82.6%) patients infected with HPV, 1622 (91.4%) had HR-HPV, 709(40.0%) had HPV 16/18, and 41 (24.9%) were infected with multiple genotypes (Figure 2A). Less than half of the patients (n=978, 45.6%) had cytological abnormalities, among whom ASC-US, LSIL, ASC-H, HSIL, and AGC accounted for 24.2% (n=520), 13.0% (n=280), 3.4% (n=72), 4.3% (n=92) and 0.7% (n=14), respectively. After being biopsied under colposcopy, 1415 patients (65.9%) were pathologically confirmed to have cervical lesions, and the majority of them were LSIL (n=1052, 49.0%) (Figure 2B).

3.2 Association between vaginal microecology and HPV infection

Based on the results of HPV genotyping, the cohort was divided into HPV-positive group (n=1774) and HPV-negative group (n=355). Univariate logistic regression analysis showed that 1,742 (98.3%) patients had vaginal microecology disorder in the HPV-positive group, which was observably higher than that in the HPV-negative group (94.9%) (χ^2=14.5, P<0.001; OR=3.00, 95% CI=1.66-5.43). Further multivariate logistic regression analysis demonstrated that clue cell (OR=1.59, 95% CI=0.99–2.54; P=0.049) and sialidase (OR=1.54, 95% CI=1.01–2.35; P=0.046) were significant risk factors for HPV infection after confounding factors such as age and cytological results were controlled (Tables 2, 3). To explore the relationships between vaginal microecology and different patterns of HPV infection, HPV-positive individuals (n=1774) were further stratified into 3 pairs of two mutually exclusive groups, namely, (1.1) infection with HR-HPV (n=1622) and (1.2) infection with LR-HPV (n =152); (2.1) infection with multi-type HPV (n=441) and (2.2) infection with single-type HPV (n= 1333); (3.1) infection with HPV 16/18 (n=709) and (3.2) infection with other HPV genotypes (n=1065). The analysis results showed that HPV 16/18 increased the incidence of vaginal microecology disorder by over nine folds (OR=9.96, 95% CI=2.37–41.80; P=0.002). Nevertheless, there were no obvious differences in vaginal microecological environment between subgroups with HR-HPV and LR-HPV patients(P=1.00),

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TABLE 1 Clinical characteristics.

	group	x ± s/n, n(%)
Age		44.6 ± 10.9
PH value	≤4.5	1260 (58.7)
	>4.5	887 (41.3)
H_2O_2	negative	330 (15.4)
	positive	1817 (84.6)
Sialidase	negative	1916 (89.2)
	positive	231 (10.8)
Leukocyte esterase	negative	197(9.2)
	positive	1950 (90.8)
Vagina cleanness	I~II	1435 (66.8)
	III~IV	711 (33.1)
	missing	1 (0.1)
Trichomonas	no	2127 (99.1)
	yes	20 (0.9)
Fungus	no	2097 (97.7)
	yes	50 (2.3)
Clue cell	no	1956 (91.1)
	yes	191 (8.9)
HPV	negative	355 (16.5)
	positive	1774 (82.6)
	missing	18 (0.8)
HPV infection	LR-HPV	152 (8.6)
	HR-HPV	1622 (91.4)
HPV infection	HPV16/18	709 (40.0)
	Non-HPV16/18	1065 (60.0)
HPV infection	single infection	1333 (75.1)
	multiple infection	441 (24.9)
Cervical cytology	NILM	1134 (52.8)
	ASC-US	520 (24.2)
	LSIL	280 (13.0)
	ASC-H	72 (3.4)
	HSIL	92 (4.3)
	AGC	14 (0.7)
	missing	35 (1.6)
Colposcopic biopsy	WNL	691 (32.2)
	LSIL	1052 (49.0)
	HSIL	324 (15.1)
	CC	39 (1.8)
	Missing	41 (1.9)

and between subgroups infected with multi-type HPV and single-type HPV(P=0.589) (Table 4).

3.3 Association between vaginal microecology and cervical lesions

Cochran-Armitage trend test showed that there was no significant correlation between the incidence of vaginal microecology disorder and the severity of the cervical lesion confirmed by histological pathology (P=0.279). However, the latter was roughly proportional to the proportions of patients with abnormal vaginal cleanness (Z=-3.31, P<0.001) and PH value (Z=-3.22, P=0.001) (Table 5, Figure 3A). It was pertinent to note that the abnormal vaginal cleanness and PH value were detected in all patients diagnosed with cervical cancer (n=39). Further analysis indicated that high PH value (OR=5.46, 95% CI=2.55-11.68; P<0.001) and abnormal vaginal cleanness (OR=2.60, 95% CI=1.36-4.00; P=0.004) of vaginal discharge were significantly more likely to be detected in patients with cervical cancer, especially in comparison with the general female population. Females with cervical cancer tended to have a cleanness grade of IV (Figure 3B). However, the chi-square test of vaginal micro-environmental factors and cytological outcomes did not identify any particular association between them (P>0.05).

4 Discussion

Cervical cancer is acknowledged the most common malignant tumor in the female reproductive tract. The persistent infection with HR-HPV, especially the HPV16/18, is considered to be associated with developing cervical lesions and their recurrence after treatment (10–12). Increasing evidence suggests that local cervicovaginal factors may relate to HPV infection and following cervical lesions to a great extent. In-depth exploration of the human microecology system demonstrates that vaginal flora and its metabolites may play a vital role in maintaining the stability of the vaginal microecological environment. In this retrospective study, vaginal micro-environmental factors were found to associate with HPV infection and the development of the cervical lesions.

Bacterial vaginosis (BV), trichomonas vaginitis (TV), and vulvovaginal candidiasis (VVC) are the most common vaginal infections, which are proven related to HPV infection in previous studies (3). The research by Wang et al. (13) that enrolled 4,449 women revealed that BV and TV were closely associated with HR-HPV infection (P<0.05). Another retrospective analysis further illustrated a statistically significant difference in the proportion of BV among subgroups with HPV16/18 and non-HPV16/18 (P<0.05) (4). Nevertheless, some studies indicated that VVC did not increase the risk of HPV infection. According to their speculation, candida infection might strongly boost the immune response by promoting T cell proliferation, but the specific mechanism was still incompletely understood (14). Our results suggested that the maladjustment of vaginal microecology, especially the

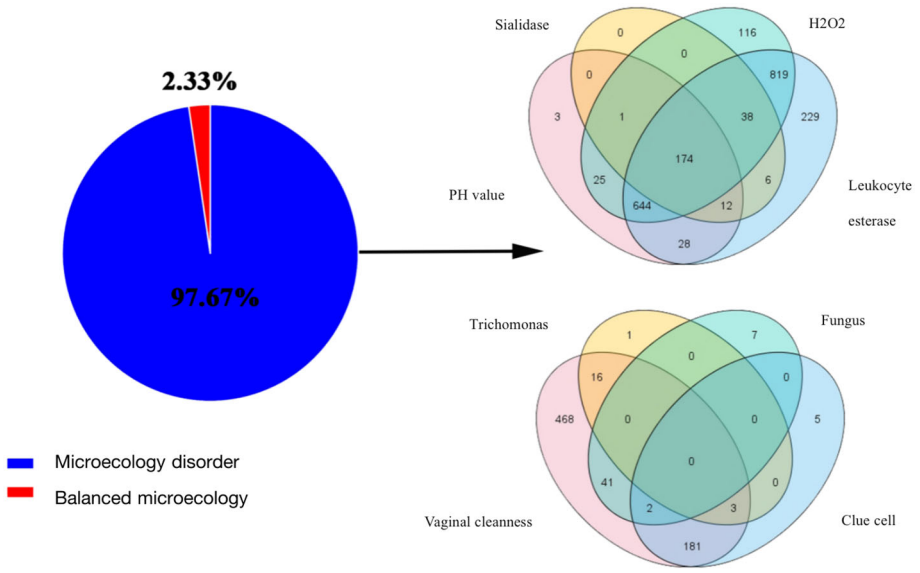

FIGURE 1
Results of vaginal microecology test. The pie chart showed the proportion of vaginal microecology disorder; The venn diagrams represented the shared and unique taxa from the perspective of function (PH, H_2O_2, sialidase, and leukocyte esterase) and morphology (vaginal cleanness, trichomonas, fungus and clue cell).

abnormalities in clue cell and sialidase, possibly, led to HPV infection, particularly HPV 16/18 genotypes. The detection rate of clue cell, one of the diagnostic indicators of BV, in HPV-positive patients was 0.51 times higher than that in HPV-negative patients. It validates that clue cell may serve as an indicator of viral infection, which is consistent with the conclusion in a previous study (15). Common recognized characteristics among women with BV and TV are the alteration of vaginal compositions, elevation of vaginal pH, and increase of bacterially produced metabolites such as sialidase, proteases, PLC and PLA_2, etc., which degrade the mucus secreted from the cervix and facilitates the HPV virus adhering to and breaching the protective epithelial barrier (16). In addition, sialidase participates in the regulation of the innate immunity of the host and thus increases the susceptibility of HPV (17). Nevertheless, the specific relationship between HPV16/18 and vaginal microecology remains unclear. Some researchers hold that the comparatively high viral load may matter, but further studies on its mechanism are required. In the vagina of HPV-positive women, the reproduction of lactobacillus is further inhibited, thus shifting the profile of microorganisms and ultimately leading to the imbalance of the vaginal micro-environment (18). Hence, we can reach the conclusion that there is a relationship between HPV infection and vaginal micro-environmental imbalance. With a knowledge that imbalanced vaginal environment favors HPV infection, we can understand the pathogenesis of this viral infection and seek alternative prophylaxis.

Emerging evidence emphasizes that the vaginal micro-environment varies in women with different precancerous diseases, and the evolution of CIN is correlated with the presence of BV, TV, and unstable vaginal PH. A recent systematic review and network meta-analysis reported that barely half of patients with CIN could be attributed to BV infection, whereas Candida albicans was not a causative agent of cervical lesions (19). The study by Mania-Pramanik et al. (20) indicated a significant association

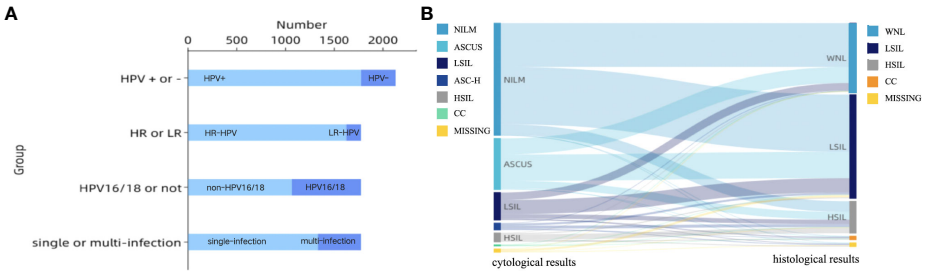

FIGURE 2
(A) Characteristics of HPV infection; HPV, human papillomavirus infection; HR-HPV, high-risk human papillomavirus infection; LR-HPV, low-risk human papillomavirus infection; **(B)** Sankey diagram of cervical lesions per- and post-colposcopy. The left column showed cytological results: NILM, ASCUS, LSIL, ASC-H, HSIL, CC, MISSING (n = 1134, 520, 280, 72, 92, 14, 35); The right column showed the histological results: WNL, LSIL, HSIL, CC, MISSING (n = 691, 1052, 324, 39, 41).

TABLE 2 Association between vaginal microecology factors and HPV infection.

Factors		HPV status/n, (n%)		X^2 [a]	P [a]	OR [a]	95%CI [a]
		HPV positive	HPV negative				
Vaginal microecology disorder [b]		1742 (98.3)	337 (94.9)	14.51	<0.001*	3.00	1.66-5.43
Morphological Evaluation	Vaginal cleanness [c]	597 (33.7)	110 (31.0)	0.96	0.327	1.13	0.88-1.45
	Trichomonas	17 (1.0)	3 (0.8)	0.05	0.812	1.14	0.33-3.90
	Fungus	40 (2.3)	9 (2.5)	0.10	0.748	0.89	0.43-1.84
	Clue cell	168 (9.5)	23 (6.5)	3.24	0.072	1.51	0.96-2.37
Functional Evaluation	PH	741 (41.8)	140 (39.4)	0.66	0.415	1.10	0.87-1.39
	H_2O_2	1505 (84.8)	295 (83.1)	0.68	0.408	1.14	0.84-1.55
	Sialidase	202 (11.4)	29 (8.2)	3.17	0.075	1.45	0.96-2.17
	Leukocyte esterase	1621 (91.4)	315 (88.7)	2.51	0.113	1.35	0.93-1.95

[a]X^2, P were calculated by Chi-square test; OR and 95%CI were calculated by univariate logistic regression analysis.
[b]The diagnosis of microecology disorder was made if any factor above was abnormal.
[c]Vaginal cleanness I~II were defined as normal and III~IV were defined as abnormal.
*P < 0.05.

TABLE 3 Factors associated with vaginal microecology disorder in HPV-positive woman.

	P [a]	OR [a]	95%CI [a]
Clue cell	0.049 *	1.59	1.66-5.43
Sialidase	0.046 *	1.54	1.01–2.35

[a]P, OR and 95%CI were calculated by multivariate logistic regression analysis. *P < 0.05.

between vaginal PH value and cervical dysplasia, and women with vaginal PH>4.5 were predisposed to cervical diseases. In our study, the severity of histological lesions was strongly relevant to abnormal PH value and vaginal cleanness. Lactobacillus inhibit colonization of BV-related bacterial species through maintenance of the acidic environment and production of corresponding bacteriocins (15). Hence, imbalanced PH value can induce growth of BV-associated taxa and potential pathogens such as Chlamydia trachomatis, Chlamydia trachomatis and Gardnerella vaginitis, leading to the inflammatory syndrome which is well-documented to promote the development of HPV infection and cervical neoplasia. Besides, abnormal vagina cleanness greatly increase the probability of BV and aerobic vaginitis (AV), finally participating in the pathogenesis of HPV infection (21). The above-mentioned indicators serve as both meaningful clues to vaginitis diagnosis and latent predictors for cervical lesions, and also provide reference for the development of adjuvant therapy with probiotics.

The present study is endowed with a few limitations. First, the uniformity among participants adversely affects the popularity of the research. Second, vaginal microecology can be dynamically modulated, and imperfect acquaintance with some exogenous factors (i.e., contraception, sexual intercourse, hygiene practices, etc.) lead to incomprehensive interpretation of the data. In general, we concluded that the presence of clue cell and sialidase are risk factors for HPV infection, and abnormal PH value and vaginal

TABLE 4 Association between vaginal microecology factors and different patterns of HPV infection.

	Vaginal microecology disorder [b] (n, n%)	P [a]	OR [a]	95%CI [a]
HPV positive	1742 (98.3)	<0.001 *	3.00	1.16-5.43
HPV negative	337 (94.9)			
HPV16/18	707 (99.7)	0.002 *	9.96	2.37-41.80
Non-HPV16/18	1035 (97.2)			
HR-HPV	1593 (98.2)	1.00	1.15	0.34-3.81
LR-HPV	149 (98.0)			
Single infection	1310 (98.3)	0.589	0.81	0.37-1.30
Multiple infection	432 (98.0)			

[a]P, OR, and 95%CI were calculated by univariate logistic regression analysis.
[b]The diagnosis of microecology disorder was made if any factor above was abnormal.
*P < 0.05.

TABLE 5 Association between cervical histology and vaginal microecology factors.

Factors		Cervical histology/n, (n%)				Z [a]	P [a]
		WNL	LSIL	HSIL	CC		
Vaginal microecology disorder [b]		676 (97.8)	1019 (97.0)	322 (99.4)	39 (100.0)	-1.08	0.279
Morphological Evaluation	Vaginal cleanness [c]	199 (28.8)	363 (34.5)	116 (35.8)	20 (51.3)	-3.34	<0.001 **
	Trichomonas	8 (1.2)	6 (0.6)	5 (1.5)	0 (0.0)	0.12	0.902
	Fungus	15 (2.2)	21 (2.0)	12 (3.7)	1 (2.6)	-1.13	0.259
	Clue cell	53 (7.7)	105 (10.0)	27 (8.3)	5 (12.8)	-1.05	0.296
Functional Evaluation	PH	262 (37.9)	433 (41.2)	137 (42.3)	30 (76.9)	-3.22	0.001 *
	H_2O_2	576 (83.4)	896 (85.2)	271 (83.6)	35 (89.7)	-0.74	0.461
	Sialidase	70 (10.1)	123 (11.7)	30 (9.3)	5 (12.8)	-0.12	0.902
	Leukocyte esterase	628 (90.9)	950 (90.3)	297 (91.7)	36 (92.3)	-0.33	0.74

[a]Z and P were calculated by Cochran-Armitage trend test.
[b]The diagnosis of microecology disorder was made if any factor above was abnormal.
[c]Vaginal cleanness I~II were defined as normal and III~IV were defined as abnormal.
*P < 0.05 **P < 0.001.

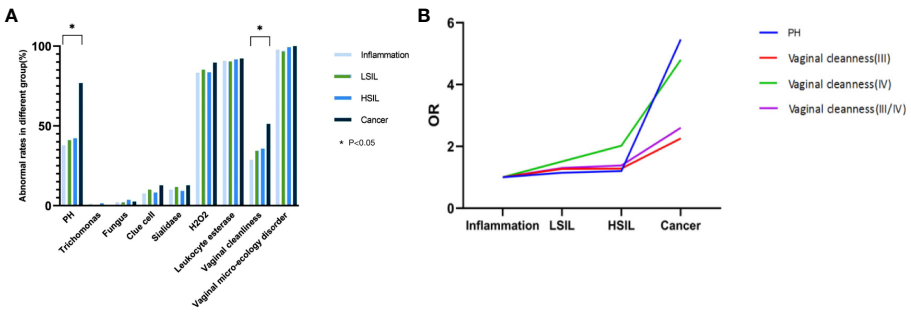

FIGURE 3
(A) Association between vaginal micro-ecology and cervical lesions (histology), *P < 0.05; **(B)** Trend of OR of vaginal cleanness and PH value with the progression of cervical histology, *P < 0.05.

cleanness may be associated with the severity of precancerous lesions. Thus, indicators of vaginal discharge can be useful microbiological predictors of HPV infection and cervical diseases in some women. Furthermore, detection of vaginal secretion may be able to help the development of targets for micro-environmental modulation with probiotics. Well-powered dense-sampling longitudinal cohorts studies are required in the further to assess the implication of regulated vaginal microecology on HPV infection, cervical lesions and even disease recurrence, thus promoting risk stratification and helping with clinical decision making.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

LZ: Writing – review & editing. RF: Writing – original draft. YTH: Supervision, Writing – review & editing. JH: Data curation, Writing – review & editing. YH: Methodology, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1306376/full#supplementary-material

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Chemical immune conization of precancerous cervical lesions awakens immune cells and restores normal HPV negative and abnormal proliferation

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Background: Cervical cancer is one of the most common and deadly cancers in women, which is closely linked to the persistent infection of high-risk human papillomavirus (HPV). Current treatment of cervical cancer involves radical hysterectomy, radiotherapy, and chemotherapy or a combination.

Objective: We investigated if hapten-enhanced intratumoral chemotherapy (HEIC) was effective in boosting immunity for effective treatment of precancerous cervical lesions and HPV infection.

Study design: We used single-cell RNA sequencing (scRNA-Seq) to obtain transcriptome profiles of 40,239 cells from biopsies of precancerous cervical lesions from the cervix directly from one patient before the start of HEIC and approximately 1 week after HEIC. The blood samples were taken at the same time as biopsies. We compared the expression characteristics of malignant epithelial cells and immune cells, including epithelial cells, endothelial cells (ECs), fibroblasts, mural cells, T cells, B cells, T and NK neutrophils, mast cells, microparticles (MPs), and platelets, as well as the dynamic changes in cell percentage and cell subtype heterogeneity.

Results: Intratumoral injection of chemotherapy drug plus hapten induces an acute immune response in precancerous cervical lesions with HPV and further awakens immune cells to prevent the abnormal proliferation of the precancerous cells.

Conclusion: HEIC provides a potential treatment method for cervical cancer and HPV infection tailored to each patient's condition.

KEYWORDS

cervical cancer, intratumoral chemotherapy, biopsy, H_2O_2, penicillin, single-cell RNA sequencing, differentially expressed genes, single-cell copy number variation

Introduction

Cervical cancer is one of the most common cancers threatening women's health, which is closely linked to the persistent infection of high-risk human papillomavirus (HPV) (1, 2). The correlation between high-risk HPV infection and precancerous lesions and cervical cancer is extremely high and can be largely preventable. Approximately 90% of cervical cancer occurs in low-income and middle-income countries due to the lack of organized screening and HPV vaccination programs in these countries. In high-income countries, the implementation of screening and vaccination has reduced the incidence rate and mortality of cervical cancer by more than half in the past 30 years.

Treatment of cervical cancer depends on the severity of the disease at the time of diagnosis and the availability of local resources, which may include radical hysterectomy, radiotherapy, and chemotherapy, or a combination of radiotherapy and chemotherapy, which has become a standard of treatment (2). Current treatment of precancerous cervical lesions (cervical intraepithelial neoplasia (CIN)) is very effective, simple, and safe. The entire conversion area of the cervix can be treated through ablation techniques (cryotherapy or thermal ablation) or resection techniques (large ring resection, cold knife conization, or conization). The choice of treatment methods depends on the size and location of the lesion, as well as the type of transformation zone (3).

Due to the lack of intervention in the form of immunotherapy, local recurrence after treatment and persistent HPV positivity are still unresolved issues. The persistence of HPV infection in patients with a high-grade squamous intraepithelial lesion (HSIL) undergoing cervical excision is strongly associated with the recurrence (4).

Inosine pranobex immunotherapy can significantly increase the clearance of viral infection with high-risk genotypes and reduce relapse of HSIL for HPV-positive patients after cervical conization (5). There are several types of immune-related drugs that have been attempted to activate the immune system to improve treatment outcomes. These include immune checkpoint inhibitors, therapeutic vaccines, engineered T cells, and antibody–drug conjugates. Checkpoint inhibitors appear to be the best treatment methods for research, with encouraging Phase II studies in established environments. Vaccines and engineered T cells that use unique immune activation mechanisms are still in the early stages of development (6, 7).

In the current study, we aimed to determine if hapten-enhanced intratumoral chemotherapy (HEIC) was effective in treating precancerous cervical lesions. We hypothesized that HEIC can induce an acute immune response to control both CIN and turning the HPV to negative, a process termed as chemical conization of precancerous cervical lesions. HEIC is used for treating several cancers by hapten-modified tumor-associated antigens or oncogenic proteins expressed in HPV-associated premalignant cervical epithelium (8–10). We carried out biopsies of precancerous cervical lesions using forceps at 12, 3, 6, and 9 o'clock of the cervix directly from one patient before the start of HEIC and approximately 1 week after HEIC. We also took blood samples from the patient before and after HEIC. We then used single-cell RNA sequencing (scRNA-Seq) to obtain transcriptome profiles of 40,239 cells. Through comparative analysis of different samples of CIN and blood samples, we comprehensively described the expression characteristics of malignant epithelial cells and immune cells, including Epithelial Cells, Ecs, Fibroblasts, Mural Cells, Tcells, Bcells, TandNK Neutrophils, Mast Cells, MPs, and Platelets, as well as the dynamic changes in cell percentage and cell subtype heterogeneity. Our results provide evidence that intratumoral co-administration of HEIC induced acute immune response in precancerous cervical lesions to prevent their abnormal proliferation.

Materials and methods

Clinical specimens

The patient had a pathological diagnosis and was diagnosed with a clinical stage of HPV-positive cervical intraepithelial neoplasia, CIN 3, in the cervix using the traditional HPV test (7, 8). The patient did not have any other therapy before this study. Before receiving treatment at Beijing Baofa Cancer Hospital, the patient's physical condition was evaluated and determined to meet the indications for HEIC. This experimental treatment was approved by the hospital ethics committee (TMBF 0010, 2015) in accordance with relevant guidelines and regulations.

After the patient has been prepared for biopsies and blood samples were collected, cleaning and disinfection of the perineum and vagina under general anesthesia were performed, and a disinfecting towel was placed. When precancerous cervical lesions were seen, four small pieces of precancerous cervical lesions (2 mm × 2 mm × 3 mm) were taken at 12, 3, 6, and 9 o'clock of the cervix as an untreated sample for scRNA-Seq analysis. This was followed by intratumoral injection at 12, 3, 6, and 9 o'clock of the cervix of a total of 10 ml containing 1.00 mg/ml adriamycin (Adr), 0.80 mg/ml of cytarabine (Ara-C), 20.0 mg/ml of H_2O_2, and 144 mg/ml of penicillin as the hapten (9–11). One week post injection, blood samples were collected again as treated samples, and biopsies of precancerous cervical lesions were carried out again using forceps at 12, 3, 6, and 9 o'clock of the cervix, taking another four small pieces of precancerous cervical lesions (2 mm × 2 mm × 3 mm) as treated samples with blood samples for scRNA-Seq analysis.

Tissue disassociation and cell collection

After small cervical lesion tissues and blood samples were collected, the fresh tissue samples were immediately stored in the sCelLiVE® Tissue Preservation Solution (Singleron, Nanjing, China) on ice. The tissues were cut into small tissue pieces and were transferred to a 15-ml centrifuge tube, followed by digestion using sCelLiVE® Tissue Dissociation Solution (Singleron) at 37°C for 15 min with shaking. The samples were then filtered using 40-μm sterile strainers and centrifuged at 1,000 rpm at 4°C for 5 min. Next, 2 ml GEXSCOPE® red blood cell lysis bufier (RCLB, Singleron) was added to lyse the red blood cells for 10 min. Finally, the single-cell

suspension was collected after re-suspension with phosphate-buffered saline (PBS), and trypan blue (Sigma) staining was used to calculate cell activity and cell count under a microscope.

Single-cell RNA sequencing

Single-cell suspensions (1~3 × 10^5 cells/ml) in PBS (HyClone, Logan, UT, USA) were loaded onto a microwell chip using the Singleron Matrix® Single Cell Processing System. Briefly, the scRNA-Seq library was constructed using the GEXSCOPE® Single Cell RNA Library Kits (Singleron). The library was lastly sequenced with 150 bp diluted to 4 nM and paired-end reads on the Illumina HiSeq X platform following an established protocol (12). Sequencing data processing and quality control were performed as described in previous publications (13).

Differentially expressed gene analysis

To identify differentially expressed genes (DEGs), genes expressed in more than 10% of the cells were selected in both the compared groups of cells and with an average log (fold changes) value greater than 1 as DEGs. The adjusted p-value was calculated using the Benjamini–Hochberg correction. The p-value of 0.05 was used as the criterion to assess the statistical significance.

Cell type annotation

The cell type identity of each cluster was determined with the expression of canonical markers found in the DEGs using the SynEcoSys database (Singleron Biotechnologies). Heatmaps/dot plots/violin plots displaying the expression of markers used to identify each cell type were generated using the Scanpy built-in functions and ggplot2.

Single-cell copy number variation analysis

The InferCNV package was used to detect the copy number alterations (CANs) in malignant cells. Non-malignant cells (T and NK cells) were used as control references to estimate the copy number variations (CNVs) of malignant cells. Genes expressed in more than 20 cells were sorted based on their loci on each chromosome. The relative expression values were centered to 1, using a 1.5 standard deviation from the residual-normalized expression values as the ceiling. A slide window size of 101 genes was used to smoothen the relative expression on each chromosome to remove the effect of gene-specific expression.

Pathway enrichment analysis

To investigate the potential functions of DEGs between clusters, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed using the "clusterProfiler" R package 3.16.1 (14). The GO gene sets including molecular function (MF), biological process (BP), and cellular component (CC) categories were used as references. Pathways with an adjusted p-value less than 0.05 were considered as significantly enriched.

Trajectory analysis

Monocle 2 algorithm (15) was used for pseudo-time trajectory analysis, and the dimensionality reduction method used was DDRTree.

Intratumoral heterogeneity score calculation

The intratumoral heterogeneity (ITH) score was defined as the average Euclidean distance between the individual cells and all other cells in terms of the first 20 principal components derived from the normalized expression levels of highly variable genes. The highly variable gene was identified using the "FindVariableGenes" function in the Seurat package, with default parameters.

Cell–cell interaction analysis (CellPhoneDB)

Cell–cell interaction (CCI) between B cells, Epithelial cells, Fibroblasts, Mononuclear phagocytes, Mast cells, Neutrophils, and T and NK cells were predicted based on known ligand–receptor pairs by CellPhoneDB v2.1.0 (16–18).

Results

Clinical benefit characteristics

In biopsies taken post-treatment, pathological examination confirmed early diagnosis as cervical intraepithelial neoplasia CIN 3. Follow-up examination every 4 weeks after the treatment during a 6-month period, physical examination, and CT of the patient showed no signs of precancerous cervical lesions in the smooth surface of the cervix, and the patient was in good health living a normal life over 1.2 years, during which traditional HPV test continued to yield negative results.

Landscape of single-cell transcriptome sequencing before and after precancerous lesion treatment

Single-cell transcriptome sequencing was performed on two cervical epithelial tissues and their paired peripheral blood

mononuclear cell (PBMC) samples before and after treatment. After dimension reduction and clustering, 10 cell types were obtained (Figure 1A), including nine in tissues and seven in the blood (Figure 1B). Cell types were annotated according to marker genes (Figure 1C) of each cell, including EpithelialCells, Ecs, Fibroblasts, MuralCells, Bcells, TandNK Neutrophils, MastCells, MPs, and Platelets.

Analysis of immune cells such as Bcells and TandNK increased in both PBMCs and cervical tissues, while the proportion of stromal cells such as Ecs, Fibroclasts, and MuralCells decreased in tissues before and after treatment. In addition, the proportion of Neutrophils cells in the blood significantly decreased (Figure 1D).

Changes in T cells before and after treatment of precancerous cervical lesions

Subdividing the T-cell subpopulations yielded a total of five cell types (Figure 2A). These cell types were annotated based on the marker genes (Figure 2B), including CD8Teff (CD8+ effector T cells), NK (natural killer cells), NaiveT (initial T cells), Tfh (follicular helper T cells), and Treg (regulatory T cells).

Analysis of the proportion of cells before and after treatment showed that after HEIC treatment, the proportion of Tfh cells in the tissue significantly increased. The changes in PBMC samples were not significant (Figure 2C).

The increase in follicular helper T cells suggests that treatment of HEIC may stimulate the immune system response and may help combat the development of precancerous cervical lesions. Follicular helper T cells are a specific type of immune cell that can help other immune cells produce stronger immune responses, thereby improving their ability to fight cancer.

Subdivision of Tfh cell subpopulations resulted in four cell types (Figure 2D). Analysis of the proportion of each cell before treatment showed that Tfh1 cells were the dominant group, while the richness of Tfh cell subpopulations increased, such as the addition of Tfh3 subpopulations (Figure 2E).

The functions of follicular helper T cell subsets show heterogeneity (Figure 2F); for example, Tfh1 significantly enriches the phagocytic pathway; Tfh2 significantly enriches the glycine, serine, and threonine metabolic pathways; Tfh3 significantly enhances the biosynthesis and metabolism-related pathways of biomacromolecules such as sugar, amino acid, and vitamin; and Tfh4 significantly stimulates the signal pathway of stem cell pluripotency.

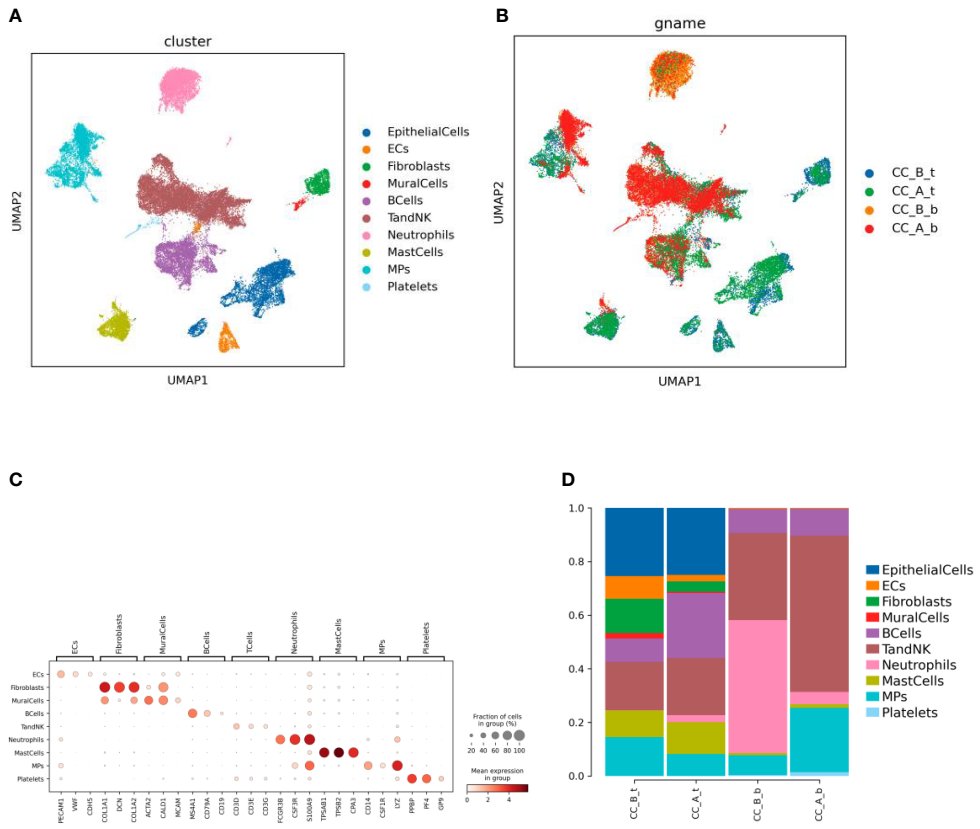

FIGURE 1
Chemical conization enhances immunotherapy for cervical cancer. **(A)** By using dimensionality reduction clustering to form UMAP cell clustering maps, a total of 10 cell types were obtained, with different colors representing different cell types. **(B)** he distribution of various cell types in tissues and blood before and after treatment, with different colors representing different samples **(C)** Marker gene bubble diagram for each cell type. **(D)** Histogram of cell proportion of each cell type before and after treatment.

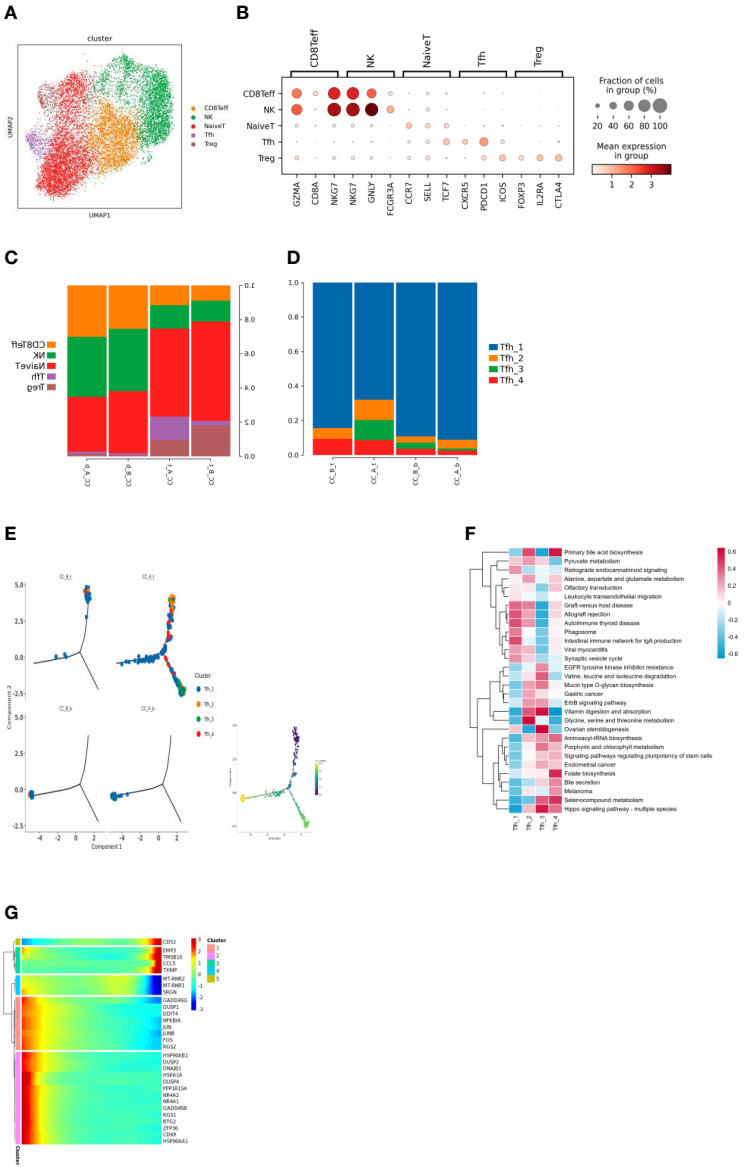

FIGURE 2
(A) T cells were clustered through dimensionality reduction to form UMAP cell clustering maps, obtaining a total of 5 cell types, with different colors representing different cell types. **(B)** Bubble plots of Marker genes in various T cell subpopulations, with color depth representing the average expression of the gene, and circle size representing the percentage of cell expression of the gene in the cell. **(C)** Histogram of the proportion of T cell subpopulations in each sample. **(D)** Histogram of cell proportion of Tfh cell subpopulations in each sample. **(E)** A pseudo time distribution map of a single sample, where one point represents one cell and different colors represent different cell types. **(F)** GSVA analysis of Tfh cell subpopulations, the redder the color, the more enriched the pathway is in the cell. **(G)** Heat map of gene expression changes during the simulated time process, with colors ranging from blue to red representing gene expression from low to high.

Time series analysis shows T-cell changes in precancerous cervical lesions before and after treatment

There were differences in the differentiation of Tfh cells in tissues before and after treatment. Before treatment, the cells in the sample were overall located in the early stage of the trajectory. After treatment, the cells were overall located in the early, middle, and late stages (Figure 3I).

Before treatment, the main cells present in the tissue sample were Tfh1 cells located at the beginning of differentiation. After treatment, the cells at the beginning of differentiation in the sample were mainly Tfh1. As differentiation progressed, Tfh1 differentiated in two directions: 1) Tfh3 and Tfh4 cells and 2) Tfh1 cells in a differentiated state (Figures 2G–K). Changes in gene expression were observed during the differentiation of Tfh1 into Tfh3 and Tfh4 cells (Figure 2J): high expression of MT-RNR2, MT-RNR1, and SRGN genes in the middle stage of differentiation. High expression of MT-RNR2 and MT-RNR1 genes indicated that cells were in a differentiation transition state with strong metabolic ability, which changes in gene expression during Tfh1 cell differentiation toward the end of Tfh1 differentiation (Figure 2J).

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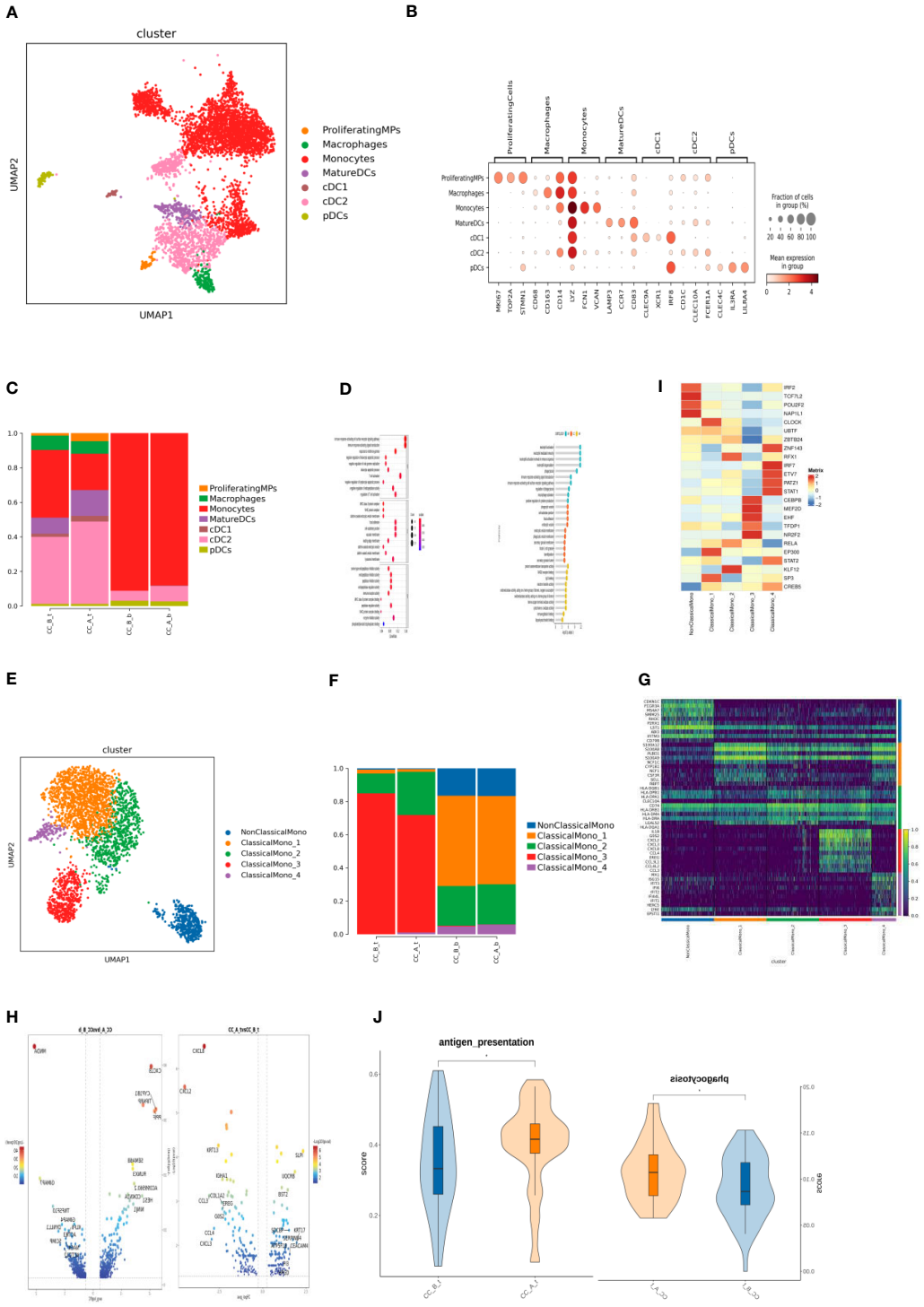

FIGURE 3
(A) MPs cells formed UMAP cell clustering maps through dimensionality reduction clustering, obtaining a total of 7 cell types, with different colors representing different cell types. **(B)** Bubble plots of Marker genes in various MPs cell subpopulations, with color depth representing the average expression of the gene, and circle size representing the percentage of cell expression of the gene in the cell. **(C)** Histogram of cell proportion of MPs cell subpopulations in each sample. **(D)** Bubble diagram (left) and lollipop diagram (right) of GO enrichment analysis results of differentially up-regulated genes of MatureDC cells (left) and cDC2 cells (right) between groups (CC_A_t vs CC_B_t). **(E)** Monocytes cells were clustered through dimensionality reduction to form a UMAP cell clustering map, obtaining a total of 5 subpopulations, with different colors representing different cell types. **(F)** Histogram of the cell proportion of Monocytes cell subpopulations in each sample. **(G)** Top10 differential gene heatmap of Monocytes cell subpopulations. **(H)** Differential gene analysis of ClassicaMono-2 cells between groups, with the left being in the tissue (CC_A_t vs CC_B_t) and the right being in the PBMC sample (CC_A-b vs CC_B-b). **(I)** Transcription factor analysis of various cell subpopulations in Monocytes. **(J)** Antigen presenting ability and cell phagocytosis related gene set scores in tissue samples before and after treatment with ClassicaMono-2 subgroup.

Changes of MP cells in precancerous cervical lesions before and after treatment

By subdividing T-cell subsets, seven cell types were obtained (Figure 3A). According to the marker gene (Figure 3B) of each cell, they include proliferating monocyte phagocyte, monocytes, macrophages, mature dendritic cells, type 1 classic dendritic cells (cDC1), type 2 classic dendritic cells (cDC2), and plasma cell-derived dendritic cells (pDCs).

Analysis of the proportion of cells after drug treatment showed that the monocytes in the tissue significantly decreased, while the proportion of cells in MatureDCs and cDC2 increased; the changes in PBMC samples were not significant (Figure 3C).

Analysis of intergroup differences in tissues showed that MatureDCs upregulated T-cell activation; MHC II protein complexes, negative regulation of white blood cell apoptosis γ-interferon response, and other related pathways (Figure 3D) suggest that MatureDCs can activate and enhance the immune response ability of T cells.

cDC2 upregulates neutrophil activation, macrophage activation, phagocytosis, cytokine production, and RAGE receptor binding-related pathways (Figure 3E), suggesting that cDC2 may regulate the activation status of neutrophils, macrophages, and other immune cells, thus participating in the immune response process cooperatively. Five cell types were obtained by subdividing the monocyte cell subpopulations (Figure 3F): non-classical monocytes (NonClassicalMono), ClassicalMono_1, ClassicalMono_2, ClassicalMono_3, and ClassicalMono_4.

Analysis of the proportion of cells found that the dominant cell populations in the tissue before treatment were ClassicalMono_2 and ClassicalMono_3, and the proportion of ClassicalMono_3 cells significantly decreased after treatment. In PBMCs, there was little change in cell composition before and after treatment, and the dominant cell population was ClassicalMono_1, which contained more non-classical monocytes than in tissues (Figure 3G), indicating heterogeneity between PBMCs and monocytes in tissues.

In tissues and PBMC samples, inflammatory chemokines such as CXCL8, CXCL2, CCL3, CCL4, and CXCL3 were downregulated in the ClassicalMono_2 subgroup, especially CXCL8 (Figure 3I), indicating that the inflammatory response may weaken after treatment. The RFX1 transcription factor is specifically overexpressed in the ClassicalMono_2 subgroup (Figure 3J).

The ClassicalMono_2 subgroup highly expresses the MHC class II gene (Figure 3H). RFX1 transcription factor may regulate the high expression of the MHC class II gene in the ClassicalMono_2 subgroup, thereby enhancing its antigen presentation ability.

Changes in fibroblast group before and after treatment for precancerous cervical lesions

Subdividing the subpopulations of fibroblast cells gave rise to a total of four cell types (Figure 4A): Fibroblasts_LUM, Fibroblasts_POSTN, Fibroblasts_ACTA2, and Fibroblasts_IGFBP2 cell subpopulations. Analysis of the proportion of each cell found that after drug treatment, fibroblasts were in the tissue. The proportion of IGFBP2 cells significantly increased. The proportion of cells in the ACTA2 subgroup significantly decreased (Figure 4B). Heterogeneity exists between various subpopulations of fibroblasts (Figure 4C).

Further analysis of Fibroblasts_Differential gene of IGFBP2 cells between groups found that fibroblasts after treatment of IGFBP2 cells overexpress multiple chemokines and interleukins IL24, IL19, and CCL8 (Figure 4E) and upregulate the receptor signaling pathway of JAK-STAT γ-interferon response and NIK/NF-κ inflammatory-related pathways such as B signaling pathway and type I interferon signaling pathway (Figure 4F).

Time series analysis showed that there were differences in the differentiation of fibroblasts in tissues before and after treatment. Before treatment, the cells in the sample were overall located in the early and middle stages of the trajectory, while after treatment, the cells were overall located in the middle and late stages (Figure 4I).

Before treatment, Fibroblasts_ACTA2 is in the early stage of differentiation, Fibroblasts_IGFBP2 is in the middle and late stages of differentiation, and after treatment, Fibroblasts_ACTA2 cell reduction and Fibroblasts_IGFBP2 increase (Figures 4G–H), indicating that fibroblasts after treatment ACTA2 may differentiate into Fibroblasts_IGFBP2 subgroup.

Further score the characteristic gene sets of adipose derived fibroblasts, inflammatory fibroblasts, myofibroblasts, and epidermal promoting fibroblasts for each subgroup of fibroblasts Figure 4G).

Changes in cellular communication before and after treatment of precancerous cervical lesions

Cell interaction analysis showed that the signal communication between cells decreased overall in the tissues before and after treatment (Figure 5A). Compared to that in PBMC samples before and after treatment, ClassicalMono_1 enhanced communication with other immune cells (Figure 5B). Cell interaction analysis in the tissues showed that before and after treatment, ClassicalMono_2/ClassicalMono_3, ClassicalMono_2/MatureDCs, ClassicalMono_2/cDC2, CCL3 between the above cells_CCR1, CCL3. The signal communication between the CCR5 receptor gene pairs is weakened (Figure 5C), which is similar to the previous results (Figure 3I). On the contrary, Fibroblasts_IGFBP2|Fibroblasts_ACTA2:IL24_NOTCH2 signal enhancement (Figure 5C), Fibroblasts (Figure 4E). Upregulation of IL24 expression was seen in IGFBP2 cells. Compared to that in PBMC samples before and after treatment, ClassicalMono_1|cDC2 and ClassicalMono_2|cDC2:LGALS9 enhanced signal communication between HAVCR2 (Figure 5D).

Discussion

In this study, we used scRNA-Seq, demonstrated that precancerous cervical lesions (CIN) can be treated with HEIC therapy, and provided evidence supporting that HEIC

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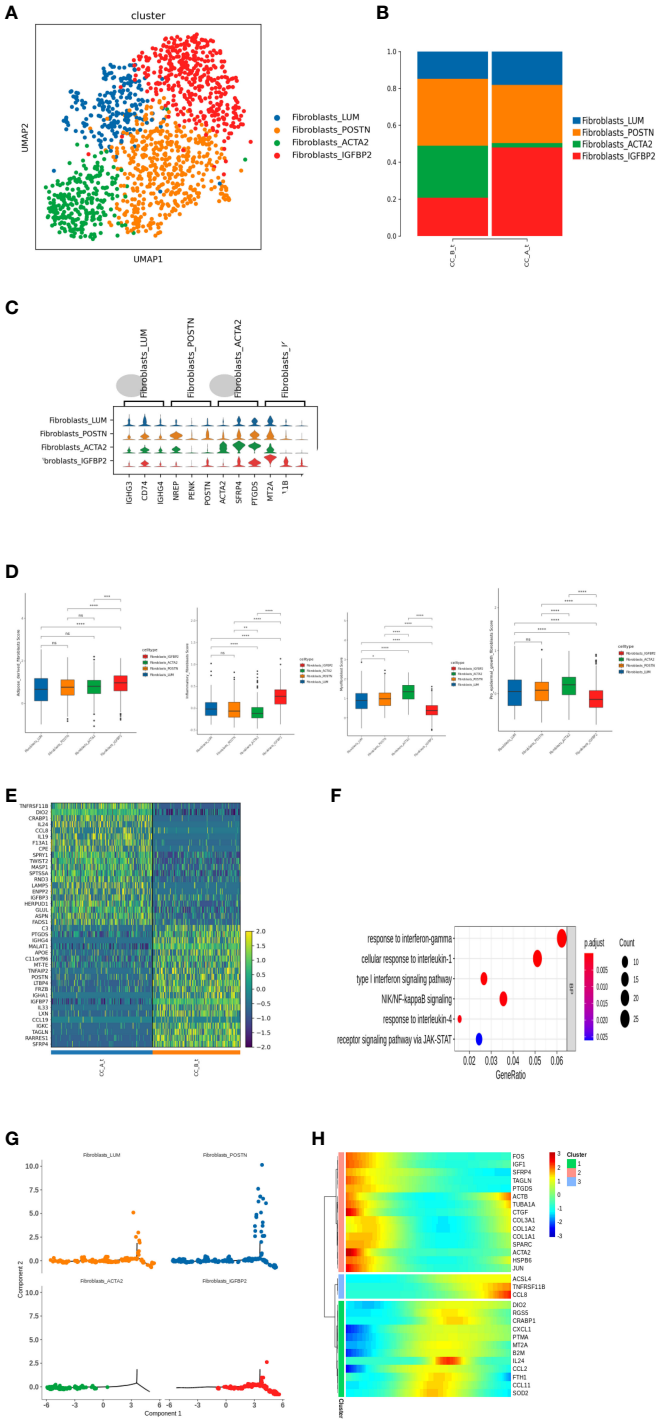

FIGURE 4
(A) Fibroblasts were clustered into UMAP cell clusters through dimensionality reduction, resulting in a total of 4 cell types, with different colors representing different cell types. **(B)** Histogram of cell proportion of fibroblast subpopulations in each sample. **(C)** Top3 differential gene violin map of fibroblast subpopulations. **(D)** The score results of the Ucell gene set of fibroblast subpopulations show the characteristics of adipogenic fibers, inflammatory fibers, myofibroblasts, and epidermal promoting fibroblasts. **(E)** Fibroblasts_ Differential gene expression heatmap of IGFBP2 cell subpopulations between groups. **(F)** Fibroblasts_ GO enrichment analysis of upregulated differentially expressed genes in IGFBP2 cell subpopulations between groups. **(G)** A pseudo time distribution map of a single sample, where one point represents a cell and different colors represent different cell types. The small image shows the pseudo time axis for fibroblast differentiation, and the dark color represents the starting point of differentiation. As the pseudo time progresses, the color from dark to light represents the differentiation from front to back. **(H)** Heat map of gene expression changes during the simulated time process, with colors ranging from blue to red representing gene expression from low to high.

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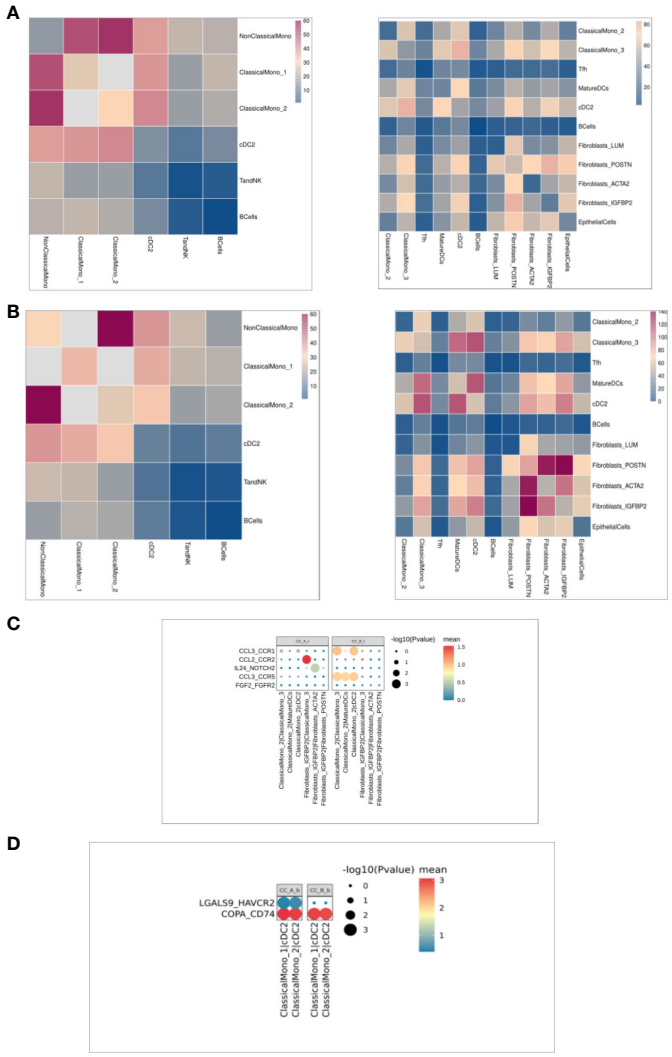

FIGURE 5
(A) The heat map of intercellular communication in the organization, with a redder color indicating stronger intercellular interactions. The left image shows before treatment and the right image shows after treatment. **(B)** The heat map of intercellular communication in PBMC shows that the redder the color, the stronger the intercellular interaction. The left image shows before treatment and the right image shows after treatment. **(C)**. Cellular communication bubble diagram in the organization, with cell pairs on the horizontal axis and ligand pairs on the vertical axis. After treatment on the left and before treatment on the right. **(D)** Cell communication bubble diagram in PBMC, with the horizontal axis representing the cell pair and the vertical axis representing the ligand pair. The left side represents after treatment and the right side represents before treatment.

precancerous cervical lesions with HPV infection induced acute immune response to control the CIN and turned the HPV negative.

Our results showed that cells such as Bcells and TandNK increased in both PBMCs and cervical tissues; the proportion of stromal cells such as Ecs, Fibroblasts, and MuralCells decreased in tissues before and after treatment; and the proportion of Neutrophils cells in the blood significantly decreased. The increase in immune cells may be due to the hapten with drugs killing precancerous cervical lesions with HPV and activating the immune cells, making it effective in activating the immune cells to attack diseased cells. The decrease in stromal cells may be due to the drug affecting the tissue structure and function around the affected cells. These changes may indicate that drug and hapten therapy have had a positive impact on the therapeutic efficacy of CIN 3, consistent in conjunction with clinical outcomes, which are HPV turning negative and cervical surface becoming smooth.

The significant clinical benefit is that one local therapy with hapten and chemotherapy drugs can kill local precancer and hapten modified with tumor-associated antigens, and the major oncogenic protein expressed in HPV-associated precancerous cervical lesions also induces an immune response to fight both precancerous cells and HPV virus.

We used scRNA-Seq and demonstrated that HEIC treatment induces the interaction among Epithelial Cells, Ecs, Fibroblasts, Mural Cells, Tcells, Bcells, TandNK Neutrophils, Mast Cells, MPs, and Platelets, promoting the expression of many genes contributing to the upregulation of immune response in precancerous cervical lesions. Since it is the first attempt to treat precancer positive for HPV, we will need a larger sample size to prove its effectiveness (19).

Detailed subdivision of Tfh cell subpopulations resulted in four cell types with Tfh1 cell in the dominant group enriching the

phagocytic pathway while Tfh cell subpopulations increased, as well as the function of follicular T-cell subsets in heterogeneity. This is consistent with the proposed roles for phagocytosis in the degradation of foreign pathogens or cell wastes, which play an important role in immune defense and metabolic regulation (20).

Our results also showed increased expression of CD52, EMP3, TMSB10, CCL5, and TXNIP in the late stage of differentiation; CD52, mainly highly expressed in B cells and T cells, which is an important immune regulatory factor for T-cell activation (21). Overexpression of CD52 leads to increased infiltration of M1 macrophages, monocytes, T-follicle helper cells, and resting memory CD4T cells. CCL5 gene encodes a chemokine ligand 5, which can promote the chemotaxis and aggregation of monocytes, eosinophils, basophils, T cells, natural killer cells, and other immune cells, thus participating in the regulation of immune response and the mediation of inflammatory response (22). TXNIP is thioredoxin that can bind to reactive oxygen species (ROS), avoiding ROS damage to cells and protecting them from oxidative stress (23, 24).

Our analysis reveals that monocytes in the cervical tissue significantly decreased, while the MatureDCs and cDC2 increased in PBMCs. MatureDCs upregulated T-cell activation, MHC II complexes, negative regulation of white blood cell apoptosis γ-interferon response, and other related pathways, suggesting that it enhanced the immune response ability of T cells (21) as well as the γ-interferon response pathway (12, 25).

ClassicalMono_2 and ClassicalMono_3 were the dominant cells in the cervical tissue before treatment, while the ClassicalMono_3 cells significantly decreased after treatment. ClassicalMono_3 overexpresses chemokines (CXCL2, CXCL3, CXCL8, CCL4, CCL3L1, CCL4L2, and CCL3), while ClassicalMono_4 overexpresses ISG56/IFIT1 family genes (ISG15, IFIT3, IFI6, IFIT2, IFI44L, and IFIT1); these genes, stimulated by interferon, play multiple regulatory roles in antiviral immunity and interferon signaling pathways (26).

Treatment decreased inflammatory chemokines such as CXCL2, CCL3, CCL4, and CXCL3, especially CXCL8, in the ClassicalMono_2 subgroup, while the RFX1 (Regulatory Factor X1) transcription factor is specifically overexpressed in the ClassicalMono_2 subgroup. Some target genes of RFX1 are known to include MHC class II genes, which encode important antigen-presenting molecules in the immune system and participate in processes such as antibody-mediated immune responses (27), which is not only for cancer immune reaction but also for HPV immune reaction. Topical application of 2,4-dinitrochlorobenzene (DNCB) as hapten was employed in the immunotherapy of HPV-associated lesions. It was previously found that hapten of DNCB treats skin expressing HPV16. E7 protein, the major oncogenic protein expressed in HPV-associated premalignant cervical epithelium, results in a hyperinflammatory response, with an associated induction of Th2 cytokines and infiltration of myeloid cells producing arginase-1, which also contributes to the hyperinflammation (11).

How does this treatment compare to laparoscopic radical hysterectomy and open approach? Due to laparoscopic radical hysterectomy and open approach treatment of precancerous cervical lesions, the positive endometrial margin is a major risk factor for predicting 5-year recurrence (19). A high risk for having a positive surgical margin, experiencing HPV persistence, positive at the inner margin of the cervix, and positive at the outer margin of the cervix (HR: 6.44 (95%CI: 2.80, 9.65); $p < 0.001$) was associated with an increased risk of persistence/recurrence. By multivariate analysis, only the inner margin of the cervix was positive, while the outer margin of the cervix was positive (HR: 4.56 (95%CI: 1.23, 7.95); $p = 0.021$) and was associated with a poorer prognosis. In this high-risk population, a positive cervical margin was the main risk factor for predicting 5-year recurrence (27). This study demonstrates the important role of awakening immune cells in combating HPV-positive precancerous cervical lesions, restoring normal HPV negative and abnormal proliferation, and preventing HPV recovery, which may be a long-term benefit for high-risk people with a positive endocervical margin and experiencing HPV persistence and "low-risk" early-stage cervical cancer (27). It is suggested that immunotherapy should be advanced before or during any treatment to wake up immune cells to recognize tumors or HPV by local administration of drugs and hapten, and PD1 or PD-L1 can be added after waking up immune cells if the patient needs it.

The results presented in our current study have never been demonstrated earlier by a single therapy that can induce an immune response like immunotherapy. Previous studies have reported that the HAVCR2-encoded protein belongs to the immunoglobulin superfamily and TIM family. This gene affects different types of T lymphocytes in the human body and participates in various immune responses, especially in tumor treatment. HAVCR2 can affect tumor growth by regulating T-cell activity and infiltration (28), indicating enhanced immune signal response in PBMCs for whole body immune response.

A significant limitation of the study is the sample size: samples from only one patient were analyzed. Given the significant cost associated with scRNA-Seq, we will seek additional funding support to extend our study to more patient samples.

Our study provides evidence supporting that hapten-mediated local chemotherapy is a safe and effective method because it induces a systematic immunity against both cancer cells and HPV by initiating an immune response from the precancerous cervical lesions to achieve desirable clinical outcomes, which may create a new field of medicine and may be called a chemical immune conization.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving humans were approved by Shandong Baofa Cancer Institute. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. The manuscript presents research on animals that do not require ethical approval for their study. Written informed consent was obtained from the individual

(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

BY: Investigation, Project administration, Supervision, Writing – original draft. XL: Data curation, Formal Analysis, Resources, Writing – review & editing. FG: Conceptualization, Methodology, Validation, Writing – review & editing. PJ: Methodology, Project administration, Writing – review & editing. PZ: Formal Analysis, Investigation, Methodology, Writing – review & editing. GZ: Formal Analysis, Investigation, Methodology, Writing – original draft. XZ: Data curation, Formal Analysis, Software, Writing – original draft.

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Conflict of interest

Author BY was employed by the company Immune Oncology Systems, Inc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Primary vaginal cancer after hysterectomy for benign conditions: a systematic review of the literature

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Background: Primary vaginal cancer is a rare condition. Some studies have revealed an increased risk of vaginal cancer among patients who have undergone hysterectomy for premalignant and malignant cervical disease. However, there is limited literature available on primary vaginal cancer following hysterectomy for benign conditions.

Objectives: This review aimed to investigate available evidence on clinical characteristics, treatments, and outcomes of primary vaginal cancer following hysterectomy for benign diseases. Additionally, we provide a case of a patient who developed primary vaginal cancer 10 years after undergoing hysterectomy for abnormal uterine bleeding.

Search strategy: We conducted a comprehensive literature search on PubMed, Scopus, Web of Science using a combination of title and abstract represented by "hysterectomy", and "vaginal cancer"; "vaginal neoplasm"; and "cancer of vagina". No article type restrictions were applied.

Main results: Eight studies with a total of 56 cases were included in this review. The main symptom observed was vaginal bleeding. Squamous cancer was found to be the most common type, followed by adenocarcinoma. The majority of vaginal cancer cases occurred approximately 10 years after undergoing hysterectomy. The most common location of the tumor was in the vaginal apex. The management approaches varied and details were available in 25 cases. Among these, 7 cases were treated with radiotherapy alone, 1 case received concurrent chemoradiation therapy, and the of rest of the cases underwent surgery as the primary treatment, with or without additional adjuvant therapy. Data of follow-up was available for 15 cases, with 2 cases resulting in death and 2 cases experiencing recurrence. The other cases were alive and well at the time of considered follow up.

Conclusion: Primary vaginal cancer after hysterectomy for benign conditions is an extremely rare condition. It is essential to have high-level evidence to guide the screening and treatment strategy for this rare condition. A part of women who have undergone hysterectomy for benign disorders can benefit from vaginal cytology evaluation. It is reasonable to postpone the initial screening after surgery and to extend the interval between subsequent screenings. Further retrospective case-control trials are expected to determine which specific

subgroups of patients mentioned above might most potentially benefit from screening. The treatment decision for vaginal cancer after hysterectomy is more favorable to radiotherapy-based management rather than surgery. Vaginal endometrioid adenocarcinoma may arise from the malignant transformation of endometriosis. More studies are expected to investigate the correlation between these two diseases.

KEYWORDS

vaginal cancer, vaginal carcinoma, hysterectomy, systematic review, endometrioid adenocarcinoma

Introduction

Primary vaginal cancer is a rare disease that affects the lower genital tract, representing 1-2% of all gynecological malignancies and 10% of all vaginal malignant neoplasms (1). In fact, vaginal cancer is more commonly secondary to malignancies from adjacent sites such as cervix, vulvar or even distant sites such as colon, breast, and pancreas (2). Primary vaginal cancer is a type of cancer that specifically occurs in the vagina, without any evidence of cervical or vulvar cancer, or a prior history of these cancers within the last five years (3). The main cause for vaginal cancer is oncogenic HPV (4), along with a few non-HPV related factors. For instance, antenatal exposure to diethylstilbestrol is associated with primary vaginal clear cell adenocarcinoma (5). The most common type of primary vaginal malignancies is squamous cell carcinoma, which is usually HPV induced, accounting for 90%. Adenocarcinoma and other rare entities like melanoma, sarcoma, and lymphoma (6, 7) are also encountered. The risk of primary vaginal cancer increases with age. More than half of the patients are over 70 years old (8).

Primary vaginal cancer can occur in patients who have had a prior hysterectomy. Researches have shown that the most common reason for a prior hysterectomy is cervical cancer or cervical intraepithelial neoplasia (9, 10), which may be explained by the consistent risk factors among primary vaginal cancer, premalignant cervical lesions, and carcinoma of the cervix. Also, scattered reports have revealed that, primary vaginal cancer occurs in patients who have undergone hysterectomy for benign diseases, which is particularly a rare condition. Due to its rarity, the management of this disease is quiet challenging. Recently, our institution admitted a case of primary vaginal adenocarcinoma which occurred 10 years after hysterectomy for benign uterine disease. This rare case inspired us to explore this specific topic further.

Our study systematically reviewed the global literature on the occurrence of primary vaginal cancer after hysterectomy for benign gynecological diseases. Only a few case reports and retrospective studies with small sample sizes provided detailed clinical information, and there is no consensus on the optimal treatment approach. As a result, we conduct a systematic review to investigate the existing evidence on clinical characteristics, management options and prognosis of primary vaginal cancer in hysterectomized patients for benign conditions. Additionally, we emphasize the need for further research to guide the screening and treatment strategy for this rare condition.

Case presentation

A 72-year-old female, who had a history of hysterectomy and bilateral salpingo-oophorectomy 10 years ago for abnormal uterine bleeding, presented with persistent vaginal spotting for one month in the gynecology department of a local hospital. Upon gynecological examination, a solid ulcerating mass measuring 2*2cm was found at the apex of the vaginal stump. The vaginal stump cytology showed high-grade squamous intraepithelial neoplasia, but human papillomavirus was not detected. Biopsy result indicated endometrioid adenocarcinoma of the vaginal stump. Subsequently, the patient was referred to our hospital for further treatment. PET-CT was scheduled to detect any potential metastatic lesions and determine the initial staging. The result showed that the mass was localized to the vagina (Figure 1), without any invasion beyond the vagina or distant spread. The patient was clinically diagnosed with stage I according to the International Federation of Gynecology and Obstetrics staging system (11). Given to the early- stage, small volume, and upper location of the tumor, our medical team planned to perform a radical vaginectomy and pelvic lymphadenectomy under laparotomy, led by an experienced gynecological oncology expert. However, even with such a talented oncologist, the surgery was still challenging. Without the uterus serving as a reliable anatomical marker, it is proved to be difficult to separate the tightly attached vaginal wall from the anterior bladder and posterior rectum. Moreover, the blood supply surrounding the vagina was abundant, and the surgical field of vision was poor, making it hard to stop bleeding. As a result, the patient experienced significant blood loss during the surgery and required a blood transfusion. Eventually, the mass was completely removed along with the vagina and pelvic lymph-nodes. The microscopic examination of the

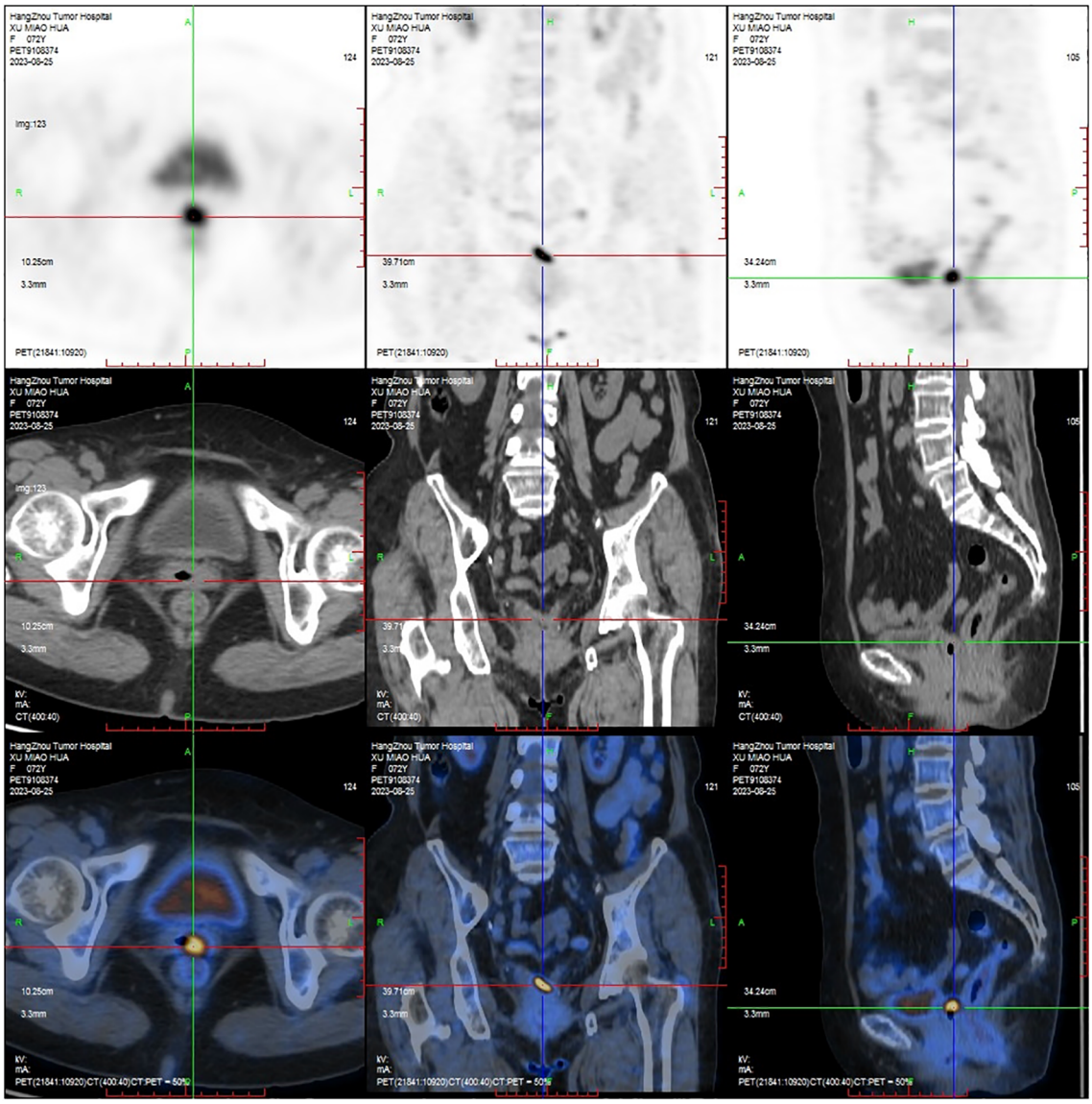

FIGURE 1
PET-CT depicted the tumor was confined to the apex of vagina.

surgical specimen confirmed a highly differentiated endometrioid adenocarcinoma measuring approximately 2.5*1*0.4 cm and infiltrating about half of the vaginal wall (Figures 2A, B), without pelvic lymph-nodes involvement. No additional treatment was scheduled after the surgery. The patient remained free of disease during the 3-month follow -up and was recommended to undergo surveillance regularly for evaluation.

Materials and methods

We performed a systematic search of literature indexed on PubMed, Scopus, and Web of Science (from their inception to September 30, 2023). Our search involved using specific terms in the title and abstract, such as "hysterectomy", "vaginal cancer", "vaginal neoplasm", and "cancer of vagina". A complete search strategy is provided in Appendix S1. Two reviewers (JQ and KG) independently evaluated the titles and abstracts of the records that were retrieved through the database search. The type of articles was not restricted. We only considered articles written in English. We also performed a manual search to include additional relevant articles, by referring to the lists of references in key articles. Full texts of records recommended by at least one reviewer were independently screened by the same two reviewers and assessed for inclusion in the systematic review. Any disagreements between the reviewers were resolved through consensus. Data selection and extraction were carried out according to study type, prior hysterectomy history, histology, intervention, and outcome, using a specifical designed form for capturing information on study characteristics. Data were extracted independently by two authors (JQ and KG) to ensure accuracy and consistency.

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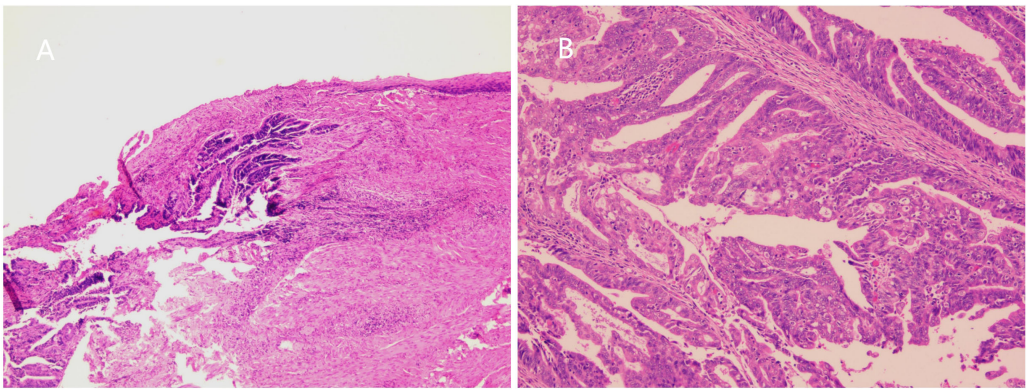

FIGURE 2
(A) Microscopic image showed tumor cells invading vaginal wall (H and E, ×100). **(B)** Microscopic image showed highly differentiated endometrioid adenocarcinoma. (H and E, ×200).

Statistical analysis

For the analysis of outcomes, we calculated the proportions of stage I patients and proportions of vaginal bleeding as the main symptoms amongst all cases. We computed the logarithm of the ratio and its corresponding standard error for each of the studies. A single proportion meta-analysis with inverse-variance weighting was performed using a fixed effects model. Forest plots were created for each outcome, displaying individual study proportions with confidence intervals (CIs) as well as the overall estimate. Heterogeneity was statistically evaluated using the I^2 test. Statistical analysis was conducted using R packages (v4.1.3).

Results

Study assessment

The electronic database search yielded a total of 874 results (Figure 3). After removing duplicates, there were 839 citations left. Among them, 759 were deemed irrelevant to the review based on title and abstract screening. Eighty studies were considered for full-text assessment, and seventy-two were excluded for the following reasons. Two papers were excluded due to being in languages other than English. Seventy papers did not address the main topic or lacked detailed clinical information. In total, 8 studies met the

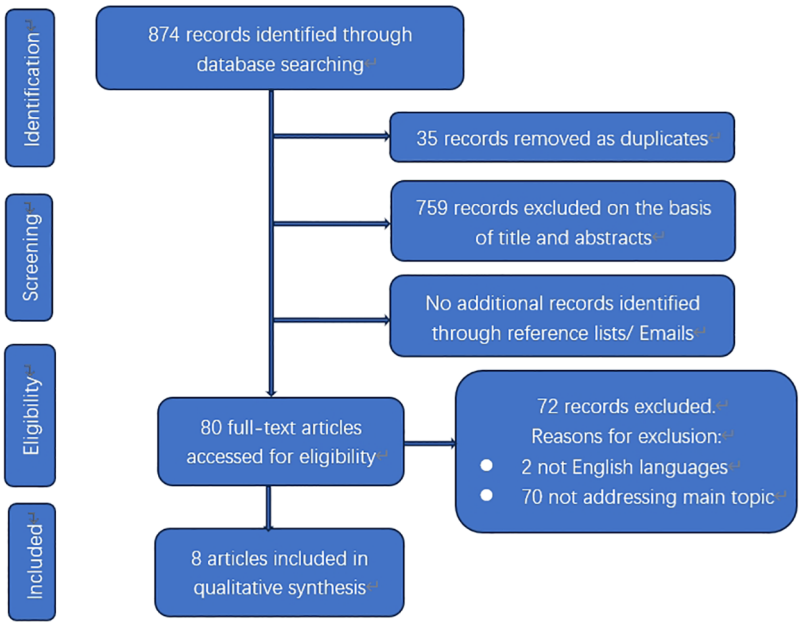

FIGURE 3
Flowchart of literature selection process.

inclusion criteria and were incorporated into the review process (Table 1). These papers consist of 5 case reports and 3 small sample sized retrospective analyses published from 1953 to 2022 (12–19).

Main findings

The papers considered included a total of 56 patients. The main characteristics of these studies are listed in Table 1. The patients' age at presentation ranged from 33 to 86 years old. The main symptoms were vaginal bleeding either with vaginal discharge, as well as other uncommon presentations, such as dysuria, obstipation, lower backache, and pelvic pain. Pooling of results from three studies (n = 47 women in whom main symptoms were reported) rendered a summary proportion of 68% (95% CI 26–54) for vaginal bleeding as the main symptom with no significant variation across the studies ($I^2 = 60\%$, $p = 0.08$) (Figure 4). Vaginal bleeding was the most common chief complaint of the target population and can occur at any stage. However, pelvic pain; backache and obstipation usually presented in late-stage patients. Moreover, some patients may be entirely asymptomatic and were diagnosed during routine examination.

Histopathology showed heterogeneous patterns. By reviewing the study of Bell et al, which included the largest number of patients (31 cases), we found that squamous cell cancer was the most common type of primary vaginal carcinoma in patients who underwent hysterectomy for benign diseases (23 cases) (17), followed by adenocarcinoma (7 cases), and rhabdomyosarcoma (1 case). Another retrospective study with a relatively large sample size by Staats et al. reported 18 cases of primary endometrioid adenocarcinoma of the vagina. Among these cases, 13 had prior hysterectomy for benign diseases, 1 had prior hysterectomy for ovarian endometrioid carcinoma, 2 had prior hysterectomy for unknown reason, and 2 did not have a history of hysterectomy (18). Therefore, 13 cases were included in our review. Stuart et al. reported 29 cases of primary squamous cell carcinoma following hysterectomy. Out of these cases, only 5 had previous hysterectomy for benign reasons and were included in our review (14). The remaining 5 studies in our review were all case reports, which contained 2 cases of endometrioid adenocarcinoma, 1 case of mesonephric carcinoma and 1 case of small-cell carcinoma (12, 13, 16, 19). The report of Dunster et al. was published in 1953, and the pathological type mentioned as "epithelioma" was ambiguous (15).

Among the 56 cases, 16 cases of vaginal cancer occurred within 10 years after the initial hysterectomy. In contrast, 31 cases occurred more than 10 years after. The rest cases did not mention the detailed information.

The most common site of the tumor was the vaginal apex, with 22 out of 56 patients specifically identifying the tumor in that location. The majority of the lesions were located in the upper half of vagina (38 cases). Only 4 cases were presented in the lower half. Valid data was not available for the remaining cases.

Pooling of results from three studies (n = 47 women with reported stage of vaginal cancer) rendered a summary proportion of 40% (95% CI 26–54) for stage I, showing no significant variation across the studies ($I^2 = 0.0\%$, $p = 0.835$) (Figure 5).

Treatment strategies were heterogeneous, with relevant details available in 25 cases. Surgery was performed in 17 women (8 case of stage I, 4 case of stage II, 4 case of stage IV, and 1 case without detailed data) followed by additional treatment in 7 cases (4 radiotherapy, 1 chemotherapy, 1 combined radiotherapy and chemotherapy, and 1 combined neoadjuvant chemotherapy and post-operation chemotherapy). Radiotherapy was the primary treatment for eight cases (five squamous cancers, and two were epitheliomas) (14, 15). In one case of small-cell carcinoma, concurrent chemoradiation therapy was performed as the initial treatment (16).

Data on follow-up was available in 15 cases, with follow-up periods ranging from 4 months to 9 years. Overall, 2 deaths were reported (1 due to lung metastasis 11 months later, and 1 due to bowel obstruction 9 years later). Recurrence was reported in 2 patients (1 with bony metastases and pelvic recurrence 3 years later, and 1 with vaginal recurrence 19 months later). All the other women included in the study remained alive and in good health during the follow-up period.

Discussion

Primary carcinoma of the vagina is a comparatively rare condition, which accounting for only 1-2% of all female reproductive tract cancers (1). In general, the risk factors for primary vaginal carcinomas are the same as those for cervical cancers, with most cases being caused by HPV infection (20). Hysterectomy is one of the most commonly performed surgical procedures in women, with approximately one in nine women undergoing the procedure during their lifetime (21). Although hysterectomy is commonly used as a treatment for gynecologic malignancies, the majority of hysterectomies are actually undertaken for benign gynecologic diseases. Recently, an extremely rare case of primary adenocarcinoma of the vaginal stump was treated in our institution, which emerged 10 years after hysterectomy for benign uterine disease. This case has highlighted our unfamiliarity with the rare disorder and has sparked our interest in getting a deeper understanding of the topic. Therefore, we conducted this systematic review to summarize the relevant literature reports. To the best of our knowledge, this is the first systematic review focusing on the occurrence of primary vaginal cancer after hysterectomy for non-malignant disease. The major strength of our analysis is the robust methodology. However, there are certain limitations that should be acknowledged. Since this topic represents a rare condition, the population of interest is small. Moreover, this systematic review heavily relies on isolated case reports and retrospective analyses with small sample sizes, which may limit the generalizability and robustness of the conclusions.

The role of vaginal vault smears in follow-up of hysterectomized women for reasons other than malignancy has been controversial. The purpose of performing vault smears on asymptomatic hysterectomized women is to detect vaginal intraepithelial neoplasia and prevent vaginal cancer. Opinions regarding the necessity of vault smear have changed over time. There has been

TABLE 1 Review of primary vaginal cancer after hysterectomy for benign conditions in the literature.

First author	Year	Country	Study design	No. of cases	Age	History of hysterectomy	Symptoms	FIGO stage	Histology	Location	Treatment	Follow-up, months
Nomoto (12)	2010	Japan	Case report	1	57	TH + RSO 15 y before (endometriosis, uterine fibroids)	vaginal discharge	I	Endometrioid adenocarcinoma	Apex	Total abdominal vaginectomy+ PLND + LSO	12months: NED
Kumar (13)	2022	India	Case report	1	40	TH 5 y before (uterine fibroids)	vaginal bleeding	N/A	Mesonephric carcinoma	Apex and anterior	Local resection + brachytherapy	N/A
Stuart (14)	1981	Canada	RA	5	33 to 86	TH an average of 13.1 y before (2 for uterine fibroids, 2 for uterine prolapse, 1 for pelvic inflammatory disease)	Asymptomatic, vaginal bleeding, dysuria	N/A	Squamous cancer	upper third of the vagina (the most common site)	RT	N/A
Dunster (15)	1953	England	Case report	3	49 42 40	TH 6 y before (uterine fibroids) TH 8 y before (uterine fibroids) TH 12 y before (Pelvic inflammatory disease)	Pelvic pain, vaginal bleeding Low backache, vaginal bleeding Pelvic pain, vaginal bleeding	III IV I	Grade I epithelioma Well-differentiated epithelioma well-differentiated epithelioma	Apex Apex Apex	Radium+ X-ray therapy Bilateral excision of the parametrium + BSO + total vaginectomy+ cystectomy Radium	Bony metastases and pelvic recurrence 3 years later N/A 36months: NED
Kusunoki (16)	2018	Japan	Case report	1	54	TH 14 y before (uterine fibroids)	vaginal bleeding	III	Small-cell carcinoma	Apex	Concurrent chemoradiation therapy	12months: NED
Bell (17)	1984	USA	RA	31	37 to 80	27 for TAH 4 for TVH all for benign disease, no detailed description Vaginal cancer occurred: <6 y in 3 patients 6-10 y in 8 patients >10 y in 19 patients Unknown in 1 patient	vaginal bleeding (most common), discharge, pain, asymptomatic	I (11) II (9) III (7) IV (1) Unknown (3)	Squamous cancer (23) Adenocarcinoma (7) Rhabdomyosarcoma (1)	Apex (8) Anterior (4) Posterior (2) Lateral (5) Unknown (12) Upper half (18) Lower half (4) Unknown (9)	N/A	N/A
Staats (18)	2007	USA	RA	13	49 to 81	All patients accepted TAH (4 for uterine fibroids, 4 for endometriosis 2 for abnormal uterine bleeding 3 for other benign diseases) Vaginal cancer occurred: <10 y in 2 patients >10 y in 8 patients Unknown in 3 patients	vaginal bleeding (most common), discharge, obstipation, asymptomatic	I (7) II (4) III (0) IV (2)	Endometrioid adenocarcinoma	Apex (6) Anterior (1) Posterior (2) Lateral (2) Upper (1) Posterior/ Apex/ Lateral (1)	Local resection (7) + RT(2)/Chemo(1)/RT and chemo (1)/None (3) Radical resection (6) + RT(1)/NAC and chemo (1)/ None (2)/ N/A(2)	NED (8) : followed up from 4 months to 6 years Dead of disease (2): 1 for lung metastasis 11 months later 1 for bowel obstruction 9 years

(Continued)

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TABLE 1 Continued

First author	Year	Country	Study design	No. of cases	Age	History of hysterectomy	Symptoms	FIGO stage	Histology	Location	Treatment	Follow-up, months
												later Local recurrence (1): 19 months later N/A (2)
Wolf (19)	2020	USA	Case report	1	72	TH+BSO 25 y before (endometriosis)	vaginal bleeding, discharge	IVA	Endometrioid adenocarcinoma	Apex	pelvic exenteration	N/A

RA, Retrospective analysis; TH, Total hysterectomy; TAH, Total abdominal hysterectomy; TVH, Total vaginal hysterectomy; RSO, Right salpingo-oophorectomy; LSO, Left salpingo-oophorectomy; BSO, Bilateral salpingo-oophorectomy; N/A, not available; PLND, Pelvic lymph node dissection; RT, Radiotherapy; NAC, neoadjuvant chemotherapy; NED, no evidence of disease.

a shift from previous enthusiasm to current skepticism, due to that the vaginal intraepithelial neoplasia is 150 times less common than cervical intraepithelial neoplasia, and vaginal cancer is one of the rarest gynecological malignancies (22). A systematic review by Stokes-Lampard et al., which included 6546 hysterectomies for benign diseases elaborated that 1.8% of patients had an abnormal smear, while only 0.12% had an abnormal biopsy, and no vaginal cancers were identified (23). Another evidence-based report by Aldrin et al. revealed that the rate of vaginal cancer and vaginal intraepithelial neoplasia was very low in women with previous hysterectomy for benign conditions (24). Although vaginal intraepithelial neoplasia rate increased in patients with previous cervical intraepithelial neoplasia, even in these patients, vaginal cancer rate was low to 0.01% (24). In 2009, The American College of Obstetricians and Gynecologists stated that, in women who have had a total hysterectomy for benign indications and have no previous history of high-grade CIN, routine cytology screening should be discontinued (25). In our review, 68% of the patients sought for medical help due to vaginal bleeding and were confirmed by biopsy. There's still a small group of patients who were asymptomatic and presented with abnormalities through vaginal smears. Interestingly, only 40% of patients were diagnosed at stage I. Most cases occurred more than 10 years after hysterectomy. Vaginal vault smear plays a crucial role in the early detection of vaginal cancer. In our opinion, women who have undergone hysterectomy for benign disorders should still receive vaginal cytology evaluation since vaginal cancer can be asymptomatic in its early stages. However, the initial time for screening can be appropriately prolonged after hysterectomy and the intervals between screenings can be lengthened. It might be worthwhile to investigate retrospective case-control trials to determine which specific subgroups of these patients would most potentially benefit from screening.

We observed an attractive finding throughout our review. Among the 56 cases included, 15 cases were histologically diagnosed with endometrioid adenocarcinoma, and the most common reason for a prior hysterectomy among them was endometriosis. We are curious about the potential connection between vaginal endometrioid adenocarcinoma and endometriosis. Previous studies have indicated that women with endometriosis have an increased risk of developing endometrial cancer (26–28). The criteria for defining a cancer arising from endometriosis are as follows: the presence of benign endometrial tissue and cancer in the same site, histology of the tumor consistent with an endometrial origin, and exclusion of metastasis from another primary site (29). Based on these criteria, the vaginal lesion can be diagnosed as vaginal endometrioid carcinoma associated with endometriosis. Staats et al. conducted a study involving 18 cases of primary endometrioid adenocarcinoma of the vagina and identified endometriosis in the tissue adjacent to the carcinoma in 13 cases (18). A review on the malignant transformation of vaginal endometriosis revealed that endometrioid adenocarcinoma (17 out of 37) was the most frequent malignancies arising from endometriosis (30). Therefore, we hypothesize that the residual extrauterine lesion in the vagina after hysterectomy for endometriosis may undergo malignant transformation, most likely leading to endometrioid adenocarcinoma, even though this condition is extremely rare.

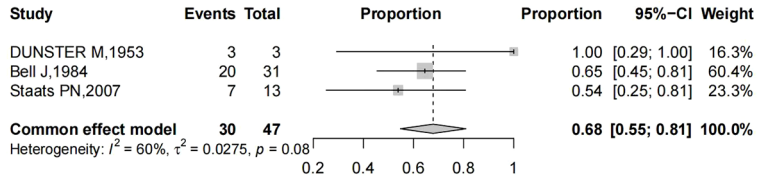

FIGURE 4
Forest plot showed the proportions of women (with 95% Confidence Intervals) with vaginal bleeding as main symptom among hysterectomized women for benign diseases.

Further studies are expected to explore the correlation between endometriosis and primary vaginal endometrioid adenocarcinoma.

Given the rarity of vaginal cancer, there are no randomized control trials to guide treatment decisions. The treatment is individualized and depends primarily on histology, tumor volume, anatomical localization of the lesion, stage of the disease, and age of the patient. Different managements can be considered, including surgery, radiotherapy, chemotherapy, or a combination of these approaches. However, the role of surgery in the treatment of vaginal cancer is limited due to the proximity of the vagina to vital organs such as the bladder, urethra, and rectum (31). Therefore, surgery is considered in selected cases as follows: small early-stage tumors that are confined to the upper posterior vagina, late-stage disease with recto-vaginal or vesico-vaginal fistulas, and central recurrence after radiotherapy (1, 7, 32). The type of surgery varies and includes options such as local excision, partial vaginectomy, radical hysterectomy, and pelvic exenteration, usually combined with lymph node assessment. Zhou et al. compared the effectiveness of local excision and vaginectomy for early-stage vaginal carcinoma. They found that vaginectomy resulted in significantly prolonged survival compared to local excision (33). According to FIGO guidelines, for stage I patients with previous hysterectomy involving the upper posterior vagina, a radical upper vaginectomy and pelvic lymphadenectomy are more appropriate (1). Yang et al. elucidated that patients with stage I and II disease had similar survival rates whether treated with surgery or radiation. However, a significant portion of the population required adjuvant radiation therapy after surgery (34). Radiotherapy using external beam and/or brachytherapy is a standard treatment for vaginal cancer, especially in cases that are locally advanced (1, 31, 34, 35). The principal advantage of radiation is organ preservation. A systematic review conducted by Guerri et al. reported that factors associated with better outcomes in the radiotherapy group included early stage of disease, small tumor size (<4 cm), previous hysterectomy, high pre-treatment hemoglobin levels and younger age (35). Another two retrospective studies demonstrated excellent outcomes with definitive radiotherapy, either with external-beam radiation therapy alone or in combination with brachytherapy. These studies also emphasized the importance of individualizing radiotherapy based on patient's specific factors (36, 37). Nowadays, with the advancements in radiation therapy, image-guided radiotherapy is being used more frequently for the treatment of vaginal cancer, leading to a significant reduction in dose to normal tissue and a decrease in toxicities (38). Chemotherapy is seldom adopted alone in the treatment of vaginal cancer, but rather in combination with other management options. Chemoradiation therapy has shown a rising trend in the treatment of vaginal cancer. A large retrospective cohort study involving 8222 patients demonstrated that chemoradiation was associated with a significant improvement in median overall survival compared to radiation alone (39). Another single institution study including 71 cases highlighted concurrent chemotherapy as a significant predictor of disease-free survival (40). The treatment decisions for vaginal cancer in patients with an intact uterus are not well-established, let alone for those without a uterus. In our review, the managements were heterogeneous without a standard pattern. However, we can draw some insights from the recent case we encountered. The patient, who was confirmed as stage I endometrioid vaginal cancer in the vaginal stump with a small mass, underwent a challenging surgical procedure and experienced significant blood loss. We found that surgical treatment for vaginal cancer after hysterectomy is very

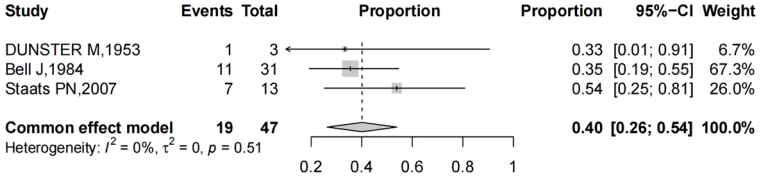

FIGURE 5
Forest plot showed the proportions of stage I (with 95% Confidence Intervals) primary vaginal cancer among hysterectomized women for benign diseases.

difficult, even with an experienced gynecologic oncologist, and can lead to numerous complications. Radiotherapy-based treatment may be a more preferable option for post-hysterectomy vaginal cancer patients.

In conclusion, the occurrence of primary vaginal cancer after hysterectomy of benign diseases is rare, and this is the first systematic review focusing on this topic. Moreover, we present a case of primary vaginal endometrioid adenocarcinoma that occurred 10 years after hysterectomy for a benign condition. Enlightenments from this study are as following: 1. Sometimes, women who have undergone hysterectomy for benign disorders can benefit from vaginal cytology evaluation. But the initial screening time can be properly prolonged after hysterectomy, and the intervals between screenings can be lengthened. Further retrospective case-control trials are expected to determine which specific subgroups of these patients would benefit the most from screening. 2. Vaginal endometrioid adenocarcinoma may arise from malignant transformation of endometriosis. More studies are expected to investigate the correlation between these two diseases. 3. The treatment decision for vaginal cancer after hysterectomy is more favorable to radiotherapy-based management rather than surgery.

Author contributions

JQ: Conceptualization, Writing – original draft. KG: Data curation, Writing – review & editing. LC: Methodology, Writing – review & editing. SX: Conceptualization, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1334778/full#supplementary-material

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Predicting the recurrence of usual-type cervical adenocarcinoma using a nomogram based on clinical and pathological factors: a retrospective observational study

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Background: Usual-type cervical adenocarcinoma is the most frequent type of adenocarcinoma, and its prevalence is increasing worldwide. Tumor recurrence is the leading cause of mortality; therefore, recognizing the risk factors for cervical cancer recurrence and providing effective therapy for recurrent cervical cancer are critical steps in increasing patient survival rates. This study aimed to retrospectively analyze the clinicopathological data of patients with usual-type cervical adenocarcinoma by combining the diagnosis and treatment records after the initial treatment and recurrence.

Methods: We retrospectively analyzed patients diagnosed with usual-type cervical adenocarcinoma who underwent radical hysterectomy and pelvic lymph node dissection at Shengjing Hospital of China Medical University between June 2013 and June 2022. We constructed a nomogram-based postoperative recurrence prediction model, internally evaluated its efficacy, and performed internal validation.

Results: This study included 395 participants, including 87 individuals with recurrence. At a 7:3 ratio, the 395 patients were divided into two groups: a training set (n = 276) and a validation set (n = 119). The training set was subjected to univariate analysis, and the risk variables for recurrence included smoking, ovarian metastasis, International Federation of Gynaecology and Obstetrics (FIGO) staging, lymphovascular space invasion, perineural invasion, depth of muscular invasion, tumor size, lymph node metastasis, and postoperative HPV infection months. The aforementioned components were analyzed using logistic regression analysis, and the results showed that the postoperative HPV infection month, tumor size, perineural invasion, and FIGO stage were independent risk factors for postoperative recurrence ($p<0.05$). The aforementioned model was represented as a nomogram. The training and validation set consistency indices, calculated using the bootstrap method of internal validation, were 0.88 and 0.86, respectively. The model constructed in this study predicted the postoperative recurrence of usual-type cervical cancer, as indicated by the receiver operating

characteristic curve. The model demonstrated good performance, as evidenced by the area under the curve, sensitivity, and specificity values of 0.90, 0.859, and 0.844, respectively.

Conclusion: Based on the FIGO staging, peripheral nerve invasion, tumor size, and months of postoperative HPV infection, the predictive model and nomogram for postoperative recurrence of usual-type cervical adenocarcinoma are precise and effective. More extensive stratified evaluations of the risk of cervical adenocarcinoma recurrence are still required, as is a thorough assessment of postoperative recurrence in the future.

KEYWORDS

usual-type cervical adenocarcinoma, nomogram, retrospective, observational, recurrent cervical cancer

1 Introduction

According to the International Agency for Research on Cancer, cervical cancer is the fourth leading cause of cancer-related deaths in women, with 604,000 new cases and 342,000 deaths reported worldwide in 2020 (1). Most patients with cervical cancer have a fair prognosis after standard treatment; however, some patients have a poor prognosis owing to recurrence or specific pathological types. Squamous carcinoma (75%) and adenocarcinoma (20%) are the most common histological types of cervical cancer.

Cervical adenocarcinoma is difficult to diagnose using cervical cytology because the lesions are hidden, the heterogeneous changes in the nucleus of exfoliated cells are not as prominent as in squamous carcinoma, and some adenocarcinoma results are negative in human papillomavirus (HPV) screening. In recent years, the incidence of squamous carcinoma has decreased but that of adenocarcinoma and other types of cervical cancer has gradually increased, with the usual type being the most prevalent (2).

Despite standardized initial therapy, 10–50% of patients with cervical cancer experience recurrence (3, 4), and the long-term survival rate for patients with recurrence is only 10–20% (5). Tumor recurrence is the leading cause of death in patients with cervical cancer. Predicting the risk factors for cervical cancer recurrence; monitoring management, such as regular re-examination of HPV mRNA; and effectively treating cervical cancer recurrence are critical for improving patient survival. The 5-year survival rates of patients with cervical cancer recurrence range from 15 to 50% (6, 7). The risk factors for usual-type cervical adenocarcinoma recurrence after surgery are not currently the subject of any research. This study aimed to retrospectively analyze the clinicopathological data of patients with usual-type cervical adenocarcinoma by combining the diagnosis and treatment records after the initial treatment and recurrence. Statistical methods were used to explore the risk factors for the postoperative recurrence of usual-type cervical adenocarcinoma. Additionally, a model for postoperative recurrence and prognosis after recurrence was constructed. This model can provide a basis for the long-term management of postoperative patients with usual-type cervical adenocarcinoma.

2 Materials and methods

2.1 Study design

We identified the independent risk factors for eventual recurrence by retrospectively evaluating data of patients with usual-type cervical adenocarcinoma diagnosed and treated at Shengjing Hospital, monitoring their postoperative recurrence, and dividing them into the recurrence and non-recurrence groups based on follow-up outcomes.

2.2 Study participants

This study included patients who underwent radical hysterectomy and pelvic lymph node dissection at Shengjing Hospital of China Medical University between June 2013 and June 2022 and were diagnosed with usual-type cervical adenocarcinoma by pathologists.

The inclusion criteria were as follows: (1) pathological diagnosis of usual-type cervical adenocarcinoma at our hospital and confirmation by two pathologists for examination, (2) treatment at our hospital with complete case data, (3) surgical standardization of treatment according to the National Comprehensive Cancer Network (NCCN) guidelines and staging (Ia2 and above) performed according to the 2018 International Federation of Gynaecology and Obstetrics (FIGO) staging, and (4) postoperative recurrence defined as the reappearance of a tumor

lesion of the same histological type after 6 months of surgical treatment to achieve clinical recovery, as established in this study by histology or imaging (computed tomography [CT]/positron emission tomography-CT).

The exclusion criteria were as follows: (1) loss of visits, (2) coexistence with other malignant tumors, and (3) coexistence with other serious medical or surgical diseases.

2.3 Methods

We mainly conducted follow-ups via telephone and gathered treatment data from the hospital's information system. From Shengjing Hospital's information system, 442 individuals with usual-type cervical cancer were selected. After excluding 14 patients whose clinical records were incomplete, 428 patients were contacted for follow-up. Of these, three declined to be followed up, one died in an accident, 29 were lost to follow-up, and 395 were eventually included, yielding an 89% follow-up rate. The final study participants were randomly divided into a training set and a validation set at a 7:3 ratio. Subsequently, the two groups were compared. The training set was subjected to univariate analysis, and significant items were included in a multifactor analysis for additional examination. The validation set was subjected to internal validation.

In this study, the Virus Research Laboratory of Shengjing Hospital detected HPV DNA using hybrid capture technology on cervical exfoliated cytology. Researchers conducted testing and reported the results within three months before surgery and three to six months after surgery, in accordance with our unit's clinical testing guidelines. Currently, 18 high-risk HPV subtypes (HPV16, 18, 26, 31, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82) and 11 low-risk HPV subtypes (HPV6, 11, 40, 42, 44, 54, 61, 70, 72, and 81) can be identified.

The annual NCCN guidelines served as the foundation for adjuvant therapy, with particular attention to the high-risk characteristics of lymph node positivity, positive resection margins, and parauterine invasion. If any one of these conditions is met, postoperative concurrent chemotherapy with cisplatin and further external pelvic irradiation is administered. Adenocarcinoma, tumor diameter >3 cm, lymphovascular space invasion (LVSI), and tumor invasion of the outer one-third of the cervical stroma were medium-risk factors. Two of these criteria were met. Therefore, concomitant chemotherapy and external irradiation were administered.

2.4 Data collection

The patients who met the inclusion criteria were divided into two groups: those who experienced recurrence and those who did not. Data were retrospectively analyzed to gather demographic information, pathological tumor characteristics, treatment data, and survival rates.

The collected data included the following:

(1) Demographic data, including age, menopause status, body mass index(BMI), smoking status, gravidity, and parity.

(2) Treatment information, including surgical procedures, ovary retention, postoperative adjuvant therapy, targeted therapy, HPV infection, and postoperative HPV persistent infection months (from the day of surgery to the postoperative review of vaginal edge HPV-negative time).

(3) Tumor pathology features, including FIGO stage, degree of differentiation, LVSI, nerve invasion, parametrial invasion, vaginal margin, myometrial invasion, and tumor size.

(4) Survival outcomes, with patients who experienced recurrence after surgery, recording both the type and diagnosis of tumor recurrence.

2.5 Statistical analyses

To determine the optimal cutoff value for continuous variables (age, gravidity, parity, and postoperative persistent HPV infection duration), the X-tile software was used. To screen out independent risk variables for recurrence of usual-type cervical cancer, the Statistical Package for the Social Sciences version 26.0 software was used for data analysis. The χ^2 test was used for univariate analysis, and logistic stepwise backward regression analysis was performed for multivariate analysis. The R.4.3.0 software was used to construct a nomogram and test for predicting the probability of recurrence of usual-type cervical cancer following surgery, as well as for conducting a goodness-of-fit test. The internal validation method was used to recalculate the consistency index (C-index) using bootstrap self-sampling (1000 times) to demonstrate the model's repeatability.

2.6 Ethical consideration

This study was approved by the hospital's ethics committee (Ethics No. 2023PS890K), and the participants provided informed consent.

3 Results

3.1 Patients' basic information

The best age cutoff value was 35 years, the best gravidity cutoff value was 2, the best parity cutoff value was 2, and the number of months of persistent HPV infection after surgery was 9 months. A total of 395 patients were included in this study based on the inclusion and exclusion criteria, with 40 (10.1%) patients aged <35 years and 355 (89.9%) aged >35 years. There were 156 postmenopausal (39.5%) and 239 premenopausal (60.5%) women. 330 (83.5%) women had two or more pregnancies, while 65 (16.5%) women had fewer than two pregnancies. In total, 278 (70.4%)

individuals had parity <2 times, whereas 117 (29.6%) individualshad parity ≥2 times. In total, 373 patients were nonsmokers (94.4%) and 22 (5.6%) were smokers. A total of 57 (14.4%) patients underwent laparoscopic surgery, and 338 (85.6%) patients underwent transabdominal surgery.290 patients had cervical exfoliated cell HPvDNA screening performed as part of preoperative HPV testing; 219 (75.51%) had positive results and 71 (24.48%) had negative results. Of the individuals in the recurrence group, 21 individuals, or 36.84% (21/57), tested negative for HPV. Of the non-recurrent group, 50 individuals (21.46%, or 50/233) tested negative for HPV. Recurrence rates were 29.58% (21/71) for negative patients and 16.44% (36/219) for the HPV positive group, respectively. This difference in recurrence rates was statistically significant (p=0.0157). In accordance with the standard, 308 patients did not experience recurrence, and 87 patients experienced recurrence (Table 1). Follow-up was allowed for a maximum of 123 (mean, 53) months. In this study, the recurrence rate was 22.0% (87/395) (Table 1).

3.2 Logistic regression analysis on postoperative recurrence of usual-type cervical adeno-carcinoma

A total of 395 patients were randomly divided at a 7:3 ratio into two groups: a training set (n = 276) and a validation set (n = 119). Subsequently, the two groups were compared. There were no significant differences between the two groups in any of the variables (p>0.05), except for age (p<0.05). Univariate analysis was performed on the training set to identify the risk factors for postoperative recurrence, including smoking, ovarian metastasis, FIGO staging, LVSI, perineural invasion, tumor size, lymph node

TABLE 1 Demographic characteristics of patients with usual-type cervical adenocarcinoma.

Variable	recurrence group (n = 87)	non-recurrence group (n = 308)	Total (n = 395)	Statistic	P
age, n (%)				χ^2=6.206	0.013
>35	72 (82.76)	283 (91.88)	355 (89.87)		
≤35	15 (17.24)	25 (8.12)	40 (10.13)		
gravity, n (%)				χ^2=1.998	0.158
<2	10 (11.49)	55 (17.86)	65 (16.46)		
≥2	77 (88.51)	253 (82.14)	330 (83.54)		
parity, n (%)				χ^2=0.220	0.638
<2	63 (72.41)	215 (69.81)	278(70.38)		
≥2	24 (27.59)	93 (30.19)	117 (29.62)		
BMI[1], n (%)				χ^2=0.229	0.632
≥28	10 (11.49)	30 (9.74)	40 (10.13)		
<28	77 (88.51)	278 (90.26)	355 (89.87)		
menopause, n (%)				χ^2=0.430	0.512
no	50 (57.47)	189 (61.36)	239 (60.51)		
yes	37 (42.53)	119 (38.64)	156 (39.49)		
smoke, n (%)				χ^2=8.961	0.003
no	76 (87.36)	297 (96.43)	373 (94.43)		
yes	11 (12.64)	11 (3.57)	22 (5.57)		
Types of HPV[2], n (%)				χ^2=16.319	0.022
16+	11 (19.30)	78 (33.48)	89 (30.69)		
16+18+	1 (1.75)	10 (4.29)	11 (3.79)		
16+18+and other	3 (5.26)	2 (0.86)	5 (1.72)		
16+and other	1 (1.75)	8 (3.43)	9 (3.10)		
18+	12 (21.05)	63 (27.04)	75 (25.86)		
18+and other	2 (3.51)	12 (5.15)	14 (4.83)		

(Continued)

TABLE 1 Continued

Variable	recurrence group (n = 87)	non-recurrence group (n = 308)	Total (n = 395)	Statistic	P
other+	6 (10.53)	10 (4.29)	16 (5.52)		
negative	21 (36.84)	50 (21.46)	71 (24.48)		
Not done	30 (–)	75 (–)	105 (–)		
surgical methods, n (%)				χ^2=1.508	0.219
transabdominal	78 (89.66)	260 (84.42)	338 (85.57)		
laparoscopic	9 (10.34)	48 (15.58)	57 (14.43)		
Ovary, n (%)				χ^2=0.318	0.573
resection	80 (91.95)	277 (89.94)	357 (90.38)		
retain	7 (8.05)	31 (10.06)	38 (9.62)		
Ovarian metastasis, n (%)				χ^2=24.128	<.001
no	75 (86.21)	304 (98.70)	379 (95.95)		
yes	12 (13.79)	4 (1.30)	16 (4.05)		
Degree of differentiation, n (%)				χ^2=4.882	0.087
low	35 (40.23)	87 (28.25)	122 (30.89)		
median	17 (19.54)	82 (26.62)	99 (25.06)		
high	35 (40.23)	139 (45.13)	174 (44.05)		
FIGO, n (%)				-	<.001
I	21 (24.14)	231 (75.00)	252 (64.8)		
II	15 (17.24)	46 (14.94)	61 (15.44)		
III	48 (55.17)	31 (10.06)	79 (20)		
IV	3 (3.45)	0 (0.00)	3 (0.76)		
LVSI[3], n (%)				-	0.517
no	72 (82.76)	268 (87.01)	340 (86.08)		
yes	15 (17.24)	40 (12.99)	55 (13.92)		
Perineural invasion, n (%)				χ^2=10.385	0.001
no	77 (88.51)	300 (97.40)	377 (95.44)		
yes	10 (11.49)	8 (2.60)	18 (4.56)		
The depth of myometrial invasion, n (%)				χ^2=22.261	<.001
<1/2	22 (25.29)	166 (53.90)	188 (47.59)		
≥1/2	65 (74.71)	142 (46.10)	207 (52.41)		
Tumor size, n (%)				χ^2=21.587	<.001
<4cm	50 (57.47)	251 (81.49)	301 (76.2)		
≥4cm	37 (42.53)	57 (18.51)	94 (23.8)		
Lymph node metastasis, n (%)				χ^2=89.871	<.001
no	38 (43.68)	277 (89.94)	315 (79.75)		
yes	49 (56.32)	31 (10.06)	80 (20.25)		
Parametrial involved, n (%)				-	0.048
no	85 (97.70)	308 (100.00)	393 (99.49)		

(Continued)

TABLE 1 Continued

Variable	recurrence group (n = 87)	non-recurrence group (n = 308)	Total (n = 395)	Statistic	P
yes	2 (2.30)	0 (0.00)	2 (0.51)		
Vaginal margin, n (%)				χ^2=1.369	0.242
no	84 (96.55)	305 (99.03)	389 (98.48)		
yes	3 (3.45)	3 (0.97)	6 (1.52)		
adjuvant treatment, n (%)				-	0.001
C[4]	11 (12.64)	20 (6.49)	31 (7.85)		
C, R[5]	0 (0.00)	4 (1.30)	4 (1.01)		
CCRT[6]	16 (18.39)	46 (14.94)	62 (15.7)		
R[7]	10 (11.49)	43 (13.96)	53 (13.42)		
R,C[8]	9 (10.34)	4 (1.30)	13 (3.29)		
NO[9]	41 (47.13)	191 (62.01)	232 (58.73)		
targeted therapy, n (%)				χ^2=0.405	0.525
no	84 (96.55)	303 (98.38)	387 (97.97)		
yes	3 (3.45)	5 (1.62)	8 (2.03)		
HPV persistent infection month, (%)				χ^2=67.219	<.001
<9	63 (72.41)	303 (98.38)	366 (92.66)		
≥9	24 (27.59)	5 (1.62)	29 (7.34)		

1: body mass index; 2: preoperative human papillomavirus infection type; 3: lymphovascular space invasion; 4: chemotherapy; 5: chemotherapy then radiotherapy; 6: concurrent chemoradiotherapy; 7: radiotherapy; 8: radiotherapy then chemotherapy; 9: not done.

metastasis, month following HPV infection, and depth of myometrial invasion. After applying logistic stepwise regression analysis to the aforementioned data, the following factors were considered the independent risk factors for postoperative recurrence of d usual-type cervical adenocarcinoma ($p<0.05$): tumor size, perineural invasion, FIGO staging, and month of postoperative HPV infection (Table 2). The nomogram for the visualization of the aforementioned model is presented in Figure 1 and Table 3. Furthermore, 1000 internal samples were drawn from the training and validation sets using the bootstrap method of internal validation; this produced C-indices of 0.88 and 0.86, respectively. There was no collinearity interference issue across the variables according to the prediction model constructed in this study.

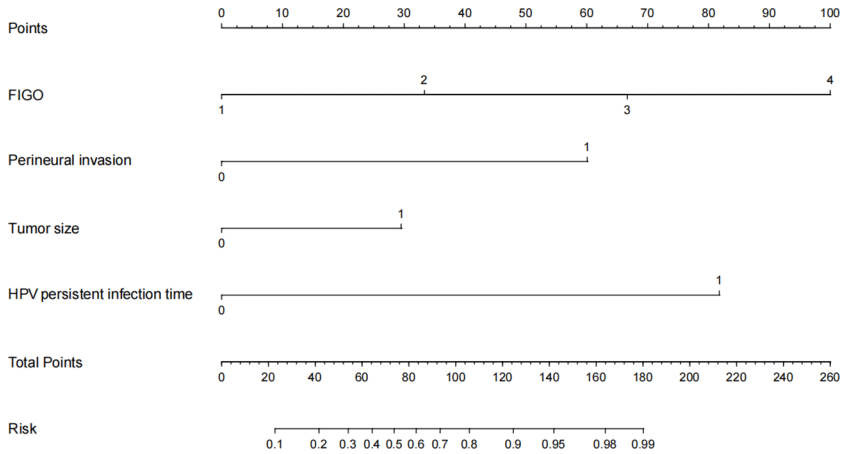

FIGURE 1
A nomogram for predicting postoperative recurrence of usual-type cervical adenocarcinoma.

TABLE 2 Univariate and logistic multivariate regression analysis of the influencing factors of post-operative recurrence of usual-type cervical adenocarcinoma.

Variables	univariate		P	multivariate		P
	Beta	OR (95%CI)		Beta	OR (95%CI)	
Smoke						
no		1.00 (Reference)			1.00 (Reference)	
yes	1.61	5.02 (1.67 - 15.07)	0.004	1.49	4.44 (0.96 - 20.53)	0.056
Ovarian metastasis						
no		1.00 (Reference)			1.00 (Reference)	
yes	2.46	11.67 (3.05 - 44.57)	<.001	1.61	4.98 (0.89 - 27.86)	0.067
FIGO						
I		1.00 (Reference)			1.00 (Reference)	
II	1.30	3.68 (1.59 - 8.50)	0.002	0.99	2.69 (0.95 - 7.61)	0.062
III	2.89	18.00 (8.31 - 38.99)	<.001	2.72	15.18 (5.81 - 39.62)	<.001
IV	17.88	58297855.21 (0.00 - Inf)	0.983	18.29	87356396.05 (0.00 - Inf)	0.989
LVSI[1]						
no		1.00 (Reference)			1.00 (Reference)	
yes	0.76	2.15 (1.04 - 4.44)	0.039	-0.92	0.40 (0.11 - 1.40)	0.151
Perineural invasion						
no		1.00 (Reference)			1.00 (Reference)	
yes	2.87	17.58 (3.69 - 83.76)	<.001	3.10	22.31 (2.77 - 179.70)	0.004
The depth of myometrial invasion						
<1/2		1.00 (Reference)			1.00 (Reference)	
≥1/2	1.46	4.29 (2.26 - 8.14)	<.001	0.83	2.09 (3.75 - 4.70)	0.143
Tumor size						
<4cm		1.00 (Reference)			1.00 (Reference)	
≥4cm	1.34	3.83 (2.05 - 7.15)	<.001	1.41	4.09 (1.73 - 9.70)	0.001
Lymph node metastasis						
no		1.00 (Reference)			1.00 (Reference)	
yes	2.48	11.92 (5.97 - 23.78)	<.001	1.21	3.05 (4.75 - 6.77)	0.243
HPV persistent infection month						
<9		1.00 (Reference)			1.00 (Reference)	
≥9	3.25	25.87 (7.28 - 91.95)	<.001	3.67	39.24 (9.05 - 170.19)	<.001

1: lymphovascular space invasion.

3.3 Model evaluation

The constructed model had a predictive effect on the postoperative recurrence of usual-type cervical adenocarcinoma according to the receiver operating characteristic (ROC) curve (Figures 2, 3). With an area under the curve (AUC) value of 0.90, the predictive model had good clinical practical value and high discrimination. The model in the validation set also showed good discrimination. The training group's model performed well, as evidenced by the highest Jordan index of 0.608 and sensitivity, specificity, and accuracy of 0.794, 0.859, and 0.844, respectively. There was no significant difference in the model's AUC value prediction between the training and validation sets, suggesting that the column chart prediction model had a high degree of repeatability. The calibration curves of the training and validation set prediction models (Figures 4, 5) demonstrated a strong degree of agreement between the predicted outcomes and actual values of the model. The clinical decision curves of the predictive model in the training and validation sets were better than the two extreme end lines, as could be observed from the clinical decision curve analysis

TABLE 3 Assignment description of the nomogram.

Variables	Description of valuation
FIGO	1: I 2: II 3: III 4: IV
Perineural invasion	0: no 1: yes
Tumor size	0: <4cm 1: ≥4cm
Postoperative HPV infection time	0:<no><9</no> months 1: ≥9 months

(Figures 6, 7), suggesting that the model has high clinical practical value. There was a net benefit in clinical application for the training group when predicting postoperative recurrence of usual-type cervical cancer under the effective threshold of >0.19, and the validation group also reported that the model performed well.

3.4 Patient information after recurrence

After an additional follow-up of 87 patients with surgical recurrence, the average survival period after recurrence was 15.7 months. Of them, eight patients experienced both internal and

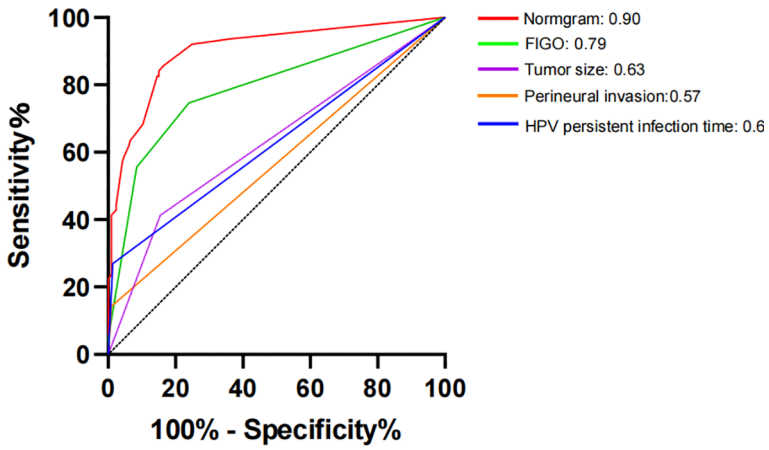

FIGURE 2
Receiver operating characteristic (ROC) curve of the training set.

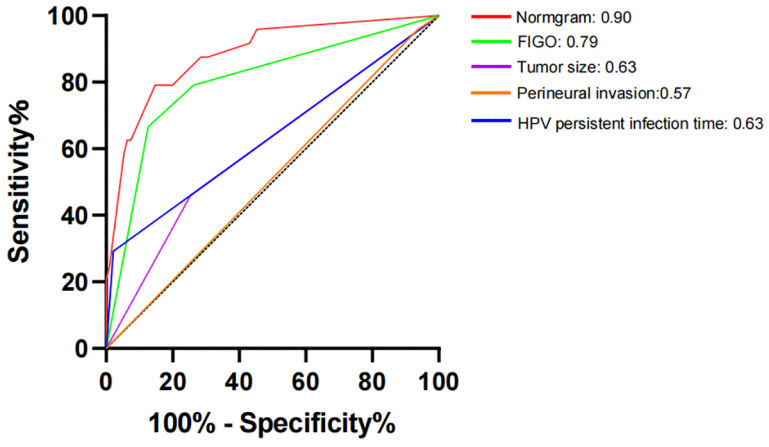

FIGURE 3
Receiver operating characteristic (ROC) curve of the validation set.

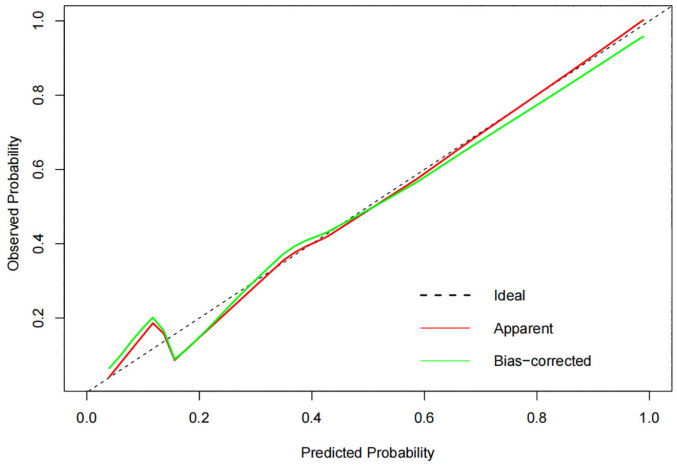

FIGURE 4
The calibration curves of the training set.

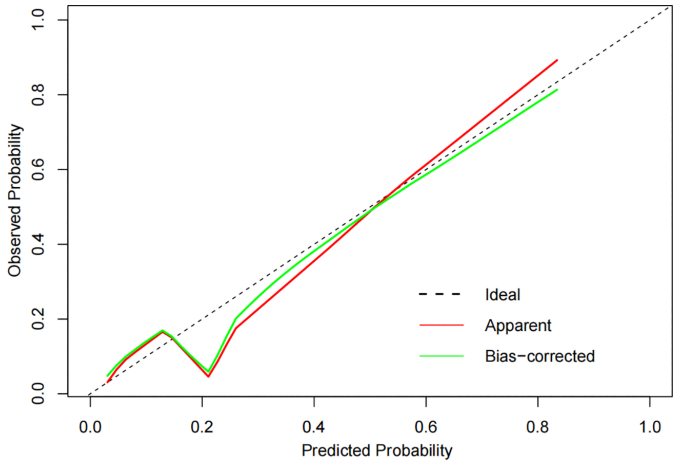

FIGURE 5
The calibration curves of the validation set.

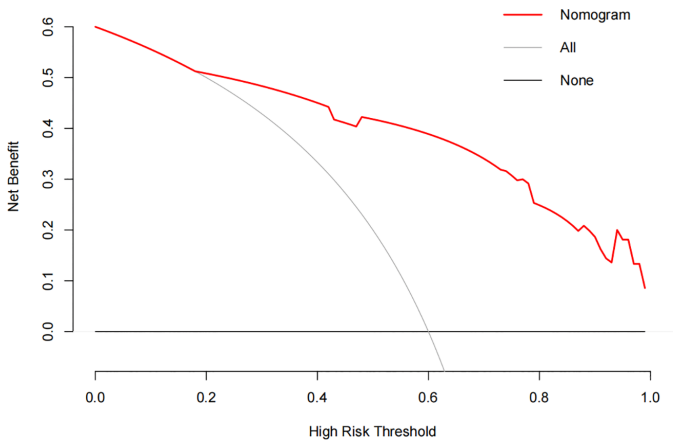

FIGURE 6
The clinical decision curve analysis of the training set.

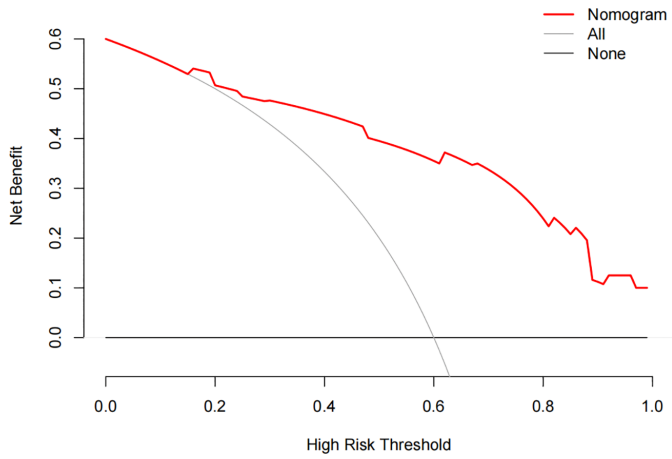

FIGURE 7
The clinical decision curve analysis of the validation set.

exterior pelvic recurrences, 46 patients experienced internal pelvic recurrence, and 33 patients experienced external pelvic recurrence.

4 Discussion

Recurrent cervical cancer is a term used to describe the clinical recovery that occurs following radical radiotherapy or standardized initial surgical treatment (radical cervical cancer surgery) and the subsequent recurrence of tumor lesions of the same histological type in the body over time. Depending on the original treatment mode, recurrent cervical cancer can be classified as recurrence after radiotherapy or surgery. Recurrence after surgery indicates the appearance of new tumor lesions after 6 months of surgical treatment, and recurrence after radiotherapy indicates the formation of new tumor lesions after 3 months of intense radiation therapy. Based on the site of recurrence, recurrence is classified as internal or external pelvic recurrence. Internal pelvic recurrence is further classified as central (limited to the uterus and vagina) or noncentral (pelvic lymph nodes and pelvic wall), whereas external pelvic recurrence refers to lymph node or long-term metastasis outside the pelvic cavity (liver, lung, and kidney).According to previous studies, 14–57% of relapses after surgical therapy occur only in the pelvis, whereas 15–61% occur as distant metastases (8). In this study, the recurrence rate was 22.0% (87/395), which was comparable to previous findings.

Cervical cancer recurrence is associated with factors such as tumor biology, nonstandard diagnosis and treatment, and individual variability. Previous studies on cervical cancer recurrence have not distinguished between histological types or used only histological types as independent variables, resulting in a relatively small number of cases of cervical adenocarcinoma. According to Rudtanasudjatum et al. (9), the risk of early cervical cancer recurrence was comparable to that of squamous cell carcinoma and adenocarcinoma. However, Mabuchi et al. (10) demonstrated that adenocarcinoma was an independent risk factor for recurrence. Furthermore, cervical adenocarcinoma responds to treatment more slowly than does squamous cell carcinoma (11), which frequently expands into the deep cervical myometrium, infiltrates the periuterine and lymphatic regions, and may be associated with a higher risk of recurrence. There is still considerable debate in current studies about whether its histology influences recurrence (12, 13).

Cervical squamous cell carcinoma (70%) and cervical adenocarcinoma (25%) are the two most common types of cervical cancer, which are further classified as cervical adenocarcinoma, cervical squamous cell carcinoma, and neuroendocrine carcinoma according to the current international classification. As there are currently numerous types of cervical adenocarcinoma, the object for this study was the most prevalent type with an increasing incidence rate. In this study, we constructed a predictive model for the recurrence of usual-type cervical cancer by excluding characteristics that could influence nonstandard diagnosis and therapy. The independent risk factors for recurrence were tumor size, postoperative HPV infection time, FIGO staging, and peripheral nerve infiltration. The model was validated and confirmed to have a high clinical practical value.

Young women with cervical cancer are more likely to experience a poor prognosis from rare types of cervical adenocarcinoma and neuroendocrine carcinoma, which increases the probability of recurrence. Additionally, young women are more likely to experience tumor recurrence and have quicker rates of cell proliferation. Moreover, HPV preferentially infects the bigger cervical ectropion and transition zone, which may contribute to the high incidence of HPV infection in young women. This is also associated with frequent contact with new sexual partners (14). However, in our study, age was an influencing factor in the univariate analysis, but in the multivariate analysis, there was no significant difference in age between the relapse and non-relapse groups.

Both viral (genotype, viral load, and integration) and host (genetics, immunosuppression, and social behavior) factors can affect the duration of HPV infection. Currently, persistent HPV infection is not well-defined. Several researchers believe that when

HPV invades host basal cells, it is called persistent HPV infection if a woman's cervical HPV test consistently shows positive results for the same type during two consecutive follow-up visits spaced 4–6 months or 6–12 months apart (15). In this study, postoperative HPV infection time was defined as follows: it is calculated from the date of surgery to the time of HPV infection detection, and the first negative conversion occurs following a follow-up examination of the vaginal stump shedding cells. A total of 105 patients were not tested for HPV during the preoperative examination for this study, and 71 results were negative. Following surgery, HPV was re-examined in all patients in this study, and several individuals with negative preoperative HPV test results tested positive for the virus after surgery. Currently, >95% of the usual types of cervical adenocarcinomas are HPV-related adenocarcinomas; therefore, it is possible that the preoperative sample selection was inadequate or that a small percentage of patients developed an infection after surgery.

The initial follow-up period following standard surgery is 3 months from the date of surgery; however, this period may significantly vary depending on the patient for personal reasons. The patient tested negative for HPV during postoperative follow-up, indicating a continuous infection duration of 0. There were three types of patients in this group: those who were infected before surgery and not infected after surgery, owing to the possibility of virus self-clearance by the body's immune system; those who were not tested before surgery and were not infected after surgery, owing to the possibility of partial non-HPV infection and partial HPV virus clearance; and those who tested negative before surgery and negative after surgery, owing to the possibility of non-HPV infection.

Numerous studies have found minor changes in the HPV model and infection time with respect to the effects of persistent HPV infection time on recurrence. Persistent high-risk HPV (HR-HPV) infection is a risk factor for HPV-associated cervical cancer recurrence. Persistent infection weakens a patient's immune system, promotes tumor growth, and leads to cervical cancer recurrence (16). According to an increasing number of studies, effective surveillance of HR-HPV infection after the initial standardized therapy is an essential predictor of recurrence. In the present study, we found that persistent postoperative HPV infection for >9 months was an independent risk factor for recurrence ($p<0.05$). Furthermore, the location of recurrence is associated with HR-HPV infection, and the sustained positive incidence of HR-HPV infection in patients with pelvic recurrence is higher than that in patients with distant recurrence. However, no comparison was made in the present study. A study of 113 patients with cervical cancer (stages I–IV) revealed that chronic HPV-18 infection could predict recurrence (17). However, in a study that involved 248 participants who were followed up for 5 years, HPV status had no effect on the recurrence rate ($p=0.384$) (18). Belkic et al. found that HPV-18 positivity during follow-up was the greatest predictor of recurrence in a cohort of 84 women with cervical adenocarcinoma in situ, with an odds ratio of 141 (19). HPV-18 positivity is reportedly the best predictor of recurrence ($p<0.005$). Positive HPV findings in two cases predicted recurrence ($p<0.02$). HPV-18 and prolonged HPV positivity are highly predictive of recurrence (19).

In this study, we discovered that the preoperative HPV-negative group had a higher recurrence rate than the preoperative HPV-positive group(36.84%, 16.44%), which was statistically significant ($p=0.0157$). Among the HPV-positive group, the recurrence rate of 18 positives was higher than that of 16 positives (5.48%, 5.02%), but the difference was not significant. The vast majority of usual-type cervical adenocarcinoma is HPV-related, and HPV-negative test results are typically explained by insufficient sampling or poor previous testing techniques. However, HPV-negative patients exhibit distinct characteristics and have a poorer prognosis than HPV-positive patients (20). According to reports, the HPV negative rate varies depending on geographical region, histological subtypes, patient age, and sampling material storage time (21). In large-scale epidemiological studies, the HPV positivity rate in usual-type cervical adenocarcinoma ranges between 72 and 90% (21). In this study, the pre-operative HPV detection rate was 73.4%, while the positive HPV rate was 75.5%. As demonstrated in this study, HPV negative is associated with a poor prognosis, including postoperative recurrence (20, 22). However, in a study of cervical adenocarcinoma, there was no significant difference in cancer-specific survival rates between HPV-positive and negative cases (23). However, in other HPV-related malignant tumors, such as head and neck cancer (24), HPV positivity is also associated with a favorable prognosis. This is due to differences between the groups, as HPV-positive tumors are thought to be more susceptible to radiation.

The three main surgical methods for radical hysterectomy in cervical cancer are minimally invasive, open, and robotic. The third edition NCCN guidelines updated in 2019 indicate that laparoscopic surgery for cervical cancer be avoided. The foundation is based on the Anderson Cancer Center's Laparoscopic Approach to Cervical Carcinoma study (25), which found that for early cervical cancer, minimally invasive surgery may have a greater postoperative recurrence risk than that by open surgery. A meta-analysis revealed no significant difference in the long-term recurrence rate between laparoscopic and robot-assisted laparoscopic surgeries (26); however, there were frequent differences in blood loss and exhaust time during surgery. In a study of 319 patients with cervical cancer who were randomly assigned to either a minimally invasive surgery or laparotomy group, the rates of postoperative adjuvant treatment were comparable between the two groups. A reduced postoperative recurrence rate was associated with less invasive radical hysterectomies (27). However, despite significant differences in univariate analysis, multivariate analysis revealed that the different surgical methods did not significantly influence recurrence (28). There was no significant difference in the postoperative recurrence of usual-type cervical cancer between open and laparoscopic surgeries in this study, which could be due to the small sample size. The use of uterine lifting devices, pneumoperitoneum, vaginal disconnection, and suturing may be associated with an increased risk of postoperative recurrence during laparoscopic surgery.

The role of clinicopathological factors in the postoperative recurrence of cervical cancer remains controversial. In the present

study, the factors that influenced postoperative recurrence were FIGO stage, tumor size, ovarian metastasis, lymph node metastasis, vascular infiltration, depth of the infiltrating muscle layer, and peripheral nerve invasion ($p<0.05$). FIGO stage and peripheral nerve invasion were independent risk factors for postoperative recurrence in usual-type cervical cancer in multivariate analysis. Compared with the 2009 FIGO staging system, the 2018 FIGO staging system has switched to a pathological staging approach for cervical cancer surgery.

The spread of tumor cells through lymphatic veins or blood causes postoperative recurrence (29). LVSI is more common in patients with recurrent cervical cancer, which may be due to LVSI generating distant hematogenous metastases (30). According to a previous study, the incidence rate of lymph node metastasis in LVSI-positive patients was higher than that in LVSI-negative patients (31). LVSI did not have a significant effect on recurrence in the multifactor analysis in this study, and this may be associated with LVSI-positive patients receiving more adjuvant treatment after surgery, which is comparable to the findings of the study by Wey (32). Perineural infiltration is defined as tumor invasion of neural tissues. This peripheral nerve infiltration also confirms the spread of malignant cells.

The lymph node status is associated with cervical cancer recurrence. Mabuchi et al. (33) analyzed 163 cases of cervical adenocarcinoma and adenosquamous cell carcinoma in FIGO 2009 stages IA2–IIB and concluded that lymph node metastasis was a significant predictive factor for cervical adenocarcinoma and adenosquamous cell carcinoma. Patients with lymph node metastases show a dramatically decreased disease-free survival. Meir et al. reported that lymph node metastasis was an independent risk factor for recurrence. The most recent 2018 FIGO staging system defines lymph node metastasis as stage IIIC, confirming that lymph node metastasis may result in a worse prognosis. This was confirmed by the results of the present study. The prognosis differs slightly in patients whose cervical cancer lymph nodes have metastasized. Pelvic lymph nodes, including the internal and external iliac lymph nodes, are not independently associated with poor prognosis in individuals with recurrence, whereas iliac lymph node metastases are. Consequently, further studies with large sample sizes are required to assess the prognostic variations and their effects on recurrence among individuals who have positive iliac common lymph nodes and positive iliac internal and external lymph nodes.

According to the latest guidelines, ovarian preservation is not an absolute contraindication for usual-type cervical adenocarcinoma, and the indications for ovarian preservation are still debated. Ovarian metastasis is considered a risk factor for cervical adenocarcinoma metastasis. Therefore, ovarian preservation is not recommended for patients with adenocarcinoma. However, in this study, preservation of the ovary and ovarian metastasis were not risk factors for postoperative recurrence of usual-type cervical adenocarcinoma in the multifactor analysis. Considering that cervical adenocarcinoma and ovaries are both glandular tissues, it is still necessary to be cautious in grasping the indications for ovarian preservation.

Currently, there are disparities in the recurrence rates of cervical cancer after surgery among different treatment approaches. Chemotherapy is a systemic treatment that can reduce distant recurrence; however, investigations have shown that this is not the case. In one trial, chemotherapy reduced local recurrence rates but had no effect on distant recurrence, which could be due to the confounding effects of adjuvant therapy such as postoperative radiation therapy (34). In a trial of 246 patients who required further postoperative chemotherapy, 182 received it, with a postoperative recurrence rate of 2.74%, whereas 64 did not, with a recurrence rate of 10.93% ($p<0.05$) (34). In the present study, adjuvant treatment had a considerable effect on postoperative recurrence. Therefore, more stratified, large-sample testing is required. According to Rotman et al. (35), pelvic radiation therapy after radical surgery can considerably reduce the incidence of recurrence and progression-free survival in women with stage Ib cervical cancer. Sakai et al. (36) divided 122 patients with early cervical cancer who underwent thorough hysterectomy into four groups: paclitaxel+cisplatin adjuvant chemotherapy (n = 82), other chemotherapy (n = 10), radiotherapy (n = 25), and no further treatment (n = 5). The results showed that there was no difference in the overall 5-year survival rate of the abovementioned patients ($p>0.05$); however, when subgroup analysis was performed only for patients with high-risk factors, recurrence-free survival (RFS) time was significantly shorter in the radiotherapy group than in the paclitaxel+cisplatin adjuvant chemotherapy group. This suggests that for patients with cervical cancer with high-risk factors, chemotherapeutic medications can improve radiation sensitivity and minimize the probability of postoperative recurrence. Takekuma et al. (37) randomly assigned 111 postoperative patients with stage IB–IIB cervical cancer and high-risk variables to one of the two groups: chemotherapy (n = 37) or synchronous radiochemotherapy (n = 74). The results showed that the chemotherapy and radiochemotherapy groups had 4-year RFS rates of 71.7% and 68.3%, respectively ($p>0.05$). According to this study, the efficacies of synchronous radiotherapy and c.

Currently, there are different adjuvant treatment methods for different stages, preoperative and postoperative stages of cervical cancer, mainly including neoadjuvant treatment for patients with stage Ib3 and above disease or radical radiotherapy for patients with disease in the later stages. The effectiveness of various adjuvant treatment approaches in patients with advanced cervical cancer is currently under debate. In a study that included patients with advanced adenocarcinoma or adenosquamous cell carcinoma, the 5-year overall survival and RFS rates of the radical hysterectomy (n = 128) and synchronous radiotherapy and chemotherapy (n = 36) groups were 83.2% and 73.3% ($p=0.164$) and 75.2% and 59.6% ($p<0.036$), respectively. Patients who underwent radical hysterectomy had a lower probability of recurrence (11.6%, $p=0.023$) (38). There is an ongoing debate regarding whether neoadjuvant therapy can improve patient survival and minimize recurrence rates. Some clinical trials have demonstrated that neoadjuvant therapy can further reduce tumor volume and improve surgical treatment effectiveness and prognosis. Chen

et al. (39) found that compared with patients who underwent surgery alone, patients who underwent neoadjuvant chemotherapy-assisted surgery had significantly better 3- and 5-year survival rates ($p<0.05$). According to a systematic review, although neoadjuvant chemotherapy reduces postoperative recurrence, there is no evidence that it influences the survival rate of patients with cervical cancer at various stages and periods (40).

This study has the following limitations: (1) It was a retrospective study; therefore, inherent biases, such as those regarding data inclusion, are possible. (2) The case data were obtained from the same institution, and the treatment techniques and environment were uniform, indicating a lack of external validation. (3) Although pathological analysis is the gold standard for detecting recurrence, some individuals have advanced illnesses that can only be detected through imaging. Currently, there are differences in the recurrence rates of cervical cancer after surgery across different treatment modalities.

5 Conclusions

The model and nomogram for predicting the recurrence of usual-type cervical adenocarcinoma after surgery are accurate and effective, with high discrimination and calibration, and have good clinical practical value, based on FIGO staging, peripheral nerve invasion, tumor size, and postoperative HPV infection months. However, a thorough assessment of postoperative recurrence is critical, and large-scale stratified assessments of the risk of cervical cancer recurrence are required in the future. In addition, further studies on the management of various types of recurrence in common cervical adenocarcinomas are required.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethics Committee of Shengjing Hospital Affiliated to China Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

YL: Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft. NZ: Writing – review & editing, Data curation, Resources. QY: Formal analysis, Funding acquisition, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer XZ, declared a shared affiliation with the authors to the handling editor at the time of the review.

Publisher's note

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Exploring the potential prompting role of cervical human papilloma virus detection in vulvar lesions: a cross-sectional study in China

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Introduction: The etiology and clinical presentation of vulvar carcinomas, especially vulvar lesions, are not fully understood. Because the vulva and cervix are anatomically connected, human papillomavirus (HPV) is the main cause of cervical lesions. Thus, this study explored the potential characteristics and effects of specific HPV infection types across vulvar lesions and concurrent cervical lesions.

Methods: This retrospective, cross-sectional study analyzed patients with cervical HPV or cytological results and concurrent vulvar biopsy who were seen in our hospital colposcopy clinic in Shanxi Province, China, between 2013 and 2023. Data on age, menopause status, vulvar manifestations, and cytology and HPV infection testing results were collected. Attributable fractions and multinominal logistic models were used to evaluate HPV genotyping and clinical characteristics across vulvar lesions.

Results: Among the 1,027 participants, 83 (8.1%) had vulvar intraepithelial neoplasia (VIN) of high grade or worse (VIN2+), and 127 (12.4%) had non-neoplastic epithelial disorders of the vulva (NNEDV). A total of 175 patients had either VIN2+ or cervical intraepithelial neoplasia (CIN) lesions of grade 2 or worse (CIN2+). The most common HPV genotypes for VIN2+ or concurrent VIN2+/CIN2+ were HPV16, HPV52, and HPV58, although attributable fractions differed among lesions. Patients with normal cytological or histopathological result were more likely to have NNEDV detected, while abnormal cervical diagnosis was associated with higher detection of VIN2+. Multinominal logistic modeling showed that age and HPV16 infection were risk factors for VIN2+ or concurrent VIN2+/CIN2+; however, only vulvar presentation with depigmentation was a risk factor for NNEDV. Among patients with low-grade CIN1/VIN1, compared with those who were HPV16 negative, those who were HPV16 positive were at 6.63-fold higher risk of VIN2+/CIN2+ [95% confidence interval (CI): 3.32, 13.21]. Vulvar depigmentation was also associated with increased risk of NNEDV (odds ratio: 9.98; 95% CI: 3.02, 33.04).

Conclusions: Chinese women may be at specific, high risk for HPV infection types associated with VIN or CIN. The use of cervical cell HPV detection along with vulvar presentation during cervical cancer screening may also contribute to vulvar lesion detection.

KEYWORDS

vulvar intrepithelial neoplasia, vulva, human papillomavirus, clinical presentation, non-neoplastic epithelial disorders of the vulva

1 Introduction

Vulvar carcinoma is a rare malignancy, representing approximately 4% of all genital cancers in women (1). According to Global Cancer Statistics 2020, among 185 countries, during that year, there were an estimated 45,240 new vulvar cancer cases (0.2% of new cancer cases) and 17,427 deaths (0.2% of all cancer deaths) (2). Over recent decades, the incidence of vulvar carcinoma has increased, particularly among younger women (3). The most common histology for vulvar carcinoma is squamous cell carcinoma (SCC) (1). Vulvar intraepithelial neoplasia (VIN) and differentiated VIN (dVIN), which originate from squamous cells, are premalignant conditions in vulvar squamous cell carcinoma (VSCC). The etiology of vulvar cancer is largely unknown. To date, human papillomavirus (HPV) infection- and non-HPV infection-related etiological pathways have been summarized. Previous studies found that persistent HPV infection is associated with long-term development of squamous intraepithelial lesion and VSCC. dVIN is more common in vulvar lichen sclerosis (VLS) and, without treatment, is more likely to progress to VSCC than to high-grade vulvar lesions. VLS is a common type of non-neoplastic epithelial disorders of the vulva (NNEDV), which represent a chronic inflammatory disease that may affect any cutaneous site (4), among which the vulvar area is most common.

Neither VIN grades 2 or 3 have an obvious, specific early clinical manifestation and can appear in any vulvar area. The most common NNDEV symptoms are itch and depigmentation, which may be why it is relatively infrequently presented at clinic for further examination and treatment. VIN2/3 are thus often diagnosed late. There also remains no robust screening method. The presence of a thick keratin layer in the vulvar area, which covers the vulvar epithelium, may lead to cytological misdiagnosis. Cytology collection at a spatula end in women with vulvar lesion, after confirmatory biopsy and histopathological examination, reveals only 32% of smears significant for VIN2/3, nor have various other techniques for vulvar cytology testing, including vulvar brush cytology, yielded adequate results, likely because of scarce cellularity. Vulvar cytology testing for VIN2/3 is therefore not currently recommended (5).

The vulva and cervix are anatomical adjacent. HPV testing is effective for cervical cancer screening, and global standards have been developed for cervical sampling and detection methods. Vulvar lesions might thus be detected simultaneous with cervical cancer screening. Identifying HPV infection and its vulvar type would aid a vulvar screening strategy. One meta-analysis suggested that HPV16 and HPV33 are the most predominant HPV genotypes in VIN and that these vary across global regions (6). However, to our knowledge, HPV infection and its vulvar types have been infrequently reported in populations in China, nor are the histological types of various vulvar lesions fully understood, especially in China. Therefore, through a retrospective analysis of a cross-sectional sample of patients in China with different vulvar lesions who were seen over a 10-year period, we aimed to describe the distribution of vulvar lesions. We also explored the potential characteristics and effects of HPV infection type across vulvar lesions and concurrent cervical lesions. The overarching goal was to determine the potential role of cervical HPV in vulvar lesion diagnosis.

2 Materials and methods

2.1 Study design and participants

This single-center, cross-sectional study was conducted at the Second Hospital of Shanxi Medical University in Shanxi Province, China. All gynecology clinic patients with abnormal cytological results or HPV-positive infection, or abnormal vulvar manifestation, are invited to receive another colposcopy examination. We collected these and other data from the records of all patients who underwent colposcopy-directed vulvar biopsy at the colposcopy outpatient clinic from 1 November 2013 to 31 October 2023. All patients were pathologically diagnosed with

Abbreviations: VIN, vulvar intrepithelial neoplasia; HPV, human papillomavirus; hrHPV, high-risk human papillomavirus; lrHPV, low-risk HPV; VSCC, vulvar squamous cell carcinoma; NNEDV, non-neoplastic epithelial disorders of the vulva; dVIN, differentiated vulvar intrepithelial neoplasia; VLS, vulvar lichen sclerosis; TCT, ThinPrep test; NILM, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; VIN2+, vulvar squamous intraepithelial neoplasia 2 and worse; CIN2+, cervical intraepithelial neoplasia 2 and worse.

vulvar and cervical lesions. Patient age, menopause status, vulvar manifestations, and cytology and HPV infection test results were also recorded by tracking the clinic records system. This study received exemption approval from the ethics committee of the Second Hospital of Shanxi Medical University (2023 YX No. 113).

Patients with vulvar lesions of non-squamous epithelial origin or metastatic vulvar tumor were excluded from analyses. Overall, 1,278 patients underwent vulvar biopsy and were pathologically diagnosed with vulvar lesions during the study period. After excluding patients evaluated more than once within a short interval and those with missing age, menopause status, or lesion information, 1,027 patients were included in analyses.

2.2 Cytology and HPV testing

At the gynecological examination, a cervical cytological sample was collected as needed, for cytology diagnosis and HPV testing by the Department of Clinical Laboratory using standard procedures. Cervical cytology was performed by ThinPrep test according to the manufacturer's protocol (Hologic Medical Technologies Co., Beijing, China; http://www.hologic.com), and results were interpreted and reported by two pathologists based on the 2001 Bethesda System (7).

HPV detection and genotyping were tested using HybriMax HPV Geno-Array kit (Hybribio Biotechnology Limited Corp., Chaozhou, China) with flow-through hybridization and gene-chip methods, according to the manufacturer's instructions. The HPV Geno-Array can determine 21 HPV types, including 15 high-risk HPV (hrHPV) types (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, and 68) and 6 low-risk HPV types (6, 11, 41, 42, 44, and CP8304) (8, 9).

2.3 Vulvar and cervical histological diagnosis

Colposcopy was performed by hospital specialists within 12 weeks, according to a standardized protocol. Vulvar tissue specimens were collected via colposcopy, with all visually abnormal vulva areas biopsied (10, 11). Biopsies were sent to the pathology department, then processed to produce hematoxylin and eosin-stained slides. All slides were evaluated in a blinded manner by two gynecologic pathologists, who were also blinded to the cervical cytological diagnosis and HPV testing results. A third senior pathologist was used to resolve conflicting diagnoses, as needed. Pathological diagnoses for vulvar lesions of squamous epithelial origin included benign inflammatory reaction of the vulva (i.e., vulvitis), low-grade VIN (VIN1), high-grade VIN (VIN2 and VIN3), VSCC, and NNEDV. Pathological diagnoses for cervical lesions included cervical intraepithelial neoplasia (CIN) 1 (CIN1), 2 (CIN2), or 3 (CIN3), and squamous cell carcinoma (SCC).

2.4 Statistical analysis

Vulvar lesion distributions across age groups (< 45 years, 45–54 years, 55–64 years, and ≥ 65 years), menopause status (yes or no), vulvar manifestation (none, vulvar itching, depigmentation, masses/vegetations, and/or other symptoms), cervical cytological (NILM or ≥ ASCUS), and histopathological results were categorized. Distribution differences in vulvar lesions among these categories were compared using chi-square or Fisher's exact probability tests.

Multiple infections required the use of weighting to evaluate the proportions of vulvar or concurrent cervical lesions attributable to specific HPV types (12, 13). Participants with multiple HPV infection types were redistributed when calculating the attributable fraction of a single-type HPV infection. First, the frequency of individual single-type HPV infection across the overall sample was calculated. Then, this proportion was used in weighting of participants with multiple-type HPV infections. For example, for 10 patients who were HPV16 and 18 positive, if there were 40 single-type HPV16 infected and 10 single-type HPV58 infected, then 8 of these 10 multi-type infected participants were HPV16 positive and 2 ($10 \times 10/[40 + 10]$) were HPV18 positive.

Multinominal logistic regressions were used to evaluate the effects of potential risk factors in the incidence of high-grade vulvar or concurrent cervical lesions. Because there was a high correlation ($r > 0.5$) between HPV and HPV16 infection, and between age and menopause status, variables including age, HPV16 infection, and other factors were included in the models. Statistical analyses were performed using SPSS Statistics for Windows, version 17.0 (SPSS Inc., Chicago, IL, USA). A two-tailed level $p < 0.05$ was considered statistically significant.

3 Results

3.1 HPV infection rate and vulvar and cervical lesion detection rates

Figure 1 shows the specific-type HPV infection rates (%) and detection rates (%) for vulvar and cervical lesions within each age group. Among patients aged ≥65 years, the prevalence of hrHPV infection in nine-valent vaccine (HPV16 + 18 + 31 + 33 + 45 + 52 + 58) was 46.7%, and the prevalence of HPV16 + 18 infection was 28.9%. Among patients aged <45 years, these rates were 60.6% and 39.9%, respectively. Detection rates of high-grade VIN or worse (VIN2+) increased with age, ranging from 5.4% to 25.6% among patients aged <45–≥65 years. There were not significant age group differences in detection rates of CIN lesions grade 2 or worse (CIN2+), which ranged from 15.4% to 20.0%. There were similar rates of concurrent VIN2+ and CIN2+ detection among patients aged ≥65 years (29.8%) and those aged <65 years (18.5–19.3%).

3.2 Distribution of vulvar lesions based on patient characteristics

Among the 1,027 patients, 186 (18.1%) were diagnosed with vulvitis, 631 (61.4%) with VIN1, 49 (4.8%) with VIN2/3, 34 (3.3%) with VSCC, and 127 (12.4%) with NNEDV. The average patient age was 47.6 years, and those with VIN2/3 (51.5 years) and VSCC (59.9 years) were older.

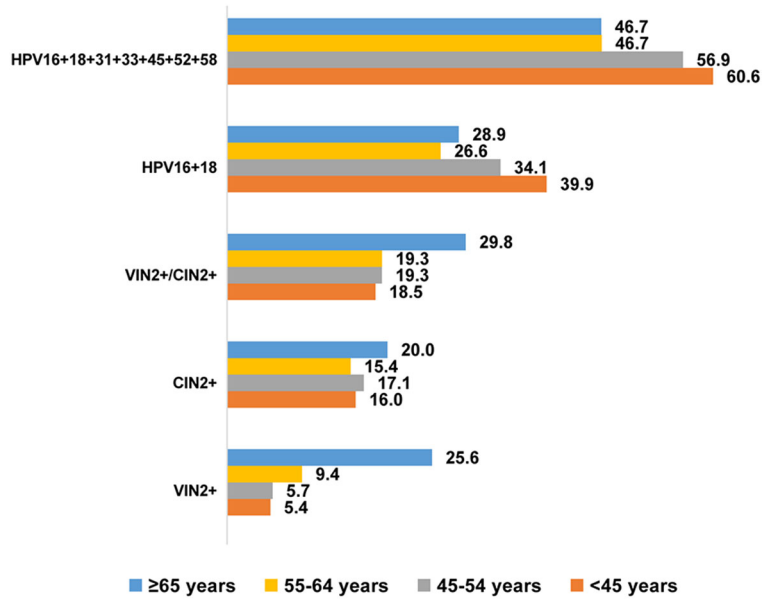

FIGURE 1
HPV specific-type infection rate (%) and detection rate (%) of vulvar and cervical lesions across different age groups. VIN, vulvar squamous intraepithelial neoplasia; VSCC, vulvar squamous cell carcinoma; VIN2+, vulvar squamous intraepithelial neoplasia 2 and worse; CIN2+, cervical intraepithelial neoplasia 2 and worse.

Table 1 shows the distribution of various vulvar lesions, patient characteristics, and clinical features. Within this sample, 58.7% of patients were older than age 45 years. With increasing age, the prevalence of VIN2/3, VSCC, and NNEDV also increased; patients aged <45 years had 3.8% VIN2/3, 1.7% VSCC, and 7.1% NNEDV, while those aged ≥65 years had 10.0% VIN2/3, 15.6% VSCC, and 25.6% NNEDV. A total of 440 (42.8%) participants were post-menopause and had a higher prevalence of high-grade vulvar lesions and NNEDV (6.1% VIN2/3, 5.9% VSCC, and 18.4% NNEDV) compared with patients who were pre-menopausal (3.8% VIN2/3, 1.4% VSCC, and 7.8% NNEDV).

The most common clinical symptoms and signs within the sample were itching or depigmentation [148 patients (14.5%)], masses/vegetations [127 patients (12.4%)] and other clinical manifestations (7.3%), including vulvar pain, vulvar ulceration, and vulvar atrophy. Patients with vulvar masses/vegetations had the highest prevalence of VIN2/3 (4.7%) and VSCC (17.3%), followed by patients with itching and depigmentation, who had 7.8% VIN2/3 and 11.8% VSCC. The clinical symptoms and signs of 566 patients were unknown; however, most (92.3%) had vulvitis or VIN1.

TABLE 1 Distribution of various vulvar lesions among basic characteristics and clinical manifestation.

	vulvitis	VIN1	VIN2/3	VSCC	NNEDV	Overall
	N=186 N (%)	N=631 N (%)	N=49 N (%)	N=34 N (%)	N=127 N (%)	N=1027 N (%)
Age						
Mean (std)	47.9 (11.4)	45.5 (11.6)	51.5 (14.3)	59.9 (14.5)	52.6 (13.0)	47.6 (12.5)
<45 years	71 (16.8)	300 (70.8)	16 (3.8)	7 (1.7)	30 (7.1)	424 (41.3)
45–54 years	61 (20.4)	185 (61.9)	13 (4.4)	4 (1.3)	36 (12.0)	299 (29.1)
55–64 years	42 (19.6)	114 (53.3)	11 (5.1)	9 (4.2)	38 (17.8)	214 (20.8)
≥65 years	12 (13.3)	32 (35.6)	9 (10)	14 (15.6)	23 (25.6)	90 (8.8)
P value			<0.001			
Menopause status						
Yes	82 (18.6)	224 (50.9)	27 (6.1)	26 (5.9)	81 (18.4)	440 (42.8)
No	104 (17.7)	407 (69.3)	22 (3.8)	8 (1.4)	46 (7.8)	587 (57.2)

(Continued)

TABLE 1 Continued

	vulvitis	VIN1	VIN2/3	VSCC	NNEDV	Overall
	N=186 N (%)	N=631 N (%)	N=49 N (%)	N=34 N (%)	N=127 N (%)	N=1027 N (%)
Menopause status						
p-value			<0.001			
Vulvar manifestation						
None	15 (13.5)	85 (76.6)	6 (5.4)	1 (0.9)	4 (3.6)	111 (10.8)
Itching	12 (29.3)	11 (26.8)	0 (0)	0 (0)	18 (43.9)	41 (4.0)
Depigmentation	14 (25.0)	12 (21.4)	3 (5.4)	1 (1.8)	26 (46.4)	56 (5.5)
Masses/vegetations	16 (12.6)	73 (57.5)	6 (4.7)	22 (17.3)	10 (7.9)	127 (12.4)
Itching and depigmentation	16 (31.4)	14 (27.5)	4 (7.8)	6 (11.8)	11 (21.6)	51 (5.0)
Others	13 (17.3)	14 (18.7)	1 (1.3)	0 (0)	47 (62.7)	75 (7.3)
Unknown	100 (17.7)	422 (74.6)	29 (5.1)	4 (0.7)	11 (1.9)	566 (55.1)
p-value*			<0.001			
TCT						
NILM	120 (18.4)	387 (59.4)	27 (4.1)	16 (2.5)	102 (15.6)	652 (63.5)
≥ASC-US	56 (16.5)	238 (70.0)	22 (6.5)	13 (3.8)	11 (3.2)	340 (33.1)
Unknown	10 (28.6)	6 (17.1)	0 (0)	5 (14.3)	14 (40.0)	35 (3.4)
p-value			<0.001			
Concurrent cervical lesions						
None	57 (17.4)	142 (43.3)	17 (5.2)	21 (6.4)	91 (27.7)	328 (31.9)
CIN1	91 (17.2)	398 (75.2)	17 (3.2)	3 (0.6)	20 (3.8)	529 (51.5)
CIN2+	27 (19.3)	84 (60.0)	13 (9.3)	6 (4.3)	10 (7.1)	140 (13.6)
Unknown	11 (36.7)	7 (23.3)	2 (6.7)	4 (13.3)	6 (20.0)	30 (2.9)

VIN, vulvar squamous intraepithelial neoplasia; VSCC, vulvar squamous cell carcinoma; NNEDV, non-neoplastic epithelial disorders of the vulva; NILM, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cells of undetermined significance; CIN 1, cervical intraepithelial neoplasia1; CIN2+, cervical intraepithelial neoplasia 2 and worse.
*Single itching, single depigmentation, and combined itching and depigmentation were combined into one group.

Among the overall sample, 340 patients (33.1%) had cervical ASC-US or higher, and 140 (13.6%) had concurrent CIN2 or higher. Patients with abnormal cytological results had higher detection rates of VIN2+ compared with patients with normal results (10.3% vs. 6.6%). Similarly, patients with CIN2+ had 13.6% VIN2+; however, patients with normal cervical histopathology also had 11.6% VIN2+. Overall, the distribution of various vulvar lesions differed significantly based on age group, menopause status, clinical symptoms, cervical cytological diagnosis, and cervical histopathological diagnosis. Figure 2 shows the colposcopic and histopathological findings based on vulvar lesion type.

3.3 HPV infection and HPV infection type across vulvar lesion types

Figure 3 lists the attributable fractions of various vulvar lesions to HPV infection and specific HPV infection type. There were 768 patients (95.3%) who were hrHPV positive and 38 (4.7%) who were hrHPV-negative; the remaining 221 cases had no HPV testing. Among the patients diagnosed with vulvitis, those with VIN2/3 or VSCC had higher rates of HPV infection than did those with NNEDV. The top 5 most common infection genotypes among the overall sample were HPV16 (36.8%), HPV52 (10.4%), HPV58 (10.3%), HPV51 (6.7%), and HPV53 (4.9%). There was 4%–4.5% of attributable fraction to HPV56, HPV39, and HPV18.

Among patients with vulvitis, the most prevalent hrHPV types were HPV16, HPV52, HPV58, HPV51, and HPV18. Among those with VIN1, similar attributable fractions of the most common hrHPV were observed; fractions for HPV16 were 33%–39%, for HPV52 and HPV58 were ~10%–12% each, and for HPV51 was 7.3%. Among those with VIN2+, the top hrHPV type was HPV16, and its attributable fraction was 77.5%; the other most common hrHPV types were HPV58 (6.0%), HPV52 (4.2%), HPV56 (3.1%), and HPV33 (2.4%). The total attributable fraction of the five hrHPV infections was 93.2%. The five most common hrHPV types among women with NNEDV differed from those with VIN2/3: HPV16 (28.8%), HPV58 (17.0%),

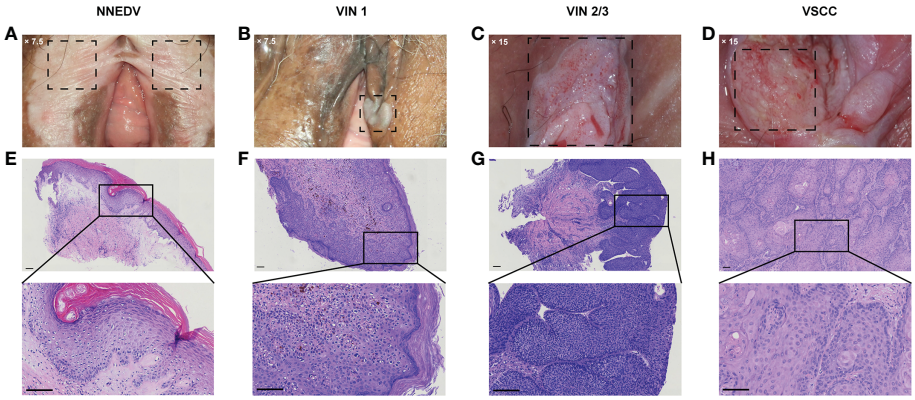

FIGURE 2
Representative cases of the vulvar lesion. **(A–D)** Images of the typical clinical manifestations of different vulval lesions under colposcopy **(A)** ×7.5; **(B)** ×7.5; **(C)** ×15; **(D)** ×15. (**E–H**) The corresponding hematoxylin–eosin staining pathological images and local magnification maps (magnification: ×100; scale bar: 100 μm). NNEDV, non-neoplastic epithelial disorders of the vulva; VIN, vulvar squamous intraepithelial neoplasia; VSCC, vulvar squamous cell carcinoma.

HPV52 (9.0%), HPV66 (8.0%), and HPV51 (5.6%). Their total attributable fraction was 68.4%.

3.4 HPV infection and HPV infection type across concurrent vulvar and cervical lesions

Figure 4 presents attributable fractions of concurrent vulvar and cervical lesions to HPV infection and specific HPV infection types. There were 564 patients with combined VIN1/CIN1 and 175 with VIN2+/CIN 2+; 27 had vulvitis and 30 had NNEDV. Patients with NNEDV also had a lower hrHPV infection rate than those with other lesion types.

Among the patients with vulvitis, the top 5 HR-HPV types were HPV16, HPV52, HPV39, HPV51, and HPV58; their attributable proportions were 33.4%, 14.1%, 7.7%, 7.1%, and 6.6%, respectively, and their accumulated attributable fraction was 68.9%. The most common type among different vulvar lesions combined with cervical lesions was HPV16, but its attributable fraction differed. The HPV16-positive rate was 30.2% among patients with VIN1/CIN1, 59.6% among patients with VIN2+/CIN2+, and 22.2%

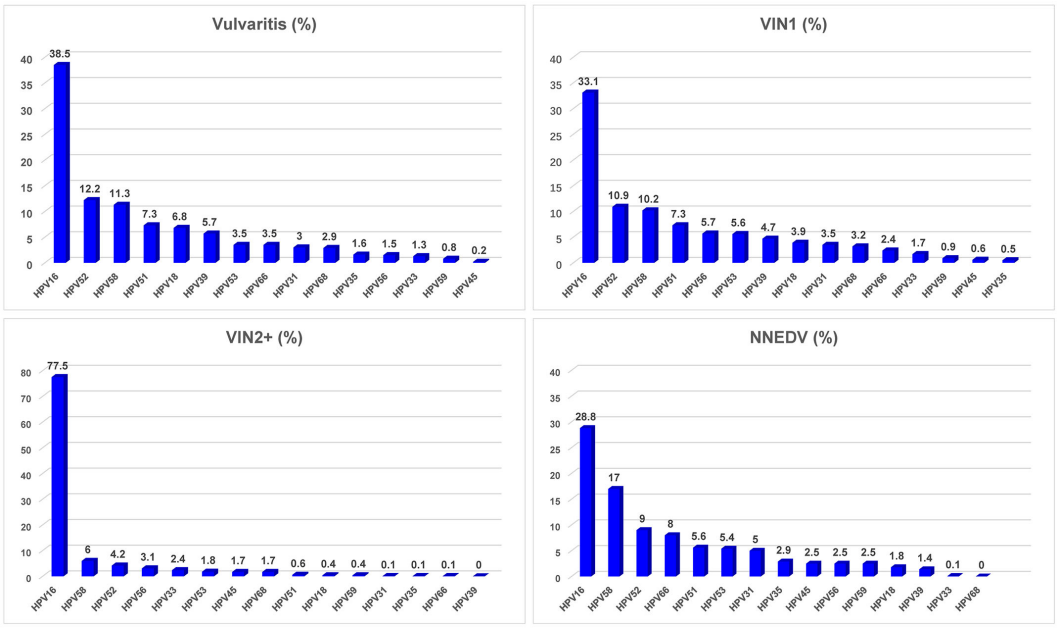

FIGURE 3
HPV infection rate and distribution of high-risk HPV type among various vulvar lesions. Abbreviations: VIN, vulvar squamous intraepithelial neoplasia; VSCC, vulvar squamous cell carcinoma; NNEDV, non-neoplastic epithelial disorders of the vulva; VIN2+, vulvar squamous intraepithelial neoplasia 2 and worse; hrHPV, high-risk human papillomavirus. *Attributable proportion was calculated using weighting method by distribution of each single HPV type across the overall subjects.

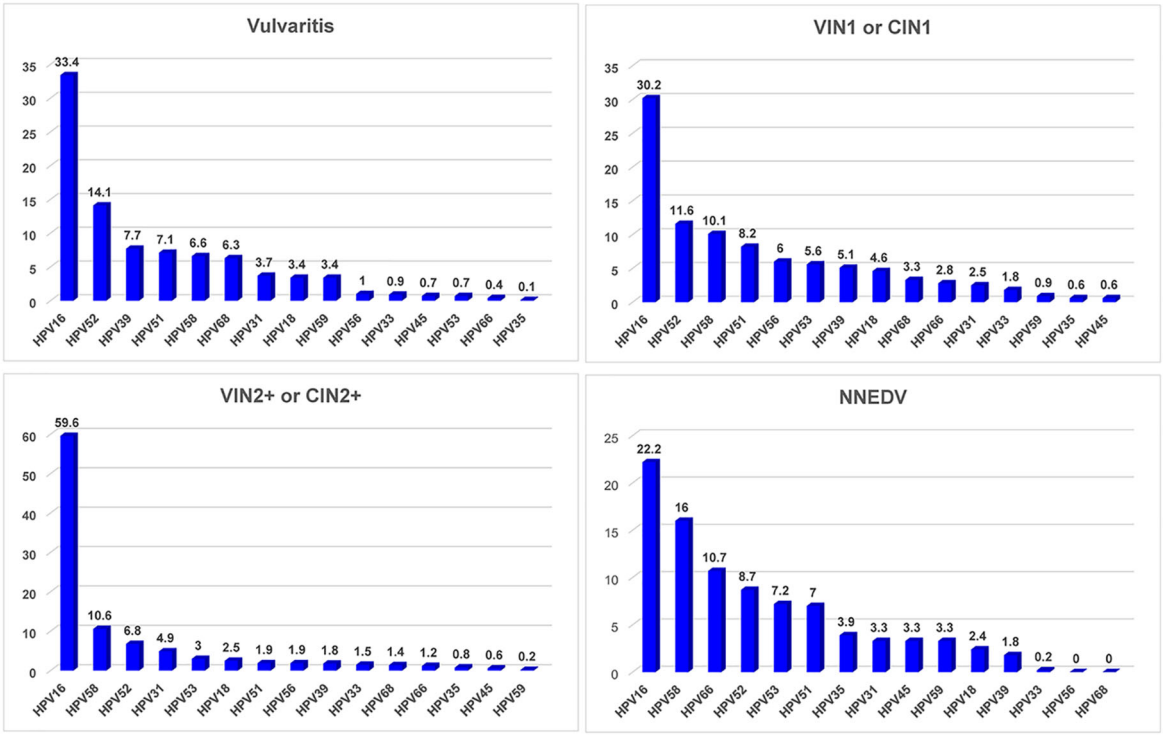

FIGURE 4
HPV infection rate and distribution of high-risk HPV type among various vulvar and cervical lesions. Abbreviations: VIN, vulvar squamous intraepithelial neoplasia; VSCC, vulvar squamous cell carcinoma; NNEDV, non-neoplastic epithelial disorders of the vulva; VIN2+, vulvar squamous intraepithelial neoplasia 2 and worse; CIN2+, cervical intraepithelial neoplasia 2 and worse; hrHPV, high-risk human papillomavirus. *Attributable proportion was calculated using weighting method by distribution of each single HPV type across the overall subjects.

among patients with NNEDV. The four other common hrHPV types differed among various vulvar or cervical lesion types. The accumulated attributable fraction of HPV16/52/58/51/56 was 66.1% among patients with CIN1/VIN1; this value was 84.9% for the attributable fraction of HPV16/58/52/31/53 among those with CIN2+/VIN2+.

3.5 Clinical characteristics related to high-grade vulvar lesions or NNEDV

Table 2 shows the potential risk factors associated with high-grade vulvar lesions and NNEDV. Multinominal logistic model showed that compared with patients with VIN1, those with depigmentation had an increased risk of vulvitis (odds ratio [OR]: 3.79; 95% confidence interval [CI]: 1.26, 11.40) compared with patients without vulvar depigmentation, and a significantly increased risk of NNEDV (OR: 17.63; 95% CI: 5.39, 57.66). Age and HPV16 infection were risk factors for VIN2+. As patient age category increased, the risk of VIN2+ also increased (OR: 2.41; 95% CI: 1.46, 3.98). Patients who were HPV16 positive had an 18.81-fold elevated risk of VIN2+ compared with those who were HPV16-negative (95% CI: 5.77, 61.32). There were no significant associations between vulvar itching or vegetation and VIN2+ or NNEDV risk.

Similar results were found for associations between the above risk factors and risk of concurrent VIN2/3 and CINs. Compared with CIN1/VIN1, those who were older and HPV16 positive had increased risk of VIN2+/CIN2+ (OR: 1.69, 95% CI: 1.17, 2.45; for age; OR: 6.63, 95% CI: 3.32, 13.21 for HPV16 infection), vulvar depigmentation was associated with increased risk of NNEDV (OR: 9.98; 95% CI: 3.02, 33.04).

4 Discussion

To the best of our knowledge, our study is the first to describe the distribution of vulvar lesions based on individual clinical characteristics and to evaluate hrHPV infections and their genotypes in patients in China with vulvar and concurrent cervical lesions. Our main findings were that the distributions of vulvar lesions differed significantly by age, menopause status, clinical presentation, and cervical cytology and pathological diagnosis. Vulvar lesions were attributable to the specific hrHPV type, with the five most frequent types being HPV16, HPV58, HPV52, HPV56, and HPV33 among patients with VIN2+. Previous studies have shown that the persistence of HPV infections is one of the most significant predictors for the risk of recurrence of HPV-related cervical and genital lesions (14); therefore, it is important and meaningful to explore the characteristics of HPV infection in vulvar lesions.

TABLE 2 Risk factors related with high-grade vulvar lesions or NNEDV.

	Cervical or vulvar lesions OR (95% CI)			
	vulvitis N=36	VIN1 N=148	VIN2+ N=27	NNEDV N=29
Risk factors				
Age	1.04 (0.67, 1.63)	Reference	2.41 (1.46, 3.98)	1.52 (0.89, 2.58)
Vulvar itching	2.60 (0.73, 9.23)	Reference	0.46 (0.04, 4.91)	3.59 (0.98, 13.13)
Vulvar depigmentation	3.79 (1.26, 11.40)	Reference	3.35 (0.70, 16.09)	17.63 (5.39, 57.66)
Vulvar vegetation	1.10 (0.47, 2.56)	Reference	2.74 (0.99, 7.58)	1.99 (0.62, 6.47)
HPV16 infection	1.47 (0.65, 3.31)	Reference	18.81 (5.77, 61.32)	1.20 (0.40, 3.60)
	Normal/ vulvitis N=10	VIN1/ CIN1 N=135	VIN2 +/CIN2+ N=65	NNEDV N=25
Risk factors				
Age	1.51 (0.77, 2.93)	Reference	1.69 (1.17, 2.45)	1.63 (0.93, 2.85)
Vulvar itching	1.17 (0.11, 12.45)	Reference	0.39 (0.09, 1.71)	2.45 (0.71, 8.53)
Vulvar depigmentation	3.24 (0.46, 23.13)	Reference	1.62 (0.57, 4.55)	9.98 (3.02, 33.04)
Vulvar vegetation	3.31 (0.73, 14.95)	Reference	0.80 (0.40, 1.64)	1.54 (0.45, 5.22)
HPV16 infection	1.01 (0.20, 5.22)	Reference	6.63 (3.32, 13.21)	1.21 (0.37, 3.99)

VIN, vulvar squamous intraepithelial neoplasia; VSCC, vulvar squamous cell carcinoma; NNEDV, non-neoplastic epithelial disorders of the vulva; NNEVD, non-neoplastic epithelial disorders.

4.1 Clinical characteristics related to vulvar lesions

Among our retrospective, cross-sectional study, in which patients had vulvar biopsy and pathological diagnosis, we observed detection rates of 4.8% VIN2/3, 3.3% VSCC, and 12.4% NNEDV among women with hrHPV or cytology results. To date, few studies have presented the distribution of vulvar lesion histopathological type, especially including NNEDV. One recent study from Germany reported on 499 women diagnosed with vulvar pathology, showing a similar VLS prevalence (56/499, 11.2%), although it did not report on other lesions (15).

The average age of our patients with VIN2/3 was 51.5 years and 59.9 for those with VSCC. This is consistent with previous research showing that the risk for VIN2/3 and VSCC increases with age (>50 years) (16). Among our patients with NNEDV, 25.6% were older than 65 years. This is consistent with another study showing that with increasing age, the risk of vulvar cancer also increases in women with NNEDV (17). Our study showed that patients who are post-menopause have higher rates of VIN2/3, VSCC, and NNEDV compared with patients who are pre-menopausal. Other prospective studies have shown that women are more likely to develop vulvar cancer after menopause.

Herein, patients with vulvar biopsy were also more likely to have vulvar itching, depigmentation, or masses/vegetations; those with VIN2+ had atypical skin vegetations, and those with NNEDV were more likely to have itching or depigmentation. Several review papers have concluded that the main manifestations of NNEDV are vulvar skin changes and abnormal vulvar sensations; the most common symptom of NNEDV is itching, while VIN2/3 presents with no obvious symptoms. The consistency among other global studies and ours bolsters confidence in these findings. After adjusting for age, multi-presentation (including vulvar itching, depigmentation, and vegetation), and HPV infection, we found that patients with vulvar vegetation were also at elevated risk of VIN2+. Overall, patients with vulvar depigmentation were at an obviously increased risk of NNEDV. NNEDV has garnered increasing attention in recent years because of the high risk of progression to VSCC. Diagnosis of vulvar lesions may be delayed due to the absence of obvious symptoms and routine screening. Therefore, vulvar depigmentation and vegetation could be critical screening signs.

4.2 HPV infection and its specific type related to vulvar lesions

Herein, hrHPV infection prevalence was >90%. Among different VIN2/3, women with VIN2+ had 100% hrHPV infection and 77.5% had HPV16 infection. Previous studies have suggested that >80% of vulvar precancerous lesions are HPV positive, while only approximately 25%–40% of VSCC are mediated by HPV-associated pathways (18). A recent meta-analysis reported that low-grade vulvar squamous intraepithelial lesions have a 61.6% pooled prevalence of HPV DNA positivity; the value was 83.3% for the high-grade vulvar squamous intraepithelial lesion, although included studies were highly heterogenous. In Asia, vulvar cancer and VIN2/3 had 48.4% and 82.3% prevalence rates for HPV infection, respectively; the HPV16-positive rate was 66.4% among women with vulvar lesions. In comparison, our study showed a relatively higher hrHPV infection rate than either the global or other regional rates, while our HPV16 infection rate was close to the global level (6). Two possible reasons may partly explain this difference. First, our participants were included based on their HPV test results, among whom the hrHPV infection rate was 95.3%. This may have led to selection bias compared with other studies. Second, different HPV DNA testing methods may alter study results.

We also found that HPV16 was the predominant infection type among VINs and NNEDV, followed by HPV52 and HPV58. Combining across cervical lesions, these three HPV types were also the most frequent overall. Consistent with these findings, another meta-analysis observed that HPV16 (45.7%) was the predominant type, followed by HPV58 (15.5%), HPV52 (11.7%), HPV33 (9.4%), HPV31 (4.3%), and HPV18 (3.5%) among women with cervical lesions (CIN2/3) in China. Another population-based study in China also reported similarly predominant HPV infection types among women with CIN2+ (12). In contrast to our study, that meta-analysis, which included global data, showed that the most common HPV

genotype in vulvar cancer was HPV16, followed by HPV33, while in other HPV types predominated by region, HPV18 was the second most frequent type in South America and Asia and HPV52 in Oceania (6). Another study provided similar findings (3). To our knowledge, no previous study has investigated the association between HPV specific-type infection and NNEDV risk, nor are we aware of any other HPV genotyping in vulvar lesions in China. Our study further confirms that Chinese women may have specific hrHPV infection types associated with vulvar or CIN lesions.

4.3 Clinical implications

Our finding that, among patients with cervical lesion diagnoses, those with normal cytological or histopathological results had a higher NNEDV detection rate, and that those with abnormal cervical diagnoses had higher VIN2/3 detection rates, suggests that the etiology of VIN2/3 and cervical CINs may be similar and distinct from NNEDV. Several studies have provided similar evidence. For example, there remained an 8% detection of CIN2+ among women treated surgically for high-grade VIN/vulvar cancer but who retained an intact cervix; approximately 25% of these patients with VIN3 had coincident or a history of CIN (19). Women with CIN3 had a 2.68-fold increased risk of vulvar cancer compared with women without CIN3 (OR 2.68; 95% CI: 1.71, 4.18) (20).

Although the etiology of vulvar lesions remains unclear, it is understood to be related to HPV infection with VIN2/3 (21). However, herein, after adjusting for multiple factors, HPV16 infection was not a risk factor for NNEDV compared with VIN2/3; that is, the etiology of VIN2/3 and NNEDV may differ. Potential mechanisms may include that susceptibility to, or opportunistic, HPV infection is increased in one of many ways [e.g., estrogen level changes among post-menopausal women, skin injury, and vulvar area stimulation from itching that causes scratches in patients with VLS (22)], after which it progresses to vulvar cancer. Our study also confirmed that hrHPV is strongly associated with VIN2+ and CIN2+ detection. HPV may thus play an important role in vulvar lesion diagnosis. As such, HPV detection in cervical cells during cervical cancer screenings may likewise aid the detection of VIN2+. We recommend that attention be paid to the vulva during routine cervical examinations and that more education on vulvar presentations and enhanced medical care awareness should be further enforced.

Although this article focuses on the value of HPV in the diagnosis of vulvar diseases, based on the current treatment status of vulvar cancer (23), it is theoretically beneficial to prevent the recurrence of vulvar cancer by clarifying the characteristics of HPV infection and increasing the intervention of HPV during the personalized treatment of HPV-related vulvar cancer. Therefore, it is also meaningful to explore the role of HPV in the treatment and follow-up of HPV-associated vulvar cancer.

4.4 Study strengths and limitations

Main study strengths included our concurrent evaluation of hrHPV infection and genotypes for individual histopathological VIN and CIN lesion types, among a single sample, using consistent histopathology, cytology, and HPV genotyping methods, in a population in China. However, the study also had several disadvantages. First, our participants were all seen at the colposcopy outpatient clinic. Most with abnormal cytological results or HPV-positive infection were transferred for colposcopy examination and then further vulvar biopsy and diagnosis. The women with HPV-negative or cytological results were thus not included, likely contributing to a higher HPV infection rate and limiting our ability to evaluate the effects of hrHPV-negative status on risk for vulvar lesions or the prevalence of VIN2/3 among the overall clinic population. Thus, we only evaluated hrHPV infection and attributable fractions of genotypes among various vulvar and cervical lesions. Second, this retrospective, cross-sectional study covered a 10-year period during which gynecologists with different levels of experience were involved in colposcopy examinations and vulvar biopsies, possibly missing some patients with atypical vulvar presentations. While the VIN2+ detection rate herein was 3.3%, reflecting a much higher VSCC incidence (0.2/10,0000) than typical, some cases may have been missed. Finally, we were unable to collect other information on potentially confounding factors, although the focus herein was objective age, menopause status, and cytological and HPV testing results, rather than factors that may influence long-term outcomes.

4.5 Conclusion

These retrospective, cross-sectional analyses revealed a NNEDV detection rate twice that of VIN2/3. Our findings suggest that VIN, including VIN1/2/3, and VSCC, compared with NNEDV, have distinct clinical presentations and etiologies. VIN2/3 may be similar to cervical cancer, regarding both etiology and other characteristics. These findings support the notion that greater attention should be paid to the vulva during cervical examination, to increase VIN2/3 detection. Chinese women may have specific hrHPV infection types that are associated with VIN or CIN. The use of HPV detection with cervical cells during cervical cancer screening may also contribute to detection of vulva VIN2+.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by the ethics committee of the Second Hospital of Shanxi Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of

kinin accordance with the national legislation and institutional requirements.

Author contributions

XD: Data curation, Writing – original draft. QL: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft. JL: Data curation, Validation, Writing – original draft. RL: Data curation, Validation, Writing – original draft. BF: Data curation, Validation, Writing – original draft. CW: Data curation, Validation, Writing – original draft. LG: Data curation, Validation, Writing – original draft. RF: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – review & editing. ZW: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Barriers to cervical cancer prevention in a safety net clinic: gaps in HPV vaccine provider recommendation and series completion among Ob/Gyn patients

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Objective: The primary objective of this study was to evaluate patients' knowledge regarding HPV vaccination and vaccine uptake in a diverse patient population. The secondary objective was to evaluate factors influencing the decision to vaccinate, potential barriers to vaccination, and to assess whether HPV vaccines were offered to or discussed with eligible patients in a safety net Obstetrics and Gynecology (Ob/Gyn) clinic.

Methods: A 28-item survey was developed using Likert scale survey questions to assess patient agreement with statements regarding HPV and the vaccine. The surveys were administered to patients in the Ob/Gyn outpatient clinics from May 2021 through September 2022. Additionally, pharmacy data were reviewed and chart review was performed as a quality improvement initiative to assess the impact of expanded HPV vaccine eligibility to patients with private insurance on vaccine uptake. Descriptive statistics were performed.

Results: 304 patients completed surveys from May 2021 through September 2022. The median age of respondents was 32 (range 18-80). 16 (5%) were Non-Hispanic White, 124 (41%) were Hispanic White, 58 (19%) were Non-Hispanic Black, 6 (2%) were Hispanic Black, 29 (9.5%) were Haitian, 44 (14%) were Hispanic Other, 7 (2%) were Non-Hispanic Other, 20 (6.6%) did not respond. 45 (14%) patients were uninsured. Many patients (62%) reported that a physician had never discussed HPV vaccination with them. Seventy nine percent of patients reported they had never received the HPV vaccine, and 69% of patients reported that lack of a medical provider recommendation was a major barrier. Among patients to whom HPV vaccination had been recommended, 57% reported that the vaccine was not available the same day in clinic.

Conclusion: Our study demonstrated that many patients never had a provider discuss HPV vaccination with them and never received the HPV vaccine. Additionally, amongst those who did initiate HPV vaccination, completion of

the series remains a key barrier. Ensuring that providers discuss HPV vaccination and that patients receive HPV vaccines, along with expanding access to and convenience of HPV vaccination are critical aspects of preventing cervical cancer.

KEYWORDS

human papilloma virus, cervical cancer, vaccination, patient education, healthcare disparities

1 Introduction

The human papillomavirus (HPV) is the leading cause of cervical cancer (1, 2). In the United States (U.S.), a virus-like particle-based vaccine has been available since 2006, with the 9-valent vaccine available since 2016 that provides protection against 9 strains of high-risk HPV (3, 4). Increasing uptake of the HPV vaccine is an important aspect of the World Health Organization's (WHO) goal of eradicating cervical cancer (5). The WHO has set a target of vaccinating 90% of women by age 15 (6). The U.S. Department of Health and Human Services has similarly set a target of 80% of adolescents receiving HPV vaccines as part of its Healthy People 2030 initiative (7). The Advisory Committee on Immunization Practices and the American College of Obstetricians and Gynecologists (ACOG) recommend HPV vaccination for all men and women between the ages of 9-26, and with shared decision-making for patients ages 27-45 (8).

However, despite the wide availability of HPV vaccination in the U.S., vaccination rates are low, with only 27% of men and 53.6% of women between the ages of 18-26 reporting vaccination (9). Furthermore, there are significant racial and ethnic disparities in knowledge of and access to the vaccine (10). Associations have been noted between HPV knowledge and factors such as race, ethnicity, nation of origin, level of education, and primary language (11, 12). Racial disparities are significant barriers in women's healthcare, likely contributing to these differences (13). Both incidence and death rates from cervical cancer reflect racial and ethnic disparities (14). From 2016-2020, the U.S. incidence rate among Hispanic and Non-Hispanic Black (NHB) patients was 9.3 and 8.5 respectively, compared to 7.1 for non-Hispanic White (NHW) patients (7). From 2016-2020, the death rates for NHB and Hispanic patients were 3.3 and 2.5 respectively, while the death rate for NHW patients was 2.0 (7). Hispanic women are 1.26 times more likely to die of cervical cancer than NHW women (15).

In addition to primary prevention, HPV vaccination may also be utilized in the adjuvant setting for the management of cervical dysplasia. In 2023, ACOG expanded recommendations for consideration of HPV vaccination for patients undergoing treatment of CIN 2 (16). Recent studies have demonstrated that the majority of patients who developed lower genital tract dysplasia following hysterectomy for HPV-related disease had HPV subtypes that would be covered by the HPV vaccine, supporting further investigation of adjuvant vaccination (17). The management of HPV-associated cervical dysplasia, including increased clinical surveillance, biopsies, and excisional procedures such as loop electrosurgical excision procedure (LEEP) and cervical conization, contributes to heightened patient distress and anxiety (18). Excisional procedures have also been associated with adverse pregnancy outcomes including cervical insufficiency, preterm labor, and preterm delivery (19). Counseling patients about HPV prevention is unique because HPV is not only a sexually transmitted infection, but more importantly, causes a significant burden of disease through precancer and cancer in both men and women (10). Persistence of HPV infection is one of the most significant risk factors for developing HPV-related dysplasia and malignancy (20). Assessing patients' understanding of HPV and HPV vaccination will help providers better counsel patients to accept vaccination for cancer prevention.

The primary objective of this study was to evaluate patients' knowledge of HPV vaccination and vaccine uptake in a diverse patient population. The secondary objective was to evaluate factors influencing the decision to vaccinate, potential barriers to vaccination, and to assess whether HPV vaccines were offered or discussed during clinic visits for eligible patients in a safety net Ob/Gyn clinic.

2 Materials and methods

Institutional review board approval was obtained (IRB #00058353). A 28-item survey was developed using Likert scale survey questions to assess patient agreement with statements regarding HPV and the HPV vaccine from May 2021 through September 2022. The anonymous surveys were then given to patients in the Ob/Gyn outpatient clinics while waiting for their appointments. Patients had the option of not participating by not completing the survey. Patients had to be 18 years or older, be able to read English, Spanish, or Haitian Creole and be able to self-complete the survey. Demographic data was self-reported by survey participants, and racial and ethnic categories were chosen by investigators in order to capture the diverse racial and ethnic composition of our patient population (21). Study data was

collected and managed using REDCap electronic data capture tools hosted at the University of Miami Miller School of Medicine. REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies by providing audit trails for detecting data manipulation, automated export procedures and systems for integration with external sources (22, 23).

At the beginning of the study period, only patients with county funding were able to receive HPV vaccination in the Ob/Gyn clinics. However, following significant engagement with hospital leadership, starting in September 2021, HPV vaccination was expanded to patients with commercial insurance. Thus, the timeframe, Aug 2020 to Aug 2021 was determined as standard care for patients with county insurance, while Sept 2021 to Sept 2022 as expanded access to patients having commercial insurance in addition to those with county insurance. Patients without insurance were not able to receive HPV vaccination during either time period.

Separate from the anonymous surveys as a quality improvement initiative, chart review and analysis of pharmacy data were used to assess the completion of the HPV vaccine series from August 2020 - September 2022 within the clinic. The time period of study was chosen to assess vaccination uptake in the year prior to and following expanded access to vaccination for patients with insurance. Descriptive statistical analysis was performed to evaluate frequency, range, mean, and median values.

3 Results

3.1 Demographics

A total of 304 patients presenting for an Ob/Gyn clinic visit completed surveys. Of the respondents, 124 (41%) self-identified as Hispanic White (HW), 16 (5%) as NHW, 58 (19%) as Non-Hispanic Black (NHB), 6 (2%) as Hispanic Black (HB), 29 (9.5%) as Haitian, 44 (14%) as Hispanic Other (HO), 7 (2%) as Non-Hispanic Other (NHO) and 20 (6.6%) as No Response (NR) (Table 1). With respect to insurance status, 112 (34%) patients had Medicaid, 45 (14%) patients were uninsured, 38 (12%) had hospital safety net funding, 39 (12%) had Medicare and 29 (9%) had private insurance. The median age of respondents was 32 (range 18-80).

3.2 HPV knowledge

Only 45% (n=138) of patients knew that HPV causes cancer (Table 2). While 213 (70%) patients identified at least one correct route of transmission including vaginal, anal, or oral sex and associated skin-to-skin contact, 78 (26%) patients reported that they did not know the route of HPV transmission. Over half (n=183, 60%) of patients identified at least one health consequence of HPV infection, while 106 (35%) patients reported that they did not know the health consequences of HPV infection.

Only 22 (7%) patients knew that HPV vaccination eligibility had been extended up to the age of 45. The majority (83%) of patients did not know how many doses of HPV vaccination are required. Few patients (n=113 37%) were aware that men were eligible for HPV vaccination.

3.3 HPV counseling, uptake, and vaccine availability

Many patients had never discussed HPV (n=113, 37%) or the HPV vaccine (n=189, 62%) with a healthcare provider (Table 3). Additionally, more than half (n=211, 69%) of patients reported that a healthcare provider had never recommended HPV vaccination to them.

More than half (n=173, 57%) of all patients reported that they did not believe that the HPV vaccine was available to be given the same day in the clinic, including both those who had and who had not been offered the vaccine. A majority (n=241, 79%) of patients were unvaccinated. Among patients who were not vaccinated reasons cited included not being offered vaccination (n=86, 28%), not being aware that they were eligible for vaccination (n=86, 28%), concerns about toxic ingredients in the vaccine (n=48, 16%), not believing in vaccinations (n=21, 7%), not being able to afford vaccination (n=43, 14%), and perceived inconvenience of receiving the vaccine (n=24, 8%).

3.4 Vaccine administration

HPV vaccination was not available to patients with commercial insurance in our safety net clinic prior to September 2021. From September 2021-September 2022, the number of patients receiving HPV vaccination increased compared to the 1 year prior to expansion of eligibility.

TABLE 1 Demographics.

	Participants (n=304)
Race/Ethnicity	
Hispanic White	124 (41%)
Non-Hispanic White	16 (5%)
Non-Hispanic Black	58 (19%)
Hispanic Black	6 (2%)
Haitian	29 (9.5%)
Hispanic Other	44 (14%)
Non-Hispanic Other	7 (2%)
No response	20 (6.6%)
Insurance status	
Medicaid	112 (34%)
Uninsured	45 (14%)
Hospital Safety Net Funding	38 (12%)
Medicare	39 (12%)
Private Insurance	29 (9%)
Other	16 (5%)
No response	25 (8%)
Age	
Mean	34
Median	32
Range	18-80

TABLE 2 HPV Knowledge by Race and Ethnicity.

	All (n,%)	Hispanic White	Non-Hispanic White	Black (Hispanic)	Black (Non-Hispanic)	Haitian	Other (Hispanic)	Other (Non-Hispanic)	No Response
Total	304	124 (41%)	16 (5%)	6 (2%)	58 (19%)	29 (9.5%)	44 (14%)	7 (2%)	20 (6.6%)
Knew HPV causes cancer	138 (45%)	61 (37%)	9 (56%)	4 (67%)	23 (40%)	11 (38%)	21 (47%)	2 (29%)	7 (35%)
Correctly identified at least one route of HPV transmission	213 (70%)	100 (81%)	12 (75%)	6 (100%)	29 (50%)	11 (38%)	38 (86%)	6 (86%)	11 (55%)
Not know health consequences of HPV infection	106 (35%)	35 (28%)	5 (31%)	1 (17%)	29 (50%)	13 (45%)	14 (32%)	2 (29%)	7 (35%)
Knew oldest age of vaccine eligibility	22 (7%)	7 (5.6%)	1 (6%)	0 (0%)	3 (5%)	5 (17%)	5 (11%)	0 (0%)	1 (5%)
Knew correct number of doses	52 (17%)	18 (15%)	3 (19%)	2 (33%)	12 (21%)	8 (28%)	6 (14%)	3 (43%)	0 (0%)
Knew men were eligible for the vaccine	113 (37%)	49 (40%)	7 (44%)	4 (67%)	16 (28%)	9 (31%)	20 (45%)	5 (71%)	3 (15%)

4 Discussion

Uptake and provider recommendation of HPV vaccination remains low among Ob/Gyn patients at our safety net hospital. The patterns seen in our population mirror the U.S. as a whole, where despite education and outreach efforts, vaccination rates remain below goal (9). HPV-related cancer incidence reflects this suboptimal vaccination rate, with an estimated 47,199 new cases of HPV-associated cancers annually from 2015-2019 (24). A study from 2021 found that only 47.7% NHW women, 30.9% of Hispanic women, 38.1% of Black women, and 25.9% of Asian women aged 18 to 26 years had received vaccination (25). HPV vaccination also differs by nativity, with 27.4% of adults aged 18 to 26 years born in the U.S. reporting vaccination compared to 14.3% not born in the U.S (25).

Prevention of HPV-related cancers has been cited as a priority of both international and U.S. health policy organizations (5–7)., In Australia, where HPV vaccination rates are high and supported through a national vaccination program, the HPV-associated disease burden has been substantially reduced – and is even projected to reach fewer than 4 new cases of cervical cancer per 100,000 by 2028 (26, 27).As follows, directed efforts including broader availability of HPV vaccination in clinical sites serving diverse populations are essential to increasing uptake. Studies have found that many patients receive the majority of their primary care with Ob/Gyn providers, underscoring the importance of ensuring

TABLE 3 HPV Vaccine Counseling and Uptake.

	All (n, %)	Hispanic White	Non-Hispanic White	Black (Hispanic)	Black (Non-Hispanic)	Haitian	Other (Hispanic)	Other (Non-Hispanic)	No Response
Total	304	124 (41%)	16 (5%)	6 (2%)	58 (19%)	29 (9.5%)	44 (14%)	7 (2%)	20 (6.6%)
Healthcare provider never discussed HPV infection	113 (37%)	40 (32%)	8 (50%)	0 (0%)	26 (45%)	16 (55%)	14 (32%)	1 (14%)	8 (40%)
Healthcare provider never discussed HPV vaccine	189 (62%)	76 (61%)	14 (88%)	3 (50%)	32 (55%)	20 (69%)	25 (57%)	5 (71%)	14 (70%)
Healthcare provider had never recommended HPV vaccine	211 (69%)	90 (73%)	14 (88%)	4 (67%)	39 (67%)	15 (52%)	30 (68%)	4 (57%)	15 (75%)
Did not receive the vaccine	241 (79%)	100 (81%)	14 (88%)	4 (67%)	46 (79%)	20 (69%)	39 (89%)	3 (43%)	15 (75%)

broad availability of and counselling regarding HPV vaccination in these settings (28). The success and barriers faced in implementing vaccination in our clinic highlight considerations for other sites that wish to replicate this model.

4.1 Knowledge

HPV knowledge is fundamental to increasing vaccine uptake (29–33). However, HPV and HPV vaccine knowledge in the U.S. remains low, with studies showing that only 68% of adults report having heard of HPV and the vaccine (34, 35). There are significant disparities in knowledge of and access to the vaccine (10). Associations have been noted between HPV knowledge and factors such as race, ethnicity, national origin, and primary language (11). There are multiple barriers to patient knowledge that have been identified in the literature, including language, cost, lack of information, and fear or mistrust of the healthcare system (36–38). While informational interventions can have a positive impact on vaccine uptake (29, 39, 40), educational interventions alone are likely insufficient to significantly improve vaccination rates (29, 41).

Consistent with prior literature, our study at a diverse safety net clinic found that most patients did not know basic information about the HPV vaccine, had not received HPV vaccination and that vaccination had never been discussed with them. High levels of health literacy have been linked to cervical cancer screening behaviors (42). Studies have also shown that health literacy is a stronger predictor of knowledge of risk factors for cervical dysplasia than race and ethnicity (43). Furthermore, increased information has a positive impact on vaccine uptake (39, 40, 44). Understanding the risk of HPV infection and benefits of vaccination has been associated with intention to vaccinate (45, 46).

Most patients in our study did not know that HPV infection could lead to cervical cancer, with a lower percentage of NHO, NHB, HW and Haitian patients, but a higher percentage of NHW, HB and HO patients reporting this knowledge. While this reflects findings of other studies that have demonstrated that NHB patients were less likely to report knowledge of the association between HPV and cervical cancer, it differs from findings among Hispanic patients (47). In contrast to other studies, we found proportionally higher reported knowledge of the association between HPV and cervical cancer among patients identifying as Hispanic other than HW patients (47). This discrepancy possibly reflects the unique demographic composition of Miami Dade County, which is majority Hispanic. Most patients in our study did not know basic information about the HPV vaccine such as ages of eligibility, number of doses, and whether men could receive the vaccine. Given the association between HPV and vaccine knowledge and uptake, it is imperative for healthcare providers to continue to strengthen targeted education efforts for patients and their communities.

4.2 Vaccination status

Most patients in our study had not received the HPV vaccine. NHO, HB and Haitian patients had the highest level of vaccine uptake. These results were not consistent with prior studies that have shown that misconceptions regarding HPV and vaccination are extremely common among immigrants from Haiti in the U.S (48). This discrepancy could reflect a higher level of Haitian and Haitian Creole-speaking providers in our population, but conclusions are limited by small sample size. It is important to increase education and outreach efforts, as studies have demonstrated that knowledge is a positive predictor of HPV vaccination (35).

4.3 Vaccination counselling

While many patients had discussed HPV with a healthcare provider, most had not discussed HPV vaccination or had the HPV vaccine recommended to them. This result was consistent across racial groups, with the greatest number of Hispanic patients reporting having discussed HPV and HPV vaccination. Other studies have found that many Hispanic and Haitian patients have not received vaccination recommendations from providers, and that patients with limited English proficiency were most affected (49, 50). The discrepancy in our data from these national studies likely reflects the unique patient and provider population of Miami Dade County, where the majority of the population is Hispanic. This is consistent with previous studies that have found significantly better outcomes among Hispanic patients with cervical cancer in Miami Dade County compared to nationally (51). Patient understanding and satisfaction increase in environments with culturally and linguistically concordant care (52, 53). Our data support creating clinical environments where providers and support staff have the linguistic ability and cultural competency to properly counsel patients.

Multiple studies have shown that a strong recommendation from a healthcare provider has a positive impact on vaccine uptake (50, 54–56). Furthermore, patients who report receiving HPV information from a physician have been found to have higher knowledge scores (35). In our study among those patients who were not vaccinated, lack of strong recommendation from a healthcare provider and lack of awareness of eligibility were cited as significant barriers. Additionally, previous work has shown major deficits in knowledge of the HPV vaccine among students in the health professions and healthcare providers (57). Even among patients in gynecologic oncology clinics, providers are less likely to offer vaccination to patients without HPV-related dysplasia (58). It is critical to ensure that healthcare providers are knowledgeable about HPV vaccination, up to date on current practice guidelines, and trained in counselling patients given the recent increase in vaccine hesitancy (59).

4.4 Vaccine administration

The quality improvement initiative performed in connection with our study noted an increase in HPV vaccine administration following expansion of eligibility to patients with insurance. Although many of our patients reported that they did not believe

that the HPV vaccine was available for same-day clinic administration, uptake increased with expanded eligibility. The convenience of administration is a known barrier to vaccine acceptance, administration, and series completion (60, 61). Interventions such as designated provider champions, clinical screening, financial assistance and elimination of barriers such a pregnancy testing have been shown to improve vaccine uptake (62). A limited number of patients completed the recommended 3-dose vaccine series, which is consistent with national trends (63–65). The percentage of women age 18 to 26 who received the recommended number of doses has been increasing, from 25.7% in 2013 to 35.3% in 2018, although completion rates still remain below target (9). Research has demonstrated protective benefits with even one dose of the vaccine, although the benefit increases with series completion (66). It is imperative that healthcare providers and clinic staff continue to educate patients regarding vaccination schedules and work to identify and minimize barriers to adherence with recommended follow-up. Additionally, to ensure that all patients who are eligible for vaccination have the opportunity to opt in, asking about HPV vaccine status should be included as a standard element of a gynecologic history.

4.5 Strengths and limitations

The strength of this study is the diverse patient population across a spectrum of age, race, ethnicity, and nativity. Additionally, the inclusion of Haitian identity allows for the ascertainment of unique disparities in this population. Limitations include survey administration at a single hospital site, limiting generalizability of findings to the national population. Notably, the majority of patients were Hispanic, and a comparatively low number of respondents identified as Haitian. Our surveys were anonymous, and we therefore were unable to link responses with vaccination data, series completion data or cervical cancer screening data in our clinic.

5 Conclusions

Our study demonstrates that there are significant deficits in knowledge regarding HPV and HPV vaccination and that HPV vaccination rates remain significantly below national goals in our safety net clinic population.

The lowest percentage of patients reporting knowledge that HPV causes cancer were found among NHO, NHB, HW and Haitian respondents. NHB patients had the highest percentage reporting that they did not know the health consequences of HPV infection. Additional counseling and community-based efforts are needed to address these knowledge gaps. While vaccine uptake did improve following the expansion of access, completion of the vaccination series remains challenging for our patients. Further studies are needed to identify interventions that can improve patient knowledge, vaccine uptake, and provider recommendation for vaccination, and assess barriers to vaccination series completion.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Jackson Memorial Hospital Institutional Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because Survey responses were completely anonymous and no chart review was performed. Decision was made by the Jackson Memorial Hospital Institutional Review Board that no written consent was required.

Author contributions

LF: Conceptualization, Data curation, Formal Analysis, Project administration, Writing – original draft, Writing – review & editing, Methodology. ML: Formal Analysis, Investigation, Writing – review & editing, Data curation, Methodology. AT: Data curation, Writing – review & editing, Formal Analysis, Investigation, Methodology. PJ: Conceptualization, Project administration, Validation, Writing – review & editing, Formal Analysis, Methodology, Resources, Supervision. MH: Conceptualization, Data curation, Formal Analysis, Investigation, Project administration, Resources, Supervision, Writing – review & editing, Methodology.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Trends of cervical tumours amongst women from perspectives of demographic, socioeconomic and geographic indicators: retrospective ecological study in Czechia

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Introduction: For many infectious diseases, women are at higher risk and have a more severe disease course than men for many reasons, including biological differences, social inequalities, and restrictive cultural norms. The study focuses on infections with human papillomaviruses (HPV) in the form of cervical cancer as a gender-specific disease. The main goal is to evaluate cervical tumour incidence trends in the Czech female population in the HPV vaccination period 2012–2020 in relation to selected demographic, socioeconomic, and geographic indicators.

Methods: This is a retrospective ecological study. Data from publicly available databases about the incidence and mortality of cervical tumours (C53 Malignant neoplasm of cervix uteri, D06 Carcinoma *in situ* of cervix uteri according to ICD 10) and HPV vaccination rate were analysed and compared with demographic, socioeconomic and territorial data. Associations were searched using correlation analysis.

Results: There was a decreasing trend in the incidence of cervical cancer in the observed period. Regarding cervical tumours (C53, D06) and malignant neoplasm of cervix uteri incidence (C53), the decrease was approximately 11 and 20%, respectively. Differences between regions were observed in incidences and vaccination rates. Based on correlation analysis, indicators connected with urban/rural aspects, such as a share of urban population and population density, were statistically significant. The indicators related to higher cervical cancer incidence are the high unemployment rate of women, the high number of divorces, the high number of abortions, the high share of the urban population, the high number of students, and the high number of women with only primary education. On the other hand, the indicators related to lower cervical cancer incidence are the high gross domestic product (GDP), the high average gross monthly wage per employee, the high employment rate of women, the higher average age of mothers at birth, and the high number of women with tertiary education.

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Conclusion: Results underline the problem of economically disadvantaged regions and families. Increasing vaccination rates, promoting regular screening for cervical cancer, and supporting awareness in the population, especially in regions with higher incidence rates, should be priorities for public health efforts.

KEYWORDS

human papillomavirus, cervical tumour, women, vaccination rate, indicators

1 Introduction

Diseases are not only biophysical phenomena but have social and cultural causes and consequences. These also include the availability and nature of treatment and the extent to which treatment is accepted or adhered to. Currently, the emphasis is on an interdisciplinary approach to understanding health and disease. At the same time, more and more attention is being paid to the influence of gender on health status, as well as to the individual's approach to disease prevention. For the purpose of this study, the terms "female" and "woman" are perceived from both a biological (sex) and sociological (gender) point of view. Gender refers to the cultural characteristics and models assigned to the male or female biological sex and refers to social differences between women and men (1).

Also, in infectious diseases, sexual dimorphism has been described (2). For many infectious diseases, women are at higher risk and have a more severe disease course than men (3). Health disbalance in infectious diseases between men and women is the result of interactions between biological and sociocultural factors, such as sex hormones, genetic predisposition, lifestyle, age, social inequalities, restrictive cultural norms, the geographic distribution of pathogens, and access to healthcare or comorbidities (3–5). Women are less burdened than men when it comes to developing most infectious diseases because of hormonal and chromosomal control of immunity (6). Estradiol provides immune protection, but progesterone and testosterone suppress anti-infective responses. Women demonstrate a more remarkable ability to recognise pathogens, recruit more innate immune cells, and mount stronger adaptive immune responses than men (5). Although the finding is that estradiol helps women manage infectious diseases, it is also necessary to be aware of the mentioned socioeconomic influences on the course of diseases (5). Women make up the dominant part of the population at risk of poverty, especially single mothers and pensioners. However, the social benefits system does not sufficiently consider this aspect. The consequence of infectious disease is that it will restrict various areas of a woman's life. They can only occur for a certain period, long-term or permanently, while some symptoms accompanying the disease can also negatively affect the work sphere. There can be various complications that lead to long-term incapacity for work, which is related to the financial impact of illnesses. Socioeconomic factors, both material and psychosocial, can impact infectious diseases.

Regarding infectious diseases and their impact on the female population, the following work focuses on infections with human papillomaviruses (HPV) in the form of cervical cancer as a gender-specific disease. Worldwide, cervical cancer is the fourth most common cancer in women (7). HPV is the most common sexually transmitted infection. Before age 50, genital HPV infection occurs in 80% of women and at least 50% of men (8). HPV causes asymptomatic infections in most cases but also several benign diseases with high morbidity and several other premalignant diseases and cancers in both women and men. Cervical cancer is by far the most common HPV-related disease. About 99.7% of cervical cancer cases are caused by persistent genital high-risk HPV infection (9, 10). Regarding scientific studies focused on cervical cancer and connected issues, there is still a gap. There exist studies dealing with trends of cervical cancer incidence and possible indicators which can influence both incidence and mortality.

One of such indicators is screening, vaccination, and the age of screening and vaccination. Screening and treatment of pre-cancer lesions with HPV vaccination are effective measures to eradicate cervical cancer as a global public health problem (11). According to Cancer Research United Kingdom (UK) (12), girls who are vaccinated between the ages of 12 and 13 have an 87% lower incidence of cervical cancer in their 20s compared to those who have not been vaccinated. The effectiveness decreased with the advanced age of the vaccinated. Also, vaccination of boys and men may reduce the incidence of cervical cancer and its precursors via herd immunity (13). Although there is no evidence of a clear impact on cervical cancer elimination by vaccinating boys, vaccination directly protects men from HPV-related diseases, and most high-income countries have implemented gender-neutral programmes. European Cancer Organization aims to have a gender-neutral approach all over Europe by 2030 (14). On the other hand, it is assumed that HPV vaccination rates declined as a result of the COVID-19 pandemic. According to the WHO, vaccination coverage worldwide decreased by over a quarter compared to 2019 (15).

Other important indicators are demographic and/or socio-economic factors. For example, a study from India (16) underlines the following significant risk factors for HPV infection: early age at marriage, lack of education, increased parity, early age at first pregnancy, poor sanitation, use of tobacco, and belonging to below poverty line. Buskwofie et al. (7) observed the situation in the USA and depicted the following risk factors: racial and ethnic minorities and socioeconomically disenfranchised. Concerning the situation in China, the influence of HPV-related knowledge on HPV testing also lies in the joint effects of socio-demographic factors, including residence, education, and monthly

Abbreviations: CBR, Central Bohemia Region; HKR, Hradec Králové Region; HPV, human papillomavirus; KVR, Karlovy Vary Region; LBR, Liberec Region; MSR, Moravian-Silesian Region and region with the lowest incidence of cervical tumours (C53, D06); OLR, Olomouc Region; PAR, Pardubice Region; PCC, Prague the Capital City; PLR, Plzeň Region and region with the highest incidence of cervical tumours (C53, D06); SBR, South Bohemian Region; SMR, South Moravian Region; ULR, Ústí nad Labem Region; VYR, Vysočina Region; ZLR, Zlín Region.

income (17). Besides the factors mentioned above, there are others, such as location and/or differences between urban and rural areas (7, 18, 19).

Concerning the situation in Czechia, roughly 92% of cervical, 35% of vulvar, 82% of anal and 65% of oropharyngeal tumours were associated with HPV types included in the nonvalent HPV vaccine (20). Currently, three prophylactic vaccines against HPV infection are available: bivalent Cervarix, quadrivalent Gardasil (formerly Silgard) and nonavalent Gardasil9. The insurance companies have covered HPV vaccination for girls aged 13 since 2012 and boys of the same age since 2018. According to available vaccination data, the vaccination rate of girls aged 13 represented 75.7% in 2012 and only 60.2% in 2018 (21). Currently, the vaccination rate is around 60; on the other hand, there are significant differences between regions (21). The main cause of insufficient vaccination in Czechia is "vaccine hesitancy," the distrust in vaccination caused by the spread of misinformation (22). On the other hand, the significant increase in the number of vaccinated boys, which was only 29.7% in 2018/19, is positive (23).

EUROSTAT states that cervical cancer screening coverage is 52.5% (24). All Czech women over 15 years old are screened yearly by Pap test. From 2021, the HPV screening test (examination of the presence of nucleic acid of high-risk types of HPV in cervical smear) is paid by public health insurance funds for all women aged 35 and 45.The overall prevalence of HPV in Czechia remains relatively high, with a 2020 study (24, 25) about 6.6% of women in the general population are estimated to harbour cervical HPV-16/18 infection and 79.3% of invasive cervical cancers are attributed to HPVs 16 or 18. According to the HPV information centre and its estimation for 2020 (24), about 769 new cervical cancer cases are diagnosed, and about 398 cervical cancer deaths occur annually in Czechia.

The main goal of this paper is to evaluate the incidence trends of cervical tumours in the Czech female population in the HPV vaccination period 2012–2020 in relation to selected demographic, socioeconomic, and geographic indicators. The sub-goals were to analyse: (i) the trends in the incidence and mortality of cervical tumours over the vaccination period 2012–2020; (ii) the differences in the cervical tumours incidence rate between regions, urban and rural areas; (iii) relationship of the cervical tumours incidence rate with selected demographic and socioeconomic indicators.

Unique is that this paper focuses on not only malignant cervical tumours but also carcinoma *in situ*. The incidence of all these cervical tumours (both malignant neoplasm C53 and carcinoma *in situ* D06 according to ICD 10) more closely reflects the risk of HPV exposure. Also, the unique location of Czechia in the centre of Europe and its regional diversity enables the transferability of the results of our study to other regions as well. Therefore, this data analysis that our study will bring could be used for nationwide education regarding HPV knowledge. The new perspectives on the issue of HPV, which our study offers, can significantly contribute to the development of knowledge in this area and thus support the prophylaxis of this type of disease not only in the female population but all over the world.

2 Materials and methods

2.1 Study settings

This is a retrospective ecological study based on analysis of cervical tumours (C53, D06) incidence trends in relation to demographic and socioeconomic indicators. The study population was women from Czechia between 2012 and 2020. The datasets used and/or analysed during the current study are all publicly available.

2.2 Study location

Czechia (the Czech Republic) is a country in Central Europe with a population of 10,516,707, of which 5,332,932 are women (26). The average life expectancy for women was 82.1 years in 2019 (27). Women population at risk for cervical cancer C53 (female population aged >= 15 years) is about 4.6 million (24). According to estimations for 2020, cervical cancer ranks as the 11th leading cause of female cancer and as the 8th leading cause of cancer deaths of female cancer deaths in Czechia (24). Czechia is divided into 14 regions, which are: Prague, the Capital City (PCC), Central Bohemia Region (CBR), South Bohemian Region (SBR), Plzeň Region (PLR), Karlovy Vary Region (KVR), Ústí nad Labem Region (ULR), Liberec Region (LBR), Hradec Králové Region (HKR), Pardubice Region (PAR), Vysočina Region (VYR), South Moravian Region (SMR), Olomouc Region (OLR), Zlín Region (ZLR), Moravian-Silesian Region (MSR). Furthermore, only abbreviations of regions are used for the purpose of this study.

2.3 Input data

All the data comes from the State Statistical Service, which acquires data and compiles statistical information on Czechia's social, economic, demographic, and ecological development. The primary data sources were the State Statistical Service authorities such as the Czech Statistical Office (CSO) and The Institute of Health Information and Statistics of the Czech Republic (IHIS), which administrates the National Health Information System. These authorities are governed by principles of the European Statistics Code of Practice. Data about women in 5-year age categories (age 0–85+) at the level of 14 regions of the Czechia for the period 2012–2020 was used. The following population data from publicly available databases was used as input data sources.

2.3.1 Health data

2.3.1.1 Incidence and mortality of cervical tumours

Absolute incidence and mortality of cervical tumours (C53 Malignant neoplasm of cervix uteri, D06 Carcinoma *in situ* of cervix uteri according to ICD 10) were obtained from the National Oncological Register administered by the IHIS and processed by the Institute of Biostatistics and Analyses (28).

2.3.1.2 HPV vaccination rate

HPV vaccination rate was obtained from the National Register of Reimbursed Health Services administered by the IHIS. Vaccination against HPV is identified from the documents on reported health care using the ATC code J07BM or one of the procedures 02110, 02125 in combination with diagnosis Z258. HPV vaccination rate of prime-vaccinated female patients relative to the female population aged 13 years between 2012 and 2019 is presented (the number of females vaccinated in a given year corresponds to

patients who reached the age of 13 in a given year and were vaccinated in a given or the following calendar year). The insurance companies have covered HPV vaccination for girls aged 13 since 2012 in Czechia.

2.3.2 Demographic and socioeconomic data

Demographic and socioeconomic data were obtained exclusively from the CSO. The data used are freely available and aggregated at the level of regions of Czechia. These data can be divided into:

a Data about the age distribution of the women population published yearly (29).
b Data about territorial comparison of demographic and socioeconomic indicators by regions, which concern the entire population (30). They contain indicators that are not gender specific. In correlation, there were used indicators related to the whole population about:

- population (population density, share of urban population, total population change, infant mortality, number of students)
- migration (immigration, emigration)
- socioeconomic indicators (gross domestic product, average gross monthly wage per employee, pension recipients, number of old-age pensions)

c Data about territorial comparison of demographic and socioeconomic indicators by regions, which concern only women (31). There were used indicators about:

- age
- population gain/loss (total and natural population gain/loss, births, deaths)
- marriages and divorces
- abortions
- level of education
- employment (employment and unemployment rate).

2.3.3 Geographical data

The layer Boundaries from the Topographic database of the Czech Republic (Data200) were used. The database is published under a Creative Commons CC BY 4.0 licence by the State Administration of Land Surveying and Cadastre (32).

2.4 Data processing

2.4.1 Conversion of absolute incidence and mortality to relative numbers (per 100,000 women)

The relative incidence and mortality numbers were calculated from absolute values according to the average state of the population as of the first of July of the given year according to the data from CSO. These indicators were calculated by dividing the published number by the population size for each region, and each age group was displayed as units available for 100,000 women. Furthermore, only these relative numbers were used.

2.4.2 Analysis of trends in the incidence and mortality of cervical cancer

Trends of incidence and mortality of cervical cancer were analysed during the monitored period, and a sub-analysis of age distribution in 5-year age categories (age 0–85+) was made. First, the analysis was conducted for Czechia overall and subsequently for individual regions. Relative incidence and mortality trends in the individual regions were compared with the overall trend of Czechia using correlation analysis.

2.4.3 Spatial visualisation and identification of the regions with the lowest and highest incidence

The data was visualised through analytical maps and colour scales to determine the spatial phenomenon. The maps were created using QGIS 3.26.3 software. The regions with the highest and lowest incidence were selected for the following analysis based on the data.

2.4.4 Analysis of the incidence of cervical cancer in relation to demographic, socioeconomic and geographic indicators in regions

Associations of incidence of cervical cancer with selected demographic and socioeconomic indicators (specified in Input Data) were searched using correlation analysis. The evolution of year incidence during the studied period (dependent variable Y) was compared with the trend of each regional socioeconomic and demographic characteristic specified in Input Data (independent variable X). Correlations were calculated for selected regions only. Pearson correlation coefficient was calculated using TIBCO Statistica software. The study did not include variables that were correlated or a subset of another variable. This was tested using the Correlation matrix. Statistical significance cut-off was determined at $p < 0.05$.

3 Results

3.1 Trends in the incidence and mortality of cervical tumours

Figure 1 shows overall trends of the relative incidence and mortality of cervical tumours in Czechia for the monitored period from 2012 to 2020. There is a noticeable overall decreasing trend in cervical tumours (C53, D06) incidence, only with some higher incidences in years 2015 and 2016. In the last year of the studied period, the incidence increased slightly. The relative incidence of cervical tumours (C53, D06) in 2020 has decreased by 11.07% compared to 2012. In the case of separate malignant neoplasm of cervix uteri (C53) incidence, the overall trend is slightly decreasing, with some higher incidence in 2015 and 2019. However, the relative incidence in 2020 has decreased by 20.25% compared to 2012. Relative mortality of cervical tumours (C53, D06) is persistently low with a slightly decreasing trend, and in 2020, it decreased by 19.10% compared to 2012. This mortality is caused only by malignant neoplasm of cervix uteri (C53). So cervical tumours (C53, D06) mortality, which is used further, is equal to the separate mortality of C53.

The HPV vaccination rate of prime-vaccinated female patients relative to the female population aged 13 between 2012 and 2019 in

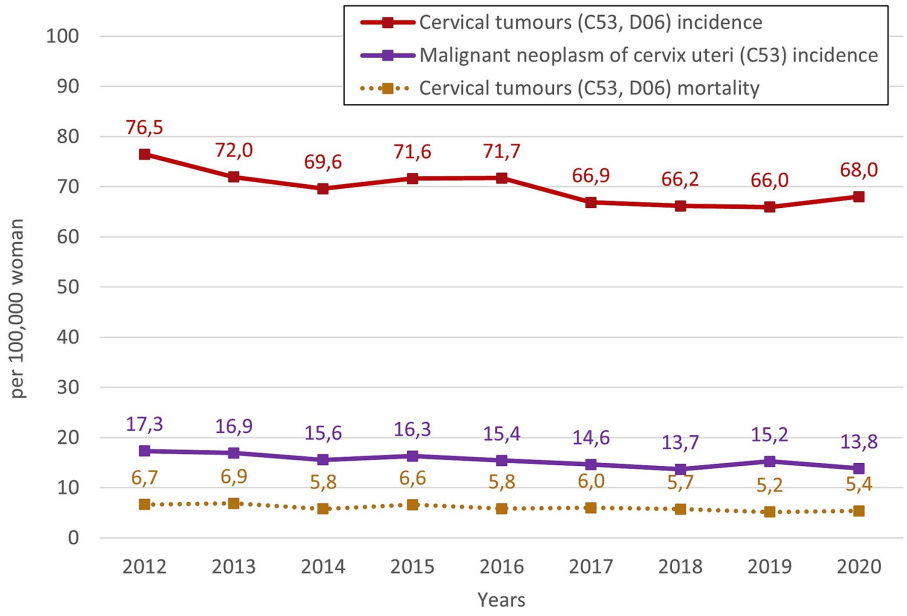

FIGURE 1
Overall trends of the relative incidence and mortality of cervical tumours in Czechia for the monitored period 2012–2020.

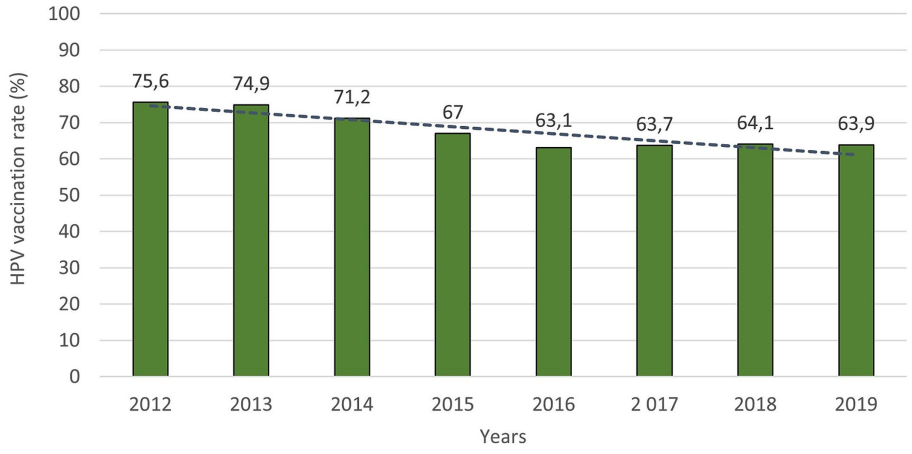

FIGURE 2
HPV vaccination rate of prime-vaccinated female patients relative to the female population aged 13 years between 2012 and 2019 (the number of females vaccinated in a given year corresponds to patients who reached the age of 13 in a given year and were vaccinated in a given or the following calendar year).

Czechia is shown in Figure 2. The number of females vaccinated in a given year corresponds to patients who reached the age of 13 in a given year and were vaccinated in a given or the following calendar year. Data about the HPV vaccination rate for the year 2020 was not published at the time of processing this paper. There has been a noticeable decrease in the vaccination rate from the start of vaccination in 2012 to 2019. Vaccination rates fell by 11.7% between those years. Figure 3 shows the HPV vaccination rate of particular regions in 2019. Differences between individual regions are evident from this visualisation. There are regions where the vaccination rate is less than 60% (ZLR, MSR, SMR, PCC).

Table 1 shows the relative incidence of cervical tumours in the studied period and age distribution analysis. All cervical tumours (C53, D06) were most often diagnosed in the age group 20–44 years. The incidence was higher than 100 per 100,000 women in these age groups. The highest incidence was in the age group 25–34 years. In separate malignant neoplasm of cervix uteri (C53), the higher incidence (over 20 per 100,000 women) started appearing from the age group 35–39 years and above. Table 2 shows the relative mortality of cervical tumours (C53, D06) in the studied period and age distribution analysis. Mortality was most frequent in the older age groups. It increased significantly from over 60 years with the highest frequency in age over 85.

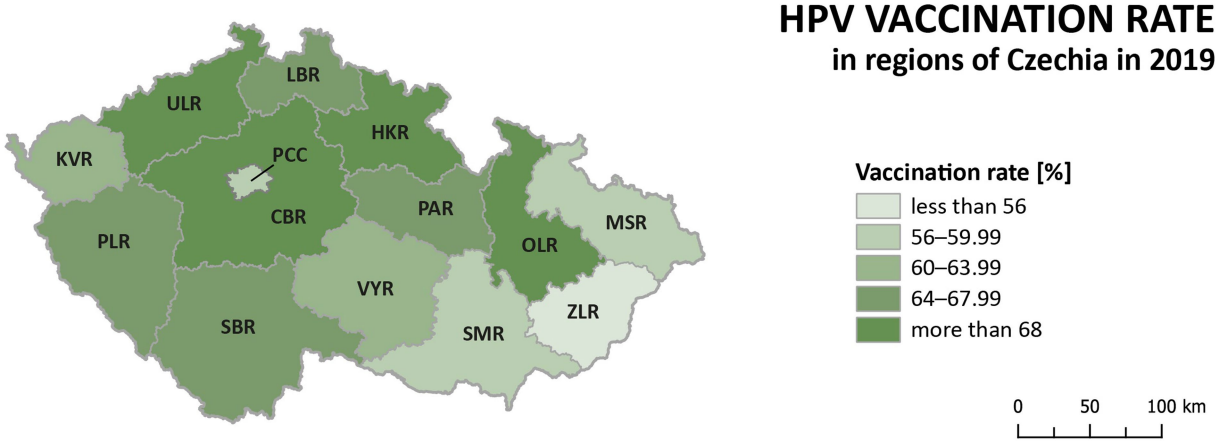

FIGURE 3
HPV vaccination rate of prime-vaccinated female patients relative to the female population aged 13 years in regions of Czechia in 2019 (the number of females vaccinated in a given year corresponds to patients who reached the age of 13 in a given year and were vaccinated in a given or the following calendar year).

3.2 Differences in the cervical tumours incidence and mortality between regions

Analysis of relative incidence trends in 14 regions of Czechia for cervical tumours (C53, D06) is shown in Figure 4 and separately for malignant neoplasm of cervix uteri (C53) in Figure 5. The data are visualised in the form of analytical maps for the initial and final years (2012 and 2020) and by colour scales for all studied years. Table 3 shows the correlation between cervical tumours' relative incidence and mortality for each region and the overall trend of Czechia. In the case of all cervical tumours (C53, D06), the difference in the individual regions is evident, even in trends through the studied period (Figure 4). As in the whole of Czechia, there is a decrease in incidence in some regions, but there are also regions where, on the contrary, there is a slight increase (PCC, VYR, CBR). Regions PLR and MSR constantly had the highest and lowest incidence, respectively, in all studied years. For both regions, the trend is correlated with the decreasing overall trend of Czechia, as shown in Table 3. There are no evident regions with consistently opposite incidence numbers in separate malignant neoplasms of the cervix uteri (C53) (Figure 5). There are differences in the individual regions. However, there are no apparent persistent trends throughout the studied period. A comparison of cervical tumours (C53, D06) mortality trends is shown in Figure 6. Higher relative mortality (over 7 per 100,000 women) is apparent in KVR and ULR regions and is persistently higher in almost all studied years. From the other point of view, the comparison does not show a region with significantly lower mortality, which would be stable through the analysed period.

So, the regions PLR and MSR are used for further analysis because of the similar trend with the overall trend of Czechia, but with diametrically different numbers of cervical tumours new cases through the studied period. Also, as already mentioned, the incidence of all cervical tumours (both malignant neoplasm C53 and carcinoma *in situ* D06) more closely reflects the risk of HPV exposure than separate malignant neoplasm C53. Trend of cervical tumours incidence in regions PRL - region with the highest cervical tumours (C53, D06) incidence and MSR-region with the lowest cervical tumours (C53, D06) incidence in comparison to the overall trend of the Czechia is shown in Figure 7. Figure 8 shows the incidence of cervical tumours (C53, D06) in selected regions in age groups. The relative incidence of cervical tumours (C53, D06) in PLR (the region with the highest incidence) decreased distinctly during the studied period. The most significant difference between 2012 and 2020 was observed in the most vulnerable age groups, the 20–44 age group. Figure 9 shows the incidence of malignant neoplasm cervix uteri (C53), where the difference is not apparent. Also, the difference between the two selected regions and the whole country is not prominent. Figure 10 shows the mortality of cervical tumours (C53, D06) in selected regions in different age groups.

3.3 The relationship of the cervical tumours incidence rate with selected demographic and socioeconomic indicators

Table 4 presents the results of the correlation analysis, focusing on demographic indicators of regions with the lowest (MSR) and highest (PLR) incidence in the period 2012–2020. The results show us the relationships between cervical tumours relative incidence and selected variables connected with the life and behaviour of women and, in some cases, the entire population. In the observed regions, there is a statistically significant positive correlation between cervical tumour relative incidence and (1) divorces (MSR, PLR), (2) abortions (PLR), (3) share of urban population (MSR, PLR), (4) the total number of students (MSR), and (5) women with primary education (MSR). These variables show positive correlations in both regions; however, they are not statistically significant in some cases. In both selected regions, we can observe a statistically significant negative correlation between cervical tumours relative incidence and (1) average age/age index (MSR, PLR), (2) live births woman (MSR, PLR), (3) average age of mother at birth (MSR, PLR), (4) marriages (MSR), (5) immigrants (MSR, PLR) and (6) woman with tertiary education (MSR, PLR). Regarding the regional aspects and differences, there are almost the same statistically significant results for the region with the lowest and

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TABLE 1 Relative incidence of cervical tumours by age group in Czechia (2012–2020).

	Age	Incidence per 100,000 women of each age group								
		2012	2013	2014	2015	2016	2017	2018	2019	2020
Malignant neoplasm (C53) and carcinoma *in situ* (D06) of cervix uteri	0–4	0	0	0	0	0	0	0	0	0
	5–9	0	0	0	0	0	0	0	0	0
	10–14	0	0	0	0	0	0	0.4	0	0
	15–19	11.7	11.2	13.1	9.4	13.9	11.6	4.0	3.5	2.6
	20–24	**105.7**	**104.3**	**100.3**	98.5	98.8	94.2	97.0	72.1	59.1
	25–29	**215.6**	**198.3**	**195.1**	**199.4**	**202.8**	**187.4**	**190.5**	**150.9**	**161.4**
	30–34	**192.0**	**173.0**	**183.0**	**190.2**	**199.3**	**185.9**	**187.5**	**188.6**	**207.0**
	35–39	**152.2**	**157.6**	**139.6**	**152.1**	**142.4**	**139.5**	**139.4**	**147.2**	**167.5**
	40–44	**124.2**	**103.3**	**105.0**	**110.3**	**116.2**	**106.2**	**109.2**	**113.7**	**117.5**
	45–49	77.6	79.0	70.9	75.0	82.6	72.6	83.1	99.4	99.8
	50–54	45.2	44.6	40.4	51.8	47.6	45.5	43.6	55.0	60.4
	55–59	41.2	38.5	38.3	31.8	37.1	32.9	37.3	38.5	35.5
	60–64	35.4	38.7	37.1	35.5	37.1	37.5	34.5	31.2	29.0
	65–69	38.2	37.1	43.4	38.9	32.7	32.2	30.9	32.2	34.3
	70–74	31.9	31.4	27.2	32.6	35.1	30.3	26.4	38.0	31.3
	75–79	35.7	30.1	26.0	29.5	27.3	31.8	20.5	30.8	25.4
	80–84	32.6	26.1	26.3	36.6	28.5	23.4	17.3	17.0	25.8
	85+	36.3	26.9	28.3	22.8	19.2	29.4	22.6	21.0	20.3
	Total	76.5	72.0	69.6	71.6	71.7	66.9	66.2	66.0	68.0
Malignant neoplasm of cervix uteri (C53)	0–4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	5–9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	10–14	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0
	15–19	0.8	0.0	0.0	0.4	0.4	0.0	0.4	0.0	0.0
	20–24	2.2	2.8	2.3	1.7	1.8	1.5	1.2	3.4	0.9
	25–29	8.6	7.9	8.8	12.1	6.8	5.4	8.8	8.1	5.2
	30–34	16.1	16.6	14.9	13.9	13.8	13.3	13.7	15.4	13.2
	35–39	**22.6**	**25.5**	**22.6**	18.3	18.1	17.6	15.7	17.6	**20.8**
	40–44	**29.5**	**23.3**	**20.2**	**23.5**	**22.5**	19.8	18.7	19.5	17.9
	45–49	**24.5**	**25.3**	**22.9**	**20.7**	**24.7**	**22.3**	**22.1**	**26.1**	**22.7**
	50–54	**20.4**	19.9	16.4	**23.1**	**25.0**	18.1	19.3	**24.0**	**21.3**
	55–59	**23.4**	**25.7**	**23.0**	**21.1**	**19.5**	**23.3**	**21.2**	**22.9**	18.8
	60–64	**23.0**	**27.7**	**23.4**	**25.2**	**24.2**	**24.9**	**21.9**	19.2	17.7
	65–69	**28.7**	**25.4**	**27.3**	**29.3**	**23.1**	**22.2**	19.4	**20.9**	**21.0**
	70–74	**23.4**	**22.1**	**20.8**	**21.7**	**23.8**	**21.9**	19.0	**27.8**	**20.2**
	75–79	**29.8**	**23.5**	**20.1**	**23.7**	**20.2**	**20.7**	16.4	**21.8**	17.2
	80–84	**28.7**	**20.2**	**24.3**	**29.3**	**23.8**	17.9	15.2	10.9	20.5
	85+	**32.9**	**26.9**	**24.4**	**22.1**	17.0	**25.1**	19.1	16.8	18.2
	Total	17.3	16.9	15.6	16.3	15.4	14.6	13.7	15.2	13.8

Bold text visualise age groups with highest incidence (C53, D06 incidence over 100 per 100,000 women, C53 incidence over 20 per 100,000 women).

highest incidence, except for population density. Focusing on data in more detail in PLR, there is an increase in population density and a decrease in the share of the urban population in the observed period. That explains the negative correlation between the incidence of cervical tumours and population density in this region.

The following Table 5 presents the results of the correlation analysis, focusing on socioeconomic indicators of regions with the lowest (MSR) and highest (PLR) incidence in the period 2012–2020. The results show us the relationships between the relative incidence of cervical tumours and selected socioeconomic indicators. In the

TABLE 2 Relative mortality of cervical tumours by age group in the Czechia (2012–2020).

Age	Mortality per 100,000 women of each age group								
	Malignant neoplasm (C53) and carcinoma *in situ* (D06) of cervix uteri								
	2012	2013	2014	2015	2016	2017	2018	2019	2020
0–4	0	0	0	0	0	0	0	0	0
5–9	0	0	0	0	0	0	0	0	0
10–14	0	0	0	0	0	0	0	0	0
15–19	0	0	0	0	0	0	0	0.4	0
20–24	0	0.3	0.6	0	0	0.8	0	0	0
25–29	0.3	1.7	0.6	0.9	1.8	0	0.3	0.6	0.7
30–34	1.0	2.3	2.2	1.1	2.0	1.4	1.1	1.1	1.2
35–39	4.2	2.2	2.4	2.7	1.4	2.7	2.1	1.1	1.9
40–44	4.5	3.5	4.1	4.6	6.0	4.2	1.8	3.5	3.2
45–49	9.5	8.4	7.7	5.6	5.2	5.9	5.7	4.1	6.2
50–54	6.0	8.9	6.5	8.4	7.6	7.6	5.6	8.6	5.3
55–59	9.0	8.7	**11.1**	9.2	7.7	8.6	8.4	**10.0**	**10.0**
60–64	11.9	**12.3**	7.9	**14.3**	**10.7**	**10.1**	**11.8**	9.9	9.5
65–69	**14.0**	**14.8**	**12.5**	**12.0**	**12.3**	**12.7**	**13.4**	**10.7**	**11.6**
70–74	**21.3**	**15.7**	**11.7**	**21.4**	**11.4**	**15.8**	**15.0**	**12.3**	**14.0**
75–79	**12.5**	**21.9**	**17.4**	**14.2**	**16.7**	**11.1**	**13.2**	**12.8**	9.8
80–84	**23.4**	**17.6**	**13.8**	**20.0**	**16.3**	**16.5**	**17.3**	**10.2**	**12.6**
85+	**24.5**	**29.3**	**20.4**	**18.3**	**17.7**	**23.0**	**20.5**	**11.9**	**17.5**
Total	6.7	6.9	5.8	6.6	5.8	6.0	5.7	5.2	5.4

Bold text visualise age groups with highest mortality (C53, D06 incidence over 100 per 100,000 women, C53 incidence over 20 per 100,000 women).

observed regions, there is a statistically significant positive correlation between cervical tumours relative incidence and the only socioeconomic variable–the unemployment rate of women (MSR). This correlation is positive in both regions; however, statistical significance is relevant only in MSR. In both selected regions, we can observe a statistically significant negative correlation between cervical tumour relative incidence and (1) gross domestic product (MSR, PLR), (2) the total number of pension recipients (PLR), (3) old age pensions (MSR, PLR), (4) average gross monthly wage per employee (MSR), and (5) employment rate (MSR).

4 Discussion

For many infectious diseases, women are at higher risk and have a more severe disease course than men for many reasons, including differences between biological and sociocultural factors. This study focuses on infections with human papillomaviruses (HPV) in the form of cervical cancer as a gender-specific disease. Before age 50, genital HPV infection occurs in 80 percent of women (8), and cervical cancer is the fourth most common cancer in women worldwide (7). Regarding scientific studies focused on cervical cancer and connected issues, there is still a gap in the knowledge of possible indicators which can influence both incidence and mortality.

Firstly, the overall trends of the incidence and mortality of cervical cancer in Czechia for the period 2012–2020 were observed. Generally, there is a decreasing trend in the incidence of cervical cancer in the observed period. Regarding cervical tumours (C53, D06), the decrease in incidence between 2012 and 2020 is approximately 11% (from 76.49 per 100,000 women in 2012 to 68.02 per 100,000 women in 2020). Focusing on the incidence of malignant neoplasm of cervix uteri (C53), the decrease is more than 20% (17.33 per 100,000 women in 2012 versus 13.82 per 100,000 women in 2020). In the case of mortality, the trend also shows a decrease in mortality rate since 2012, approximately 19% (from 6.67 per 100,000 women in 2012 to 5.40 per 100,000 women in 2020).

It is clear that vaccination is worthwhile, and the positive impact of vaccination on cervical cancer incidence and mortality should increase over time. Czechia has sufficient available vaccines and an established vaccination programme. On the other hand, the vaccination rate is decreasing despite financing the vaccines by insurance companies since 2012 for girls aged 13. Vaccination rates of girls aged 13 fell by 11.7% between 2012 and 2019 (from 75.5 to 63.9%). The leading cause of insufficient vaccination in Czechia is "vaccine hesitancy," the distrust in vaccination caused by misinformation. For HPV vaccination, written informed consent from parents and children is needed. If the girl requests an offered HPV immunisation, but the parents refuse consent, she can be immunised. However, if the parents or guardians request immunisation, but the girl objects, a court decision is needed for being vaccinated (22). Overall, trust in vaccination in Czech society is decreasing. This applies to all types of vaccinations; overall, there is a decrease. This decrease is sometimes even more than 10% (e.g., MMR vaccine). The consequence of this behaviour is the occurrence of originally

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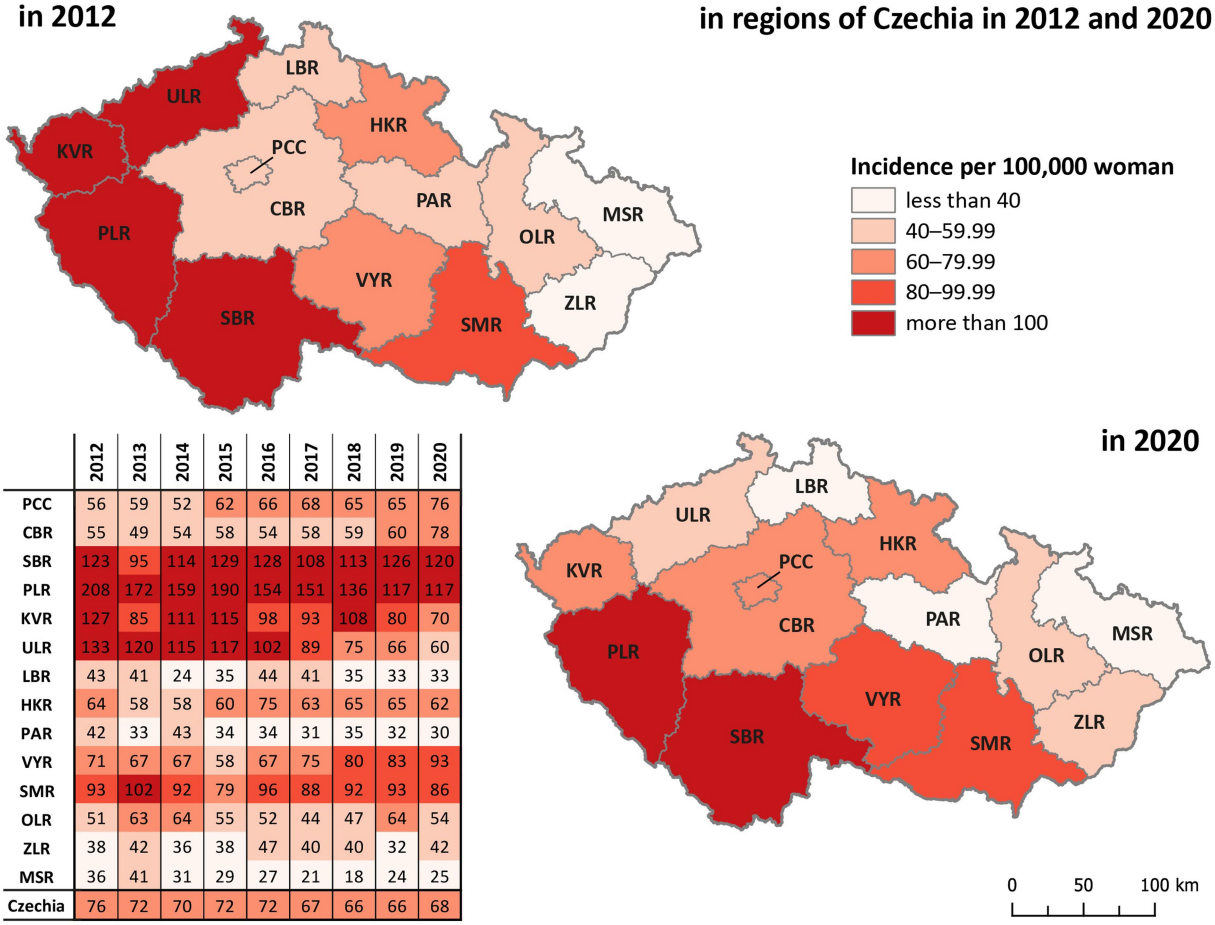


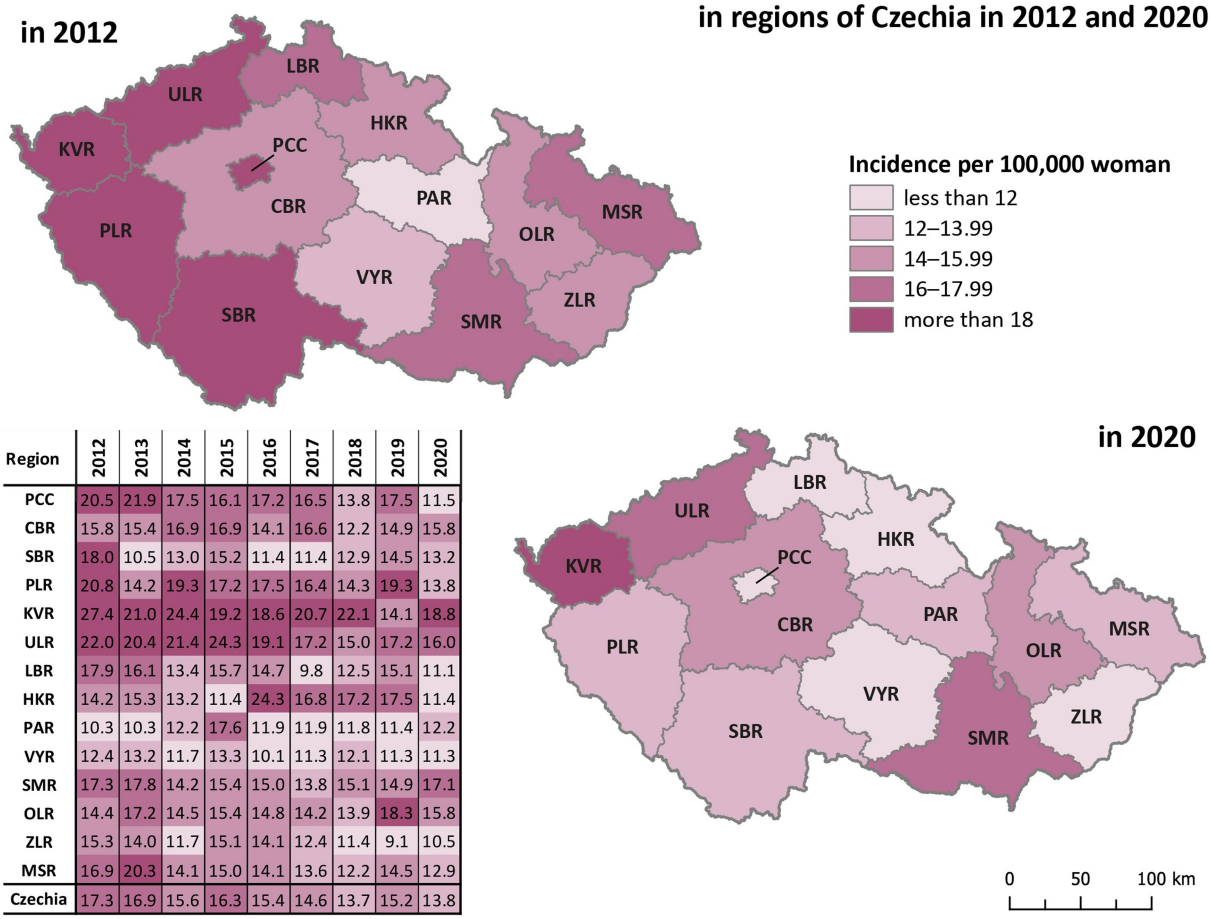
	2012	2013	2014	2015	2016	2017	2018	2019	2020
PCC	56	59	52	62	66	68	65	65	76
CBR	55	49	54	58	54	58	59	60	78
SBR	123	95	114	129	128	108	113	126	120
PLR	208	172	159	190	154	151	136	117	117
KVR	127	85	111	115	98	93	108	80	70
ULR	133	120	115	117	102	89	75	66	60
LBR	43	41	24	35	44	41	35	33	33
HKR	64	58	58	60	75	63	65	65	62
PAR	42	33	43	34	34	31	35	32	30
VYR	71	67	67	58	67	75	80	83	93
SMR	93	102	92	79	96	88	92	93	86
OLR	51	63	64	55	52	44	47	64	54
ZLR	38	42	36	38	47	40	40	32	42
MSR	36	41	31	29	27	21	18	24	25
Czechia	76	72	70	72	72	67	66	66	68

FIGURE 4
Comparison of cervical tumours (C53, D06) relative incidence in 14 regions of the Czechia (2012–2020).

eliminated diseases. Support from policy-makers, the government, and ministries is necessary. Unequivocal support of primary prevention programmes, their accentuation and highlighting of benefits, the safety of these measures, etc. Unfortunately, the misinformation scene and fake news, which are related to vaccination as such, play a significant role in this.

On the other hand, the significant increase in the number of vaccinated boys, which was only 29.7% in 2018/19, is positive (23). According to the WHO, for example, Uzbekistan achieves high HPV vaccination coverage against cervical cancer when 94% of girls aged 12–14 are now covered with a first dose of HPV vaccine (33). Globally, about 50% of countries have introduced HPV vaccination. WHO issued a call for cervical cancer elimination in 2018 and recommended the extension of HPV vaccination to boys. HPV vaccination to boys appeared more cost-effective compared with increasing vaccine uptake amongst girls in cases where vaccination coverage amongst girls is persistently lower than 75–80%. Universal HPV vaccination is likely more effective and efficient in reducing HPV virus circulation in the general population, even at lower vaccine uptake levels. In December 2021, all European Union/European Economic Area countries introduced HPV vaccination in their national programmes. Several countries (i.e., Austria, Belgium, Croatia, Czechia, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Liechtenstein, the Netherlands, Norway, Portugal, Slovakia, Slovenia, Spain, Sweden, the United Kingdom) have extended, or have decided to extend in the coming years, HPV vaccination to boys (34, 35).

In Czechia, HPV vaccination for boys aged 13 has been covered by public health insurance since 2018. In neighbouring countries, vaccination strategies for boys differ. In Austria, the HPV vaccine is offered free of charge to all children aged 9–12 years since 2014. Before 2014, the vaccine was recommended but not publicly funded. In Germany, since November 2018, HPV vaccination for all 9–14-year-olds and catch-up HPV vaccination for girls and boys 15–17-year-olds has been included in the catalogue of mandatory benefits of statutory health insurance. In Poland, by 2021, HPV vaccination was not part of the mandatory vaccination programme but was recommended for boys and girls. In Slovakia, by 2021, both females and males were offered the vaccination and it is partially funded (34). According to the HPV information centre and its estimation for 2020 (24), in Czechia, an age-standardized incidence rate (ASIR)/age-standardized mortality rate (ASMR) of cervical cancer is 9.3/3.6 (per 100,000 women per year). In Austria, Germany, Poland, and Slovakia, ASIRs/ASMRs are 5.3/1.8, 7.6/2.2, 12.3/5.9, and 16.6/5.4, respectively. Czechia has the lowest ASIR/ASMR of the Eastern European



Region	2012	2013	2014	2015	2016	2017	2018	2019	2020
PCC	20.5	21.9	17.5	16.1	17.2	16.5	13.8	17.5	11.5
CBR	15.8	15.4	16.9	16.9	14.1	16.6	12.2	14.9	15.8
SBR	18.0	10.5	13.0	15.2	11.4	11.4	12.9	14.5	13.2
PLR	20.8	14.2	19.3	17.2	17.5	16.4	14.3	19.3	13.8
KVR	27.4	21.0	24.4	19.2	18.6	20.7	22.1	14.1	18.8
ULR	22.0	20.4	21.4	24.3	19.1	17.2	15.0	17.2	16.0
LBR	17.9	16.1	13.4	15.7	14.7	9.8	12.5	15.1	11.1
HKR	14.2	15.3	13.2	11.4	24.3	16.8	17.2	17.5	11.4
PAR	10.3	10.3	12.2	17.6	11.9	11.9	11.8	11.4	12.2
VYR	12.4	13.2	11.7	13.3	10.1	11.3	12.1	11.3	11.3
SMR	17.3	17.8	14.2	15.4	15.0	13.8	15.1	14.9	17.1
OLR	14.4	17.2	14.5	15.4	14.8	14.2	13.9	18.3	15.8
ZLR	15.3	14.0	11.7	15.1	14.1	12.4	11.4	9.1	10.5
MSR	16.9	20.3	14.1	15.0	14.1	13.6	12.2	14.5	12.9
Czechia	17.3	16.9	15.6	16.3	15.4	14.6	13.7	15.2	13.8

FIGURE 5
Comparison of malignant neoplasm of cervix uteri (C53) relative incidence in 14 regions of Czechia (2012–2020).

countries. In Eastern, Western, Northern, and Southern Europe, ASIRs/ASMRs are 14.5/6.1, 7.3/2.1, 10.4/2.2, and 7.7/2.3, respectively (24).

Generally, it is evident that screening, vaccination, and the age of screening and vaccination are essential for decreasing both the incidence and mortality of cervical cancer (36). In Czechia, there is a noticeable decrease in the overall incidence of vaccination between 2012 and 2020. The most obvious decreasing trend is in all cervical tumours (C53, D06) aged 15–24 years. This reflects the targeting of the vaccination programme for girls aged 13 in Czechia, which started just in 2012 and is now manifested mainly in girls/women 10 years older. On the contrary, the highest incidence was in the age group 25–34 years, which was even over 200 per 100,000 women. In separate malignant neoplasm of cervix uteri (C53), the higher incidence (over 20 per 100,000 women) started appearing from the age group 35–39 years and above. Mortality increased significantly from the age of over 60 years, with the highest frequency at the age of over 85 years. According to a systematic review from 2022, in national immunisation programmes, most girls and boys are inoculated with the HPV vaccine by the time puberty begins; thus, it is essential to monitor the vaccine effect at least until the sexually active period in their 20s and 30s (37).

All Czech women over 15 years old are screened every year. For example, in a study from Italy (36), a bimodal shape in cancer incidence was observed, with a first peak in the 40–45 years age group, and a second, higher peak in the 75–80 years age group. Bimodality in cancer incidence was a consequence of the initiation of a screening programme covering the population only up to a given age (i.e., 70 years in Italy). In particular, the peak at high ages arises and is gradually magnified over time by the sudden increase of the population at risk of cervical cancer, which occurs at the exit of the screening age, contrasted with the cumulative success over time of diagnosis and treatment within the screened age groups.

Significant differences between regions can be observed in the incidence of cervical tumours and even in vaccination rates. For example, the HPV vaccination rate of prime-vaccinated female patients relative to the female population aged 13 years in regions of Czechia in 2019 varied from less than 56% in Prague (PCC) and ZLR region to more than 68% in OLR region and ULR region (see Figure 3). In some subregions of OLR region, the vaccination rate is more than 80% (21). Focusing on the cervical cancer incidence rate in the regions, in the case of all cervical tumours (C53, D06), the difference in the individual regions is evident (Figure 4). The region with the highest incidence in the whole analysed period 2012–2020 is

TABLE 3 Correlation of relative incidence and mortality in regions with the overall trend of Czechia (2012–2020).

Regions of Czechia	Cervical tumours (C53, D06) incidence of Czechia	Malignant neoplasm cervix uteri (C53) incidence of Czechia	Cervical tumours (C53, D06) mortality of Czechia
PCC	−0.52112	**0.88083***	0.46657
CBR	−0.39808	0.41828	0.52121
SBR	0.13310	0.36599	−0.38729
PLR	**0.87563***	0.52056	0.46535
KVR	0.57214	0.36988	0.62408
ULR	**0.85522***	**0.83779***	0.62254
LBR	0.44646	**0.85525***	0.55029
HKR	0.00145	−0.11311	0.08385
PAR	0.54542	−0.02100	0.28942
VYR	−0.58008	0.51603	0.09925
SMR	0.15830	0.41940	0.16472
OLR	0.04901	0.19525	0.52937
ZLR	0.27109	0.71208*	−0.13321
MSR	0.76808*	**0.83749***	**0.84296***

*$p < 0.05$, bold–correlation over 0.8.

PLR. On the contrary, the region with constantly lowest incidence is MSR. Regarding separate malignant neoplasm of cervix uteri (C53), there are also significant differences in the individual regions; however, no such regions consistently have the highest or lowest incidence numbers (Figure 5).

Concerning urban and rural areas, we can focus on 100% urban areas, such as Prague, the capital city (PCC). This region has the lowest vaccination rate and middle incidence rate both in all cervical tumours (C53, D06) and separate C53. In other regions, the yield of urban density varies. Urban and rural areas are divided by the number of inhabitants. A municipality of up to 3,000 inhabitants is considered a rural area, and above 3,000 as an urban area. Based on the results of correlation analysis, indicators connected with urban/rural aspects, such as a share of urban population and population density, are statistically significant. Regarding the variable "share of urban population," there is a statistically significant positive correlation with cervical cancer incidence. Therefore, the locations with a higher share of the urban population show a higher incidence rate in Czechia. Comparing our results with other studies, regional differences, especially urban/rural differences, were observed in China (18). Also, in the United States (8), were observed geographic disparities in cervical cancer incidence, particularly in rural areas. Urban and rural disparities can influence access to healthcare resources. Rural residents may face challenges such as limited healthcare facilities or transportation issues.

Other important possible indicators that can influence incidence, apart from those mentioned above, are demographic and socioeconomic factors. So, the final incidence results from the simultaneous influence of all possible indicators. Identifying only the key variables with the most important impact is difficult. Focusing on the results of the correlation analysis presented in Tables 4, 5, there are indicators that can influence the incidence positively and/or negatively.

The indicators which can cause higher cervical cancer incidence are the high unemployment rate of women, the high number of divorces, the high number of abortions, the high share of the urban population, the high number of students, and the high number of women with only primary education. Such indicators are connected with economically disadvantaged citizens (women) and women from underprivileged families. It is also associated with the low level of education and living in urban places.

On the other hand, the indicators which can have a negative impact on cervical cancer incidence are the high GDP, the high average gross monthly wage per employee (indicates the economic level of a given region), the high employment rate of women, the higher average age of mothers at birth, and the high number of women with tertiary education. Such variables indicate the middle and upper class of citizens (women), women with higher qualifications and wages, and probably more heightened awareness connected with vaccination. Various case and expert studies (38, 39) have addressed the issue of prevention and the factors that influence the willingness of the population to undergo preventive health check-ups and/or vaccination. Their results support the hypothesis that socially and economically vulnerable people attend fewer preventive check-ups. They usually lack information about vaccination or may be misinformed. For example, Brunner-Ziegler et al. (38) focused on participation in preventive health check-ups in Austria. Regarding the variables, middle-aged participants, had secondary education (women) or tertiary education (men), higher income, and were born in Austria (men) or another member state of the EU-15 (women) were more likely to have undergone a preventive health check. Another study from Germany (39) underlined the important influence of socio-economic indicators, such as education, occupation, and income.

Focusing on other scientific studies, our results underline the problem of economically disadvantaged regions and families. For example, Kapoor and Sharma (16) identified the following risk factors in India: early age at marriage, lack of education, increased parity, early age at first pregnancy, poor sanitation, use of tobacco, and belonging to below-the-poverty line. Buskwofie et al. (7) depicted the following risk factors in the USA: racial and ethnic minorities and socioeconomically disadvantaged. Concerning the situation in China, Lin et al. (17) observed the joint effects of various socio-demographic factors, including residence, education and monthly income. Except for age, residence, education, monthly income, number of sexual partners in the past 6 months, and parity, the authors noticed that high HPV knowledge level was significantly associated with HPV testing behaviour.

Due to the amount of data, it is not possible to show all mentioned indicators in Czechia in a spatial context, and it is not even the current aim of the presented study. However, how the socio-economic indicators look in this context can be found, for example, in the Statistical Atlas administered by the Czech Statistical Office (40).

Our indicators analysis is concepted as ecological epidemiological study, so causal relationships cannot be inferred. But, it can help to understand the relationship between evaluated indicators and the incidence of cervical tumours. Unique is the focus not only on the incidence of malignant cervical tumours but also on the incidence of carcinoma *in situ*, which both together more closely reflect the risk of

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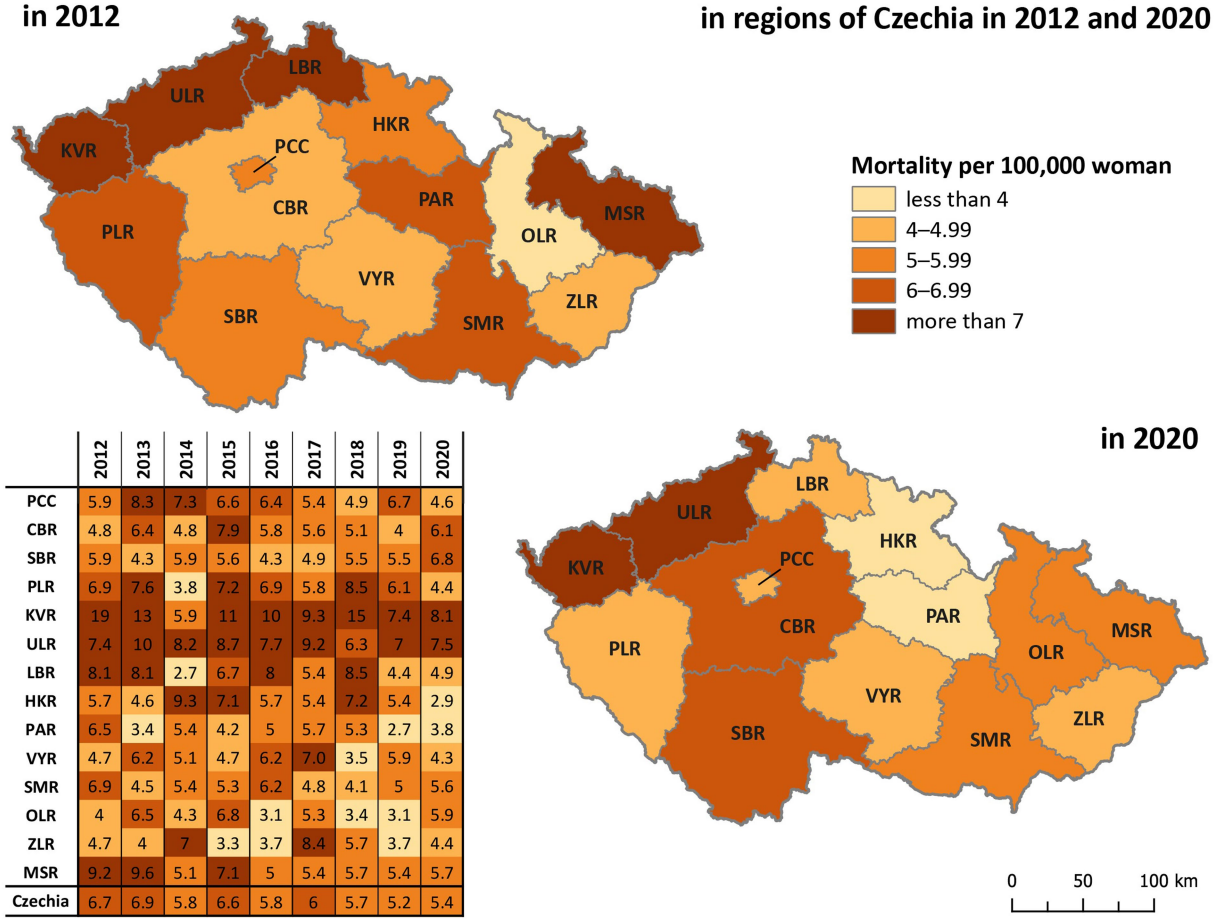

	2012	2013	2014	2015	2016	2017	2018	2019	2020
PCC	5.9	8.3	7.3	6.6	6.4	5.4	4.9	6.7	4.6
CBR	4.8	6.4	4.8	7.9	5.8	5.6	5.1	4	6.1
SBR	5.9	4.3	5.9	5.6	4.3	4.9	5.5	5.5	6.8
PLR	6.9	7.6	3.8	7.2	6.9	5.8	8.5	6.1	4.4
KVR	19	13	5.9	11	10	9.3	15	7.4	8.1
ULR	7.4	10	8.2	8.7	7.7	9.2	6.3	7	7.5
LBR	8.1	8.1	2.7	6.7	8	5.4	8.5	4.4	4.9
HKR	5.7	4.6	9.3	7.1	5.7	5.4	7.2	5.4	2.9
PAR	6.5	3.4	5.4	4.2	5	5.7	5.3	2.7	3.8
VYR	4.7	6.2	5.1	4.7	6.2	7.0	3.5	5.9	4.3
SMR	6.9	4.5	5.4	5.3	6.2	4.8	4.1	5	5.6
OLR	4	6.5	4.3	6.8	3.1	5.3	3.4	3.1	5.9
ZLR	4.7	4	7	3.3	3.7	8.4	5.7	3.7	4.4
MSR	9.2	9.6	5.1	7.1	5	5.4	5.7	5.4	5.7
Czechia	6.7	6.9	5.8	6.6	5.8	6	5.7	5.2	5.4

FIGURE 6
Comparison of cervical tumours (C53, D06) relative mortality in 14 regions of Czechia (2012–2020).

HPV exposure. Also, the location of Czechia in the centre of Europe and its regional diversity enables the transferability of the results to other similar regions and contributes to the development of knowledge in this area. The follow-up research will focus on a more detailed analysis of significant indicators in a spatial context, a series analysis of trends, more complex multivariable analyses and potential confounders.

According to the results and our findings, we can support already published statements and outcomes (14, 41–43): (i) vaccination against HPV has been demonstrated to reduce the risk of HPV-related diseases effectively; (ii) vaccination primarily aims to prevent cervical cancer (common vaccines include Gardasil and Cervarix, which protect against the most prevalent cancer-causing HPV types); (iii) administering the HPV vaccine before exposure to the virus, which typically occurs usually through sexual activity, is most effective (it is recommended around age 11 or 12); (iv) despite vaccination, regular cervical cancer screening (such as Pap smears or HPV testing) remains crucial for detecting and treating any pre-cancerous changes.

Education in the prevention of HPV through vaccination is essential. So does accentuation of the preventive programmes for both primary and secondary, as well as tertiary prevention. Ensuring the interest of the general population in the issue of HPV and the possibility of their prevention, which requires the cooperation of the media and policy-makers, is also essential. Last but not least, there is a need for education in the field of fake news regarding the usefulness and safety of preventive measures.

5 Conclusion

HPV continues to be a significant public health concern. Despite improvements in cervical cancer screening and treatment, the incidence of cervical cancer (C53) in Czechia remains relatively high, with 13.8 cases per 100,000 women in 2020. This underscores the need for increased awareness and prevention efforts, including vaccination and regular screening. Although the HPV vaccine is available and recommended for both boys and girls in Czechia, vaccination rates remain relatively low. As of 2019, only 63, 9% of girls aged 13 had the HPV vaccine. Increasing vaccination rates could help to reduce the burden of HPV-related diseases in the country.

In summary, while there have been some improvements in the prevention and management of HPV-related diseases in Czechia since 2012, there is still much work to be done to reduce the prevalence of HPV and the incidence of related cancers. Increasing vaccination rates

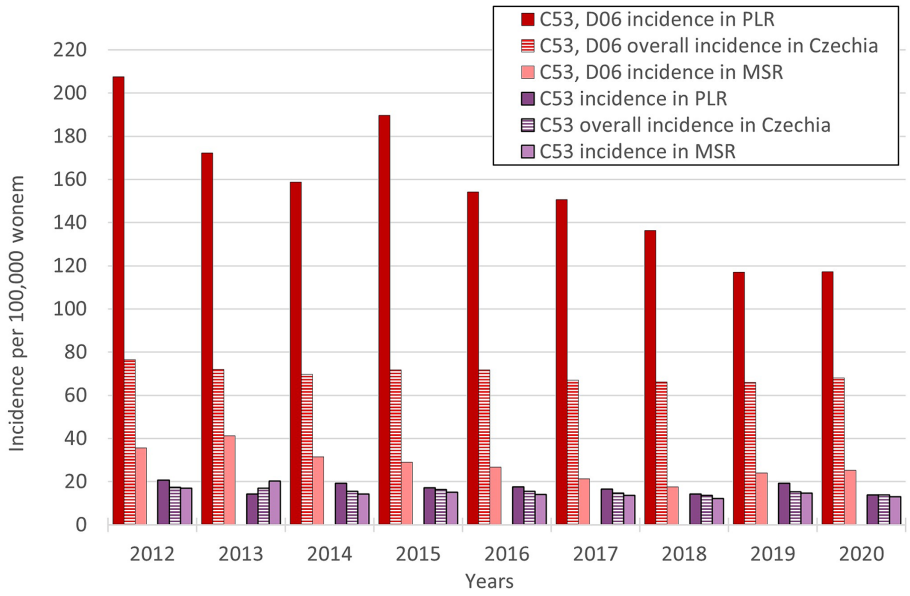

FIGURE 7
Trends of relative incidence of cervical tumours (C53, D06) in PLR and MSR in comparison to the overall trend of Czechia (PLR-region with the highest incidence of cervical tumours-C53, D06, MSR-region with the lowest incidence of cervical tumours-C53, D06).

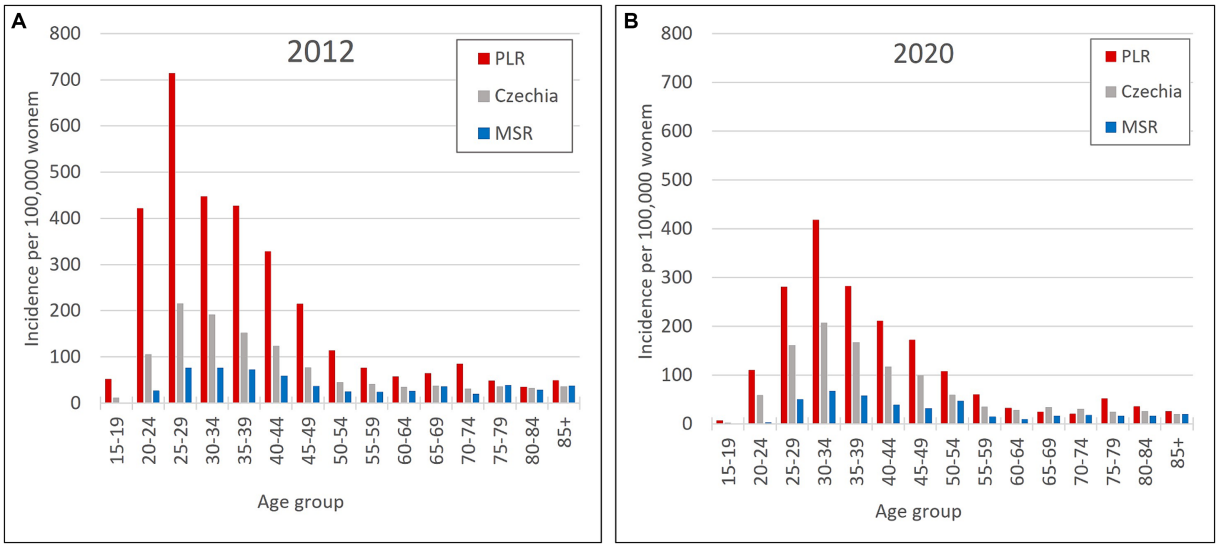

FIGURE 8
Incidence of cervical tumours (C53, D06) by age group in PLR (region with the highest incidence of C53, D06) and MSR (region with the lowest incidence of C53, D06) in comparison to the Czechia: **(A)** in 2012, **(B)** in 2020. (Incidences were calculated by dividing the absolute number by population size for each age group displayed as units available for 100,000 women).

and promoting regular screening for cervical cancer should be priorities for public health efforts in the country as well as the influence of vaccination on the decrease in the incidence rate of cervical cancer is significant, and it would be worthy to support awareness in the population, especially in regions with higher incidence rates.

There were observed differences between regions. Results underline the problem of economically disadvantaged regions and families. Based on correlation analysis, indicators connected with urban/rural aspects, such as a share of urban population and population density, were statistically significant. The indicators related to higher cervical cancer incidence are the high unemployment rate of women, the high number of divorces, the high number of abortions, the high share of the urban population, the high number of students, and the high number of women with only primary education. On the other hand, the indicators which are related to lower cervical cancer incidence are the high GDP, the high average gross monthly wage per employee, the high employment rate of women, the higher average age

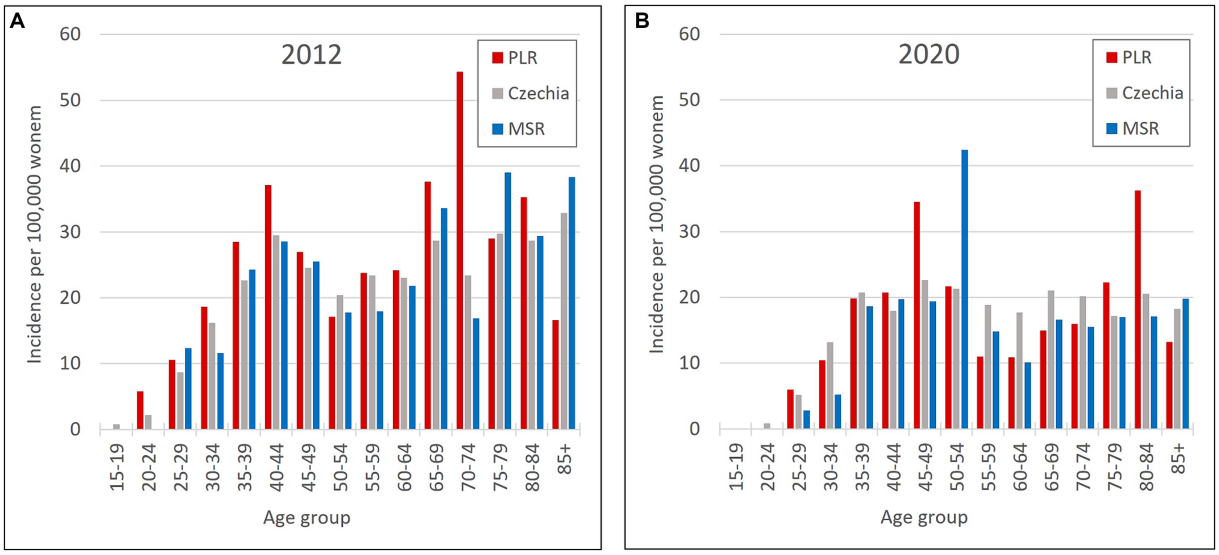

FIGURE 9
Incidence of malignant neoplasm cervix uteri (C53) by age group in PLR (region with the highest incidence of C53, D06) and MSR (region with the lowest incidence of C53, D06) in comparison to the Czechia: **(A)** in 2012, **(B)** in 2020. (Incidences were calculated by dividing the absolute number by population size for each age group displayed as units available for 100,000 women).

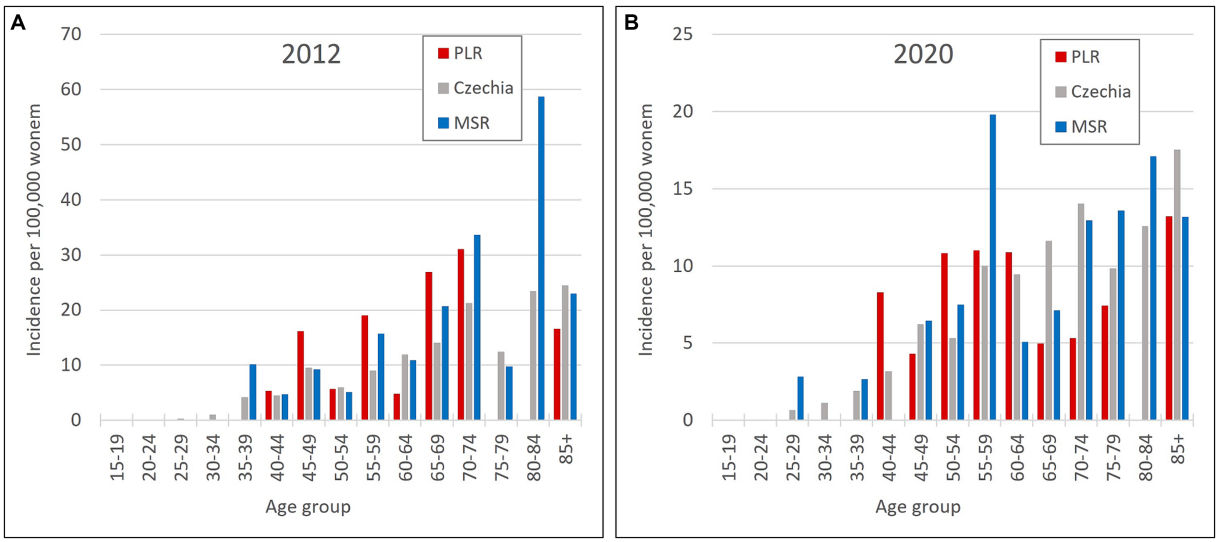

FIGURE 10
Mortality of cervical tumours (C53, D06) by age group in PLR (region with the highest incidence of C53, D06) and MSR (region with the lowest incidence of C53, D06) in comparison to the Czechia: **(A)** in 2012, **(B)** in 2020. (Mortality were calculated by dividing the absolute number by population size for each age group displayed as units available for 100,000 women).

of mothers at birth, and the high number of women with tertiary education.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: For incidence and mortality of cervical tumours in the Institute of Biostatistics and Analyses repository, http://www.svod.cz. For data about the age distribution of the women population in the Czech Statistical Office repository, https://www.czso.cz/csu/czso/age-distribution-of-the-population-2021. For data about territorial comparison of demographic and socioeconomic indicators in the Czech Statistical Office repository, https://www.czso.cz/csu/xm/mezikrajske_srovnani_vybranych_ukazatelu. For data about the territorial comparison of demographic and socioeconomic indicators only about women the Czech Statistical Office repository, https://vdb.czso.cz/vdbvo2/faces/en/index.jsf?page=uziv-dotaz. For geographical data in the State Administration of Land Surveying and Cadastre repository, https://geoportal.cuzk.cz/(S(gbqevdvghr43bw5iyj41kpss))/Default.aspx?lng=EN&menu=2291&mode=TextMeta&side=mapy_data200&metadataID=CZ-CUZK-DATA200-HRANICE-V.

TABLE 4 Correlation of cervical tumours relative incidences with demographic indicators of PLR (region with the highest incidence of C53, D06) and MSR (region with the lowest incidence of C53, D06) in 2012–2020.

Demographic indicators	Incidence of cervical tumours (C53, D06)		Incidence of malignant neoplasm cervix uteri (C53)	
	MSR	PLR	MSR	PLR
Average age	**−0.83051***	**−0.90066***	−0.73565*	−0.42274
Age index (in %)	**−0.83439***	**−0.90905***	−0.73625*	−0.44376
Total population gain/loss	−0.64035	−0.68537*	−0.47556	0.09011
Natural population gain/loss	0.06558	0.15789	0.12406	0.57649
Live births	**−0.87465***	−0.50667	−0.79804*	−0.03280
Live births woman	−0.77059*	−0.68302*	−0.76025*	−0.32278
Live birth non-marital children	**−0.89954***	−0.59018	−0.77441*	−0.29244
Average age of mother at birth	**−0.80614***	**−0.91391***	−0.74271*	−0.47224
Deaths	−0.26972	−0.45134	−0.33136	−0.64417
Marriages	**−0.85092***	−0.48586	−0.63135	0.04953
Divorces	0.77123*	0.74145*	0.73698*	−0.02856
Abortions	0.61966	**0.83304***	0.68225*	0.50334
Share of urban population[a]	0.78100*	**0.88712***	0.70459*	0.45427
Population density[a]	0.79256*	−0.74552*	0.72604*	0.00253
Immigrants[a]	**−0.86328***	**−0.84593**	−0.73900*	−0.25988
Emigrants[a]	−0.32974	−0.61883	−0.46769	0.42807
Total population change[a]	−0.22090	−0.60614	−0.03481	0.13745
Infant mortality rate[a]	−0.39217	0.25114	−0.04880	−0.58642
Students, total[a]	**0.88551***	0.68642	0.70602*	0.20610
Primary education	**0.81752***	0.59217	0.72776*	0.21121
Lower secondary education	0.58732	0.19336	0.42669	0.55877
Upper secondary education	0.66722*	−0.00347	0.55125	−0.17568
Tertiary education	**−0.86722***	−0.71189 *	−0.71066*	−0.48460

*$p < 0.05$; [a]indicators related to the entire population; bold–correlation over 0.8.

TABLE 5 Correlation of cervical tumours relative incidences with socioeconomic indicators of PLR (region with the highest incidence of C53, D06) and MSR (region with the lowest incidence of C53, D06) in 2012–2020.

Socioeconomic indicators	Incidence of cervical tumours (C53, D06)		Incidence of malignant neoplasm cervix uteri (C53)	
	MSR	PLR	MSR	PLR
Gross domestic product[a]	**−0.87380***	**−0.90928***	−0.72644*	−0.38457
Pension recipients, total[a]	−0.34093	**−0.80930***	−0.11113	−0.27193
Old-age pensions (single pensions)[a]	**−0.89198***	**−0.84954***	−0.74308*	−0.33473
Average gross monthly wage per employee[a]	−0.62028	**−0.88669***	−0.55959	−0.41085
Unemployment rate (in %)	**0.86352***	0.76345	0.76304*	0.28659
Employment rate (in %)	**−0.90332***	−0.78131	−0.72180*	−0.47529

*$p < 0.05$; [a]indicators related to the entire population; bold–correlation over 0.8.

Author contributions

OH: Conceptualization, Methodology, Supervision, Writing – review & editing. OM: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. TS: Data curation, Software, Visualization, Writing – original draft. DN: Data curation, Methodology, Writing – original draft. JZ: Conceptualization, Data curation, Methodology, Supervision, Writing – review & editing. RK: Conceptualization, Writing – original draft. JV: Conceptualization, Methodology, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Performance of P16^{INK4a} immunocytochemical stain in facilitating cytology interpretation of HSIL for HPV-positive women aged 50 and above

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Background: Few articles have focused on the cytological misinterpretation of high-grade squamous intraepithelial lesion (HSIL). Due to estrogen deficiency, cervical epithelial cells in postmenopausal women tend to show atrophic change that looks like HSIL on Papanicolaou-stained cytology slides, resulting in a higher rate of cytological misinterpretation. P16^{INK4a} immunocytochemical staining (P16 cytology) can effectively differentiate diseased cells from normal atrophic ones with less dependence on cell morphology.

Objective: To evaluate the role of P16 cytology in differentiating cytology HSIL from benign atrophy in women aged 50 years and above.

Methods: Included in this analysis were women in a cervical cancer screening project conducted in central China who tested positive for high-risk human papillomavirus (hr-HPV) and returned back for triage with complete data of primary HPV testing, liquid-based cytology (LBC) analysis, P16 immuno-stained cytology interpretation, and pathology diagnosis. The included patients were grouped by age: ≥50 (1,127 cases) and <50 years (1,430 cases). The accuracy of LBC and P16 cytology in the detection of pathology ≥HSIL was compared between the two groups, and the role of P16 immuno-stain in differentiating benign cervical lesions from cytology ≥HSIL was further analyzed.

Results: One hundred sixty-seven women (14.8%; 167/1,127) in the ≥50 group and 255 (17.8%, 255/1,430) in the <50 group were pathologically diagnosed as HSIL (Path-HSIL). LBC [≥Atypical Squamous Cell Of Undetermined Significance (ASCUS)] and P16 cytology (positive) respectively detected 63.9% (163/255) and 90.2% (230/255) of the Path-≥HSIL cases in the <50 group and 74.3% (124/167) and 93.4% (124/167) of the Path-≥HSIL cases in the ≥50 group. LBC matched with pathology in 105 (41.2%) of the 255 Path-≥HSIL cases in the <50 group and 93 (55.7%) of the 167 Path-≥HSIL cases in the ≥50 group. There were five in the <50

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group and 14 in the ≥50 group that were Path-≤LSIL cases, which were interpreted by LBC as HSIL, but negative in P16 cytology.

Conclusion: P16 cytology facilitates differentiation of Path-≤LSIL from LBC-≥HSIL for women 50 years of age and above. It can be used in the lower-resource areas, where qualified cytologists are insufficient, as the secondary screening test for women aged ≥50 to avoid unnecessary biopsies and misinterpretation of LBC primary or secondary screening.

KEYWORDS

P16 immunocytochemical stain, atrophy, cytology, high-grade squamous intraepithelial lesion (HSIL), menopause

1 Background

Diagnostics and treatment of cervical precancer for postmenopausal women are important to cervical cancer prevention in aged women because of the tendency of social aging in many countries. Cervical cytology remains the standard cervical cancer screening test worldwide for either primary or secondary screening. However, evidence shows that some atrophic changes in squamous and columnar epithelium may be misinterpreted as high-grade squamous intraepithelial lesion (HSIL) when analyzing the exfoliated cervical cells from postmenopausal women who are usually low in estrogen (1). However, many studies have evidenced that overexpression of P16 protein is positively related to transformative high-risk human papillomavirus (hr-HPV) infection and grade of cervical cell proliferation and can be an objective indicator for lesion grade. As a tumor suppressor that is highly related to HSIL and cervical cancer, P16 overexpression can be a biomarker for early diagnosis of squamous intraepithelial lesion (SIL) and evaluation of the lesion prognosis (2).

A P16 immunocytochemical stain technology was developed by Senying Biotechnology Co., Ltd. (Shenzhen, China), which uses $P16^{INK4a}$ monoclonal antibodies (sy-a01) to stain exfoliated cervical cells that were diluted at a ratio of 1:4,000 on PathCIN®$P16^{INK4a}$ automatic staining system (P16 immuno-stained cytology). This technology has been demonstrated to be more sensitive than and equally specific with liquid-based cytology (LBC) in the detection of grade II and above cervical intraepithelial neoplasia (CIN2+) (3). As it provides the cytopathologists with a more objective marker for cytology interpretation, it potentially reduces the subjective diversity in cytology interpretations from different cytologists and consequently reduces the reliance of cytology on cytologists' experiences. This study aimed to demonstrate the performance of P16 immuno-stained cytology (or P16 cytology) in facilitating the differentiation of HSILs from atrophic lesions by comparing the concordance of P16-immuno-stained cytology with the LBC and histopathology diagnoses between the groups of women ≤50 and >50 years of age.

2 Materials and method

2.1 Study design, participants, and procedures

The subjects of the study were 73,624 women living in central China who were screened for cervical cancer by primary HPV testing in a population-based municipal cervical cancer screening program in November 2019. Those women were enrolled for screening because they were eligible: 30–64 years of age, not pregnant, without uterine or cervical resection, and consented to participate in the screening and this study by signing an electronic version of the informed consent form when they registered for participation on a website (www.curekeys.com). Eligible women were primarily tested for hr-HPV with SeqHPV assay on their self-collected samples. Women with HPV-negative results were advised to undergo regular screening by HPV assay after 3 years. Those positive for hr-HPV were recalled back for management following a protocol that required a cervical sample be collected first by the physician for LBC and P16 cytology analysis for all positive women, followed by multiple biopsies on women who were positive for HPV-16 and/or HPV-18, positive for the hr-HPV types other than HPV-16 and HPV-18 (other hr-HPV type) plus positive for acetic acid test, or positive for other hr-HPV types, and negative for acetic acid test but positive for LBC (≥ASCUS). Endocervical curettage (ECC) was performed on patients whose squamocolumnar junction zone (T-zone) could not be completely visible. Pathology analysis was conducted on the biopsies and ECC specimens. Included in this analysis were 2,557 women who had complete data on the primary HPV testing, LBC analysis, $P16^{INK4a}$ immune-stained cytology (P16 cytology) interpretation, and pathology diagnosis. Women who were positive for other hr-HPV types but normal for both LBC and P16 cytology, or positive for any type but did not have results of LBC, P16 cytology, or histopathology, mainly due to sample reasons, were excluded from this study (Figure 1). The study was approved by the Institutional Review Board (IRB) of BGI Institute and the Ethics Committee of Peking University Shenzhen Hospital (PUSH, No. 2018035).

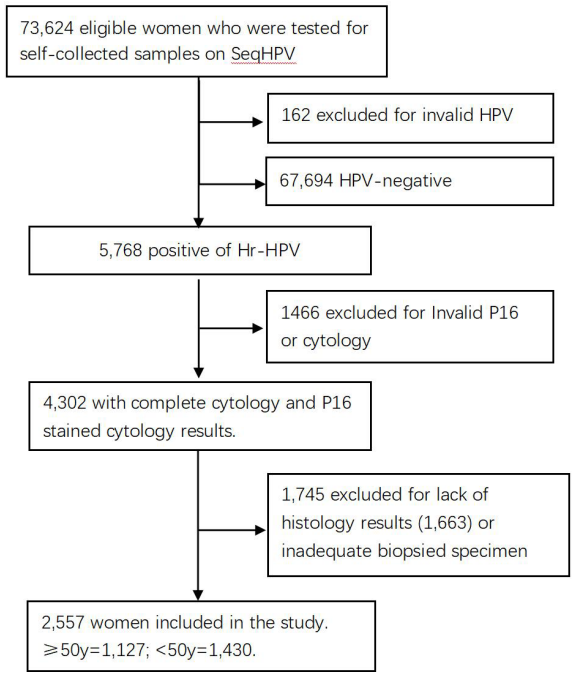

FIGURE 1
Flowchart of the study.

2.2 Sampling and HPV testing

After successful registration, which confirmed eligibility for participation in the primary screening, women were screened in the sampling sites temporarily set up in the communities according to the number of registered women in the relevant communities or nearby medical facilities (the screening sites). At the screening site, eligible women were guided to collect cervical/vaginal samples for themselves in sampling rooms by referring to the graphic self-sampling instruction with texts. A conical-shaped brush was used for self-sampling. If any woman had a problem with self-sampling, an on-site medical provider would give personal instruction. The collected sample was applied on an FTA-Illusive-card (GE) for HPV testing on SeqHPV (BGI-Shenzhen) by a reference laboratory of BGI-Shenzhen. SeqHPV is a next-generation sequencing (NGS)-based HPV testing assay that uses multi-plex PRC to amplify DNA and NGS for HPV genotyping (3). This assay can detect and report 14 hr-HPV genotypes, including HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, HPV-59, HPV-66, and HPV-68. SeqHPV had been validated to be equally sensitive and specific with Cobas4800 when tested on either provider-collected or self-collected samples (4) and to work well with FTA cards (a hard sample processing card). It has been licensed by China Food and Drug Administration (CFDA) for clinical use.

2.3 LBC and P16^{INK4a} immuno-stained cytology

LBC and the P16^{INK4a} immuno-stained cytology (Senying Biotechnology Co., Ltd., Shenzhen, China) were used for research purposes and as the secondary screening in the triage of the women who were positive for 12 other types of hr-HPV plus negative for acetic acid test. The cervical sample was collected by the provider and then put into a vial containing cell preservation liquid provided by Senying. The samples were shipped to the Senying laboratory for processing: part of each sample was processed with P16^{INK4a} immunocytochemical stain, and the remaining sample was processed for standard Papanicolaou stains. Both the P16^{INK4a} immuno- and Papanicolaou-stained cytology slides were reviewed and interpreted by two senior cytopathologists who were blinded to each other's interpretation.

Following The Bethesda System (TBS) classification standards (5), LBC interpretations were reported as negative for squamous intraepithelial lesion (NILM), atypical squamous cells of undetermined significance (ASCUS), atypical glandular cells (AGC), low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells-cannot exclude HSIL (ASC-H), HSIL, or squamous cell carcinoma (SCC) accordingly. P16 cytology positive result was reported when at least one cell was found to have P16^{INK4a} immuno-stained substance in the nucleus or cytoplasm. Quality control was conducted after the two cytopathologists completed their review of the slides, on which all cases with inconsistent interpretations by the cytologists were selected to resolve consistent interpretations via discussions between the two cytopathologists.

2.4 Colpo/biopsy and histopathology diagnostics

For women who needed biopsies according to the protocol for positive management, multiple biopsies were obtained at the site with colposcopically suspected lesions and the opposite sites, or randomly at the squamocolumnar junction zone in four quadrants of the cervix if no lesion was suspected. ECC was performed on patients whose squamocolumnar junction zone could not be completely visible under colposcopy (6).

All the pathology slides were analyzed by a senior pathologist from PUSH who performed pathology analysis for several international clinical trials. Pathology slides were analyzed while blinded to the results from both the P16 cytology and LBC tests. Histological diagnoses for cervical lesions were reported following a two-grade classification system, according to which the cervical lesions were classified as LSIL and HSIL. This system was adopted because many studies demonstrated that different grades of CIN are not the different stages of a cervical lesion development but the two obviously distinguishable pathological processes, and the two-grade classification matches with the bio-behavior of HPV that causes pathology changes in human cells and is with better duplicability (7–9).

2.5 Statistics

Results from LBC and pathology were compared to demonstrate the bias of LBC on the interpretation of HSILs in women ≥50 years of age, and P16 cytology results of the cases with LBC-LSIL and LBC-HSIL were analyzed using the relevant pathology diagnosis as the endpoint (Path-LSIL and Path-HSIL). SPSS 26.0 statistical software was used for data analysis. The chi-square test was used to compare the differences in various rates.

Differences in sensitivity and specificity along with 95% CI were calculated using McNemar's test, and a p-value of <0.05 was considered statistically significant for all analyses.

3 Results

Among the 73,624 primarily screened women, 73,462 had valid results for HPV primary testing after excluding 162 for failure of HPV testing. Of the 73,462, 5,768 were positive for primary HPV testing, and 2,557 of those positive results had complete data of HPV, LBC, P16 cytology, and histopathology results and were included in the analysis for the purpose of this study (the analytic cases). Patients who were primarily positive for 12 other types of hr-HPV but normal in cytology and P16 cytology, or abnormal in the two cytology tests but did not return for colposcopy, were excluded from this analysis.

Of the 2,557 analytic cases, 1,430 were younger than 50 years and included in the <50 group, while the remaining 1,127 were aged 50 and above and included in the ≥50 group. HSIL was pathologically confirmed (Path-HSIL) on 255 and 167 positive women in the <50 group and ≥50 group, respectively. When analyzed in age groups with LBC≥ASCUS and P16 cytology positive results as the cutoff, LBC and P16 cytology respectively detected 63.9% (163) and 90.2% (230) of the 255 Path-HSIL cases in the <50 group, while in the ≥50 group, LBC and P16 cytology detected 74.3% (124) and 93.4% (156) of the 167 Path-HSIL cases, respectively (Table 1).

When looking at the number of HSIL cases reported by LBC and pathology, we observed that HSIL from LBC (LBC-HSIL) and pathology (Path-HSIL) in the <50 group were 110 and 255 cases, respectively (Table 2A), while LBC- and Path-HSIL in the ≥50 group were 107 and 167 cases, respectively (Table 2B). However, when looking at the concordance of LBC and pathology detecting HSIL, we found that LBC matched with pathology in 41.2% (105/255) of the HSIL cases from the <50 age group and 55.7% (93/167) of such cases from the ≥50 group.

What interested us were the five (0.4%) and 14 (1.5%) Path-≤LSIL cases in the <50 and ≥50 groups, respectively, that were reported by LBC as HSIL cases (LBC-HSIL/Path-≤LSIL cases), with significant difference between the two groups (p = 0.042). Further analysis showed that all the five LBC-HSIL/Path-≤LSIL cases in the <50 group and 14 such cases in the ≥50 group were negative for P16 cytology, of which the five from the <50 group and 13 from the ≥50 group were pathologically confirmed as normal, and one from the ≥50 group was Path-LSIL (Table 3). These results indicate that P16 cytology is contributive in differentiating benign lesions from HSIL for cytology (LBC) on women ≥50 years of age.

Table 3 also shows that there were one and three LBC-HSIL/Path-HSIL cases in the <50 and ≥50 groups, respectively, that were negative for P16 cytology. All those cases were pathologically graded as CIN2.

4 Discussion

Our prior study has demonstrated that P16 cytology is better than LBC in the sensitivity and specificity for the detection of CIN2+ (10) but less dependent on cell morphology, which makes it more applicable in lower-resource areas where experienced and acknowledgeable cytologists are insufficient. In this study, we found that P16 cytology is advantageous in facilitating cytologists to differentiate benign lesions from LBC-≥HSIL in women ≥50 years of age. Due to obvious decreased levels of estrogen in women during perimenopause, some atrophic cervical cells are usually included in the cervical samples for cytology, resulting in its potential misinterpretation as HSIL (11). As the atrophic cells have smaller portions of cytoplasm, it is easy to be confused with HSIL cells on Papanicolaou-stained cytology slides, and this has always been challenging to cytologists, especially to the inexperienced ones in lower-resource regions.

Our analysis shows that the rate of LBC-reported false HSILs is significantly higher in the ≥50 group than in the <50 group. This result is consistent with many studies that reported that among the cases that returned for colposcopy/biopsies for LBC-≥HSIL, the average age of the cases' normal pathology was higher than those pathologically diagnosed as HSIL (12–14). In a study on LBC-ASC-H cases (15), Halford and coauthors reported that the rates of CIN2 were 55.8% and 37.5% among patients aged <50 and ≥50 years, respectively, with significant differences. Other studies attributed the higher rate of inconsistency between LBC-

TABLE 1 The detection rate of LBC≥ASCUS and P16+ in two groups for detection of Path-HSIL.

Groups	≥50	<50
	Path-≥HSIL	Path-≥HSIL
≥ASCUS	74.3 (124/167)	63.9 (163/255)
P16+	93.4 (156/167)	90.2 (230/255)
P	0	0

LBC, liquid-based cytology; HSIL, high-grade squamous intraepithelial lesion.

TABLE 2A LBC-LSIL/HSIL vs. Path-LSIL/HSIL in ≥50 group, n (%).

	LBC-≤LSIL	LBC-≥HSIL	Total
Path-≤LSIL	946 (98.5)	14 (1.5)	960 (100)
Path-≥HSIL	74 (44.3)	93 (55.7)	167 (100)
Total	1,020 (90.5)	107 (9.5)	1,127 (100)

TABLE 2B LBC-LSIL/HSIL vs. Path-LSIL/HSIL in <50 group, n (%).

	LBC-≤LSIL	LBC-≥HSIL	Total
Path-≤LSIL	1,170 (99.6)	5 (0.4)	1,175 (100)
Path-≥HSIL	150 (58.8)	105 (41.2)	255 (100)
Total	1,320 (92.3)	110 (7.7)	1,430 (100)

Comparison of Table 2A and Table 2B: $X^2 = 2.635$, p = 0.105 for LBC and $X^2 = 4.155$, p = 0.042 for pathology.
LBC, liquid-based cytology; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion.

TABLE 3 P16 cytology performance in detection LBC-HSIL+ cases in the two age groups.

Pathology	P16 cytology	Normal		LSIL		HSIL+	
		P16+	P16–	P16+	P16–	P16+	P16–
LBC-HSIL+ ≥50 (n = 107)		0	**13**	0	**1**	90	3*
LBC-HSIL+ <50 (n = 110)		0	**5**	0	0	104	1*

LBC, liquid-based cytology; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.
*The pathological results of the above four cases were CIN2 and Path-P16 negative. The bold values means the cases which LBC-≥HSIL/Path-≤LSIL but P16-.

HSIL and Path-Normal among women aged ≥50 years to cervical atrophic changes caused by the drop in estrogen levels, which often led to many parabasal cells and basal cells being stained dark on cytology views (16–18). LBC may possibly misinterpret cervical atrophic cells as HSIL or even cancer (19). The atrophy changes on squamous epithelium make the Papanicolaou test less precise and specific in the detection of HSIL (20, 21). Recent studies found that P16/Ki67 double-stained cytology performed high profiles for CIN2+ in postmenopausal women cytologically reported with ASCUS (22). Since misinterpretation of atrophic cells as HSIL+ would not only bring heavy psychological pressure to women but also lead to unnecessary biopsies, the performance of P16 immunocytochemical stain in the facilitation of the interpretation of cytology for women aged 50 or above is worth addressing for its clinical application.

In our study, $P16^{INK4a}$-stained cytology (P16 cytology) performs well in differentiating Path-≤LSIL from LBC-≥HSIL (Figure 2). Those findings are important for further studies and clinic services since P16 cytology helps avoid the atrophic changes of the cervical cells from aged women being misinterpreted as ≥HSIL by LBC. P16-immunostain also contributes to the indication of the invisible HSIL+ under colposcopy (23). In our study, all the LBC-≥HSIL cases that were also positive for P16 were pathologically diagnosed as ≥HSIL. Further analysis of the four Path-≥HSIL cases that were negative for P16 showed that all of them were pathologically graded as CIN2. We do not have data to confirm whether P16 negative results in those cases are potentially caused by hypermethylation (24, 25) or a regressive tendency of those CIN2 cases. The persistence of HPV infections also should be given great importance, as it is related to the persistence and recurrence of HSIL (26, 27). Further study is needed to answer those questions. The findings in our study suggest that P16 immunostain can play an important role in avoiding either overdiagnosis or misdiagnosis. For many years, investigators have endorsed finding proper technology for secondary screening that can keep enough sensitivity for the detection of HSIL but avoid unnecessary biopsies.

Our previous studies demonstrated that the detection rate of P16 cytology is as same as that of HPV testing and LBC analysis for Path-HSIL and above and can be used as the secondary screening test for positive women after primary HPV screening (28). Those studies also indicated that P16 cytology as well as cytology can find abnormal cells that may potentially progress to carcinomas and is better than HPV testing in indicating precancer (29). P16 cytology can be tested at the same time with LBC on the same sample and is advantageous as the secondary screening after primary hr-HPV testing in improving the accuracy of cytology analysis (30). Our analysis in this study further demonstrated the important advantage of P16 cytology in facilitating cytologists to differentiate atrophic changes from HSIL.

In conclusion, our study demonstrated that P16 cytology facilitates differentiation of Path-≤LSIL from LBC-≥HSIL for women 50 years of age and above. It can be used in the lower-resource areas where qualified cytologists are insufficient as the secondary screening test for women aged ≥50 to avoid unnecessary biopsies and misinterpretation of LBC primary or secondary screening.

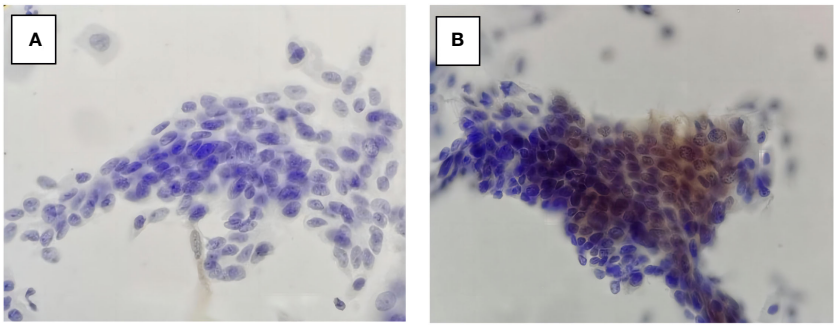

FIGURE 2
P16 cytology in LBC-≥HSIL/Path-≤LSIL **(A)** and LBC-≥HSIL/Path-≥HSIL **(B)**. LBC, liquid-based cytology; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

This study is one of the few retrospective studies on triage in women with LBC-≥HSIL with $P16^{INK4a}$ immunocytochemical stain. It contributes a basis for further studies in the relevant area. However, our study has limitations: patients were grouped by age rather than by menstruation status; thus, there is a lack of proof for the histological atrophy changes. It could be more evident if the LBC and P16 cytology results were from primary screening.

Data availability statement

The raw data supporting the conclusions of this article are available from the corresponding author on reasonable request.

Ethics statement

The studies involving humans were approved by The Ethics Committee from Peking University Shenzhen Hospital (No.2018035). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

JH: Data curation, Formal analysis, Writing – original draft. HD: Writing – review & editing. CW: Data curation, Methodology, Writing – original draft, Resources, Supervision. FS: Investigation, Methodology, Resources, Supervision, Writing – review & editing. XQ: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. RW: Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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