SURGICAL HORIZONS IN CERVICAL CANCER

EDITED BY: Giuseppe Vizzielli, Alberto Farolfi and Valerio Gallotta
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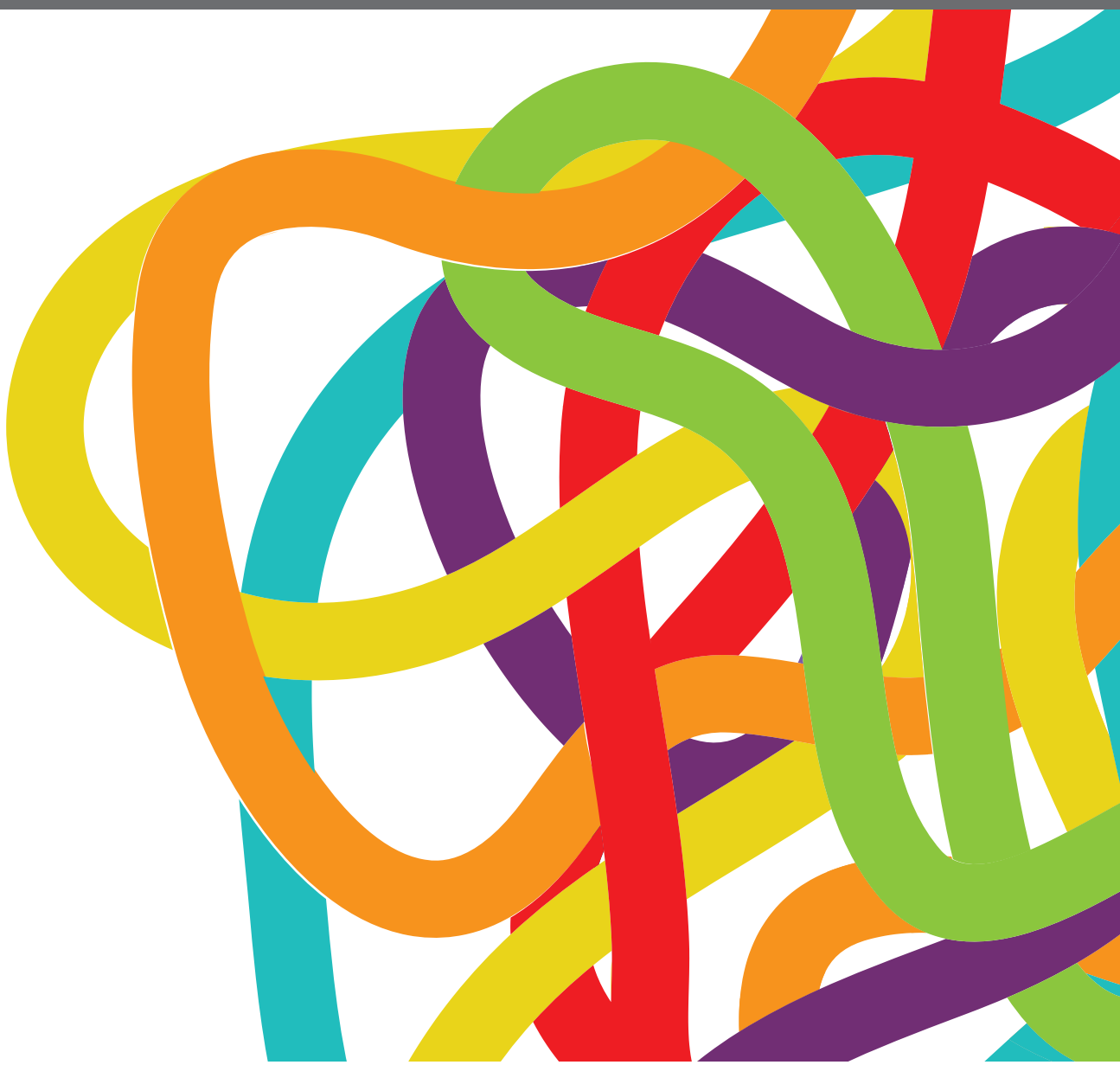

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SURGICAL HORIZONS IN CERVICAL CANCER

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Table of Contents

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Minimally-Invasive Versus Abdominal Hysterectomy for Endometrial Carcinoma With Glandular or Stromal Invasion of Cervix

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The purpose of the study was to evaluate the feasibility of laparoscopic approach versus laparotomy in endometrial cancer that extends to the cervix in the form of glandular extension and/or stromal invasion. A retrospective, single-center cohort study was conducted using data between 1995 and 2017 at an urban tertiary academic medical center. We identified patients who were diagnosed with endometrial cancer whose tumor involved the uterine cervix on final pathology. Operative and oncologic outcomes were compared between the patients who underwent minimally-invasive surgery (MIS) versus those who underwent laparotomy. A total of 282 patients with endometrial cancer were reviewed for the study. Among these patients, 76 patients underwent hysterectomy and surgical staging *via* MIS. There was no conversion from MIS to laparotomy. In the MIS group, shorter hospital stay (4.4 ± 2.3 days for MIS group *vs*. 7.1 ± 4.7 days for laparotomy group; p-value = 0.002) and less blood loss during the operations (228 mL *vs*. 478 mL, p-value < 0.001) were observed compared to the laparotomy group. The multivariate Cox regression analysis revealed that age at diagnosis, FIGO stage, histology grades, tumor size, lymph-vascular space invasion were independent prognostic markers for poor oncologic outcomes but the types of surgical approach (MIS *vs*. laparotomy) were not associated with it. The means by which colpotomy was performed (either intracorporeal or transvaginal) among the MIS group also did not affect patient survivals. Among the women with endometrial cancer that involved the uterine cervix, surgical treatment *via* MIS compared to laparotomy showed no difference in survival outcomes but better perioperative results. These findings support the use of MIS for these patient group.

Keywords: endometrial cancer, minimally-invasive surgery, laparotomy, disease-free survival, overall survival

INTRODUCTION

Endometrial cancer is the sixth most common malignancy worldwide and the most common gynecological malignancy in developed countries with new 380,000 patients diagnosed worldwide in 2018 (1). The incidence of endometrial cancer is increasing due to increasing rates of obesity and life expectancy. Risk factors of endometrial cancer include the use of hormone therapy, diabetes, having

fewer children and history of breast cancer (2, 3). In almost 80% of women, the disease is detected in the early stages, which results in cure rates greater than 90% (4).

The current standard treatment of endometrial cancer is total hysterectomy and bilateral salpingo-oophorectomy. The staging procedure encompasses pelvic and para-aortic lymph node assessment (either by dissection or sentinel lymph node mapping if feasible), omentectomy and peritoneal biopsy, depending on histologic type and stage. Traditionally, laparotomy was used for surgical treatment, but since the 2000s, the frequency of performing laparoscopic approach has increased. Many studies have demonstrated the safety and feasibility of laparoscopic approach in early stages of endometrial cancer (5). For example, studies reported that laparoscopic hysterectomy was associated with less wound infection and blood loss, shorter hospital stay compared to laparotomy and demonstrated no significant difference in overall survival (OS) and disease-free survival (DFS) (4, 6, 7). Most studies, however, were limited to patients with early FIGO (International Federation of Obstetrics and Gynecology) stages (6, 7). Therefore, the recommendation of laparoscopic approach for endometrial cancer surgery in professional guidelines is limited for those with early stages of the disease (8).

The results of the Laparoscopic Approach to Cervical Cancer (LACC) trial, a phase III multi-center randomized trial, were reported in 2018, surprisingly showing inferior survivals of minimally-invasive surgery (MIS) for early cervical cancer compared to laparotomy (9). One potential explanation to understand the inferior oncologic outcomes of MIS in early cervical cancer is related to the surgical techniques of MIS such as frequent manipulations of tumor on the cervix with uterine elevator and intra-abdominal colpotomy which might allow tumor spillage into the abdominal cavity during the procedure.

Endometrial cancer can extend to the cervix in the form of glandular extension and/or stromal invasion. Tumor extension of endometrial cancer to the cervix may also generate the same concerns for inferiority of MIS seen in the LACC trial. Unfortunately, there is a lack of studies investigating the safety of MIS in this subset of patients. In the present study, we retrospectively reviewed endometrial cancer patients who had cervical invasion on final pathology and compared the oncologic outcomes between the two surgical approaches. We also performed analysis to investigate whether either intracorporeal or transvaginal colpotomy was associated with poor survival outcomes.

MATERIALS AND METHODS

The study was approved by the Institutional Review Board (IRB number 2020-10-131-001). This was a retrospective cohort study including patients with endometrial cancer who were histologically confirmed with cervical stromal invasion and/or glandular extension on final pathology. They all underwent staging operations between January 1995 and December 2017 at an urban academic tertiary medical center in Seoul, South Korea (Samsung Medical Center).

Women with biopsy-confirmed or clinically suspicious endometrial cancer underwent either laparotomic or laparoscopic hysterectomy for staging. Radical hysterectomy could be performed if cervical stromal invasion was highly suspicious on computed tomography (CT), magnetic resonance imaging (MRI) or by physical examination. From 2006, laparoscopic staging was introduced in the present institution, and in 2009, more than half of endometrial cancer surgeries were done with laparoscopy. However, the decision on the type of hysterectomy (Type I *vs.* II *vs.* III) and the route of hysterectomy (MIS *vs.* laparotomy) was decided at the surgeons' discretion. If there was a conversion from MIS to laparotomy, we considered it a case of laparotomy. MIS included laparoscopy-assisted vaginal hysterectomy (LAVH), laparoscopy-assisted radical vaginal hysterectomy (LARVH), total laparoscopic hysterectomy (TLH), and laparoscopic radical hysterectomy (LRH). Robot surgery was also considered as MIS.

The demographic parameters evaluated were age at diagnosis, body mass index (BMI), and parity. Information about the types of surgery, conversion rates, the duration of surgery (from skin incision to skin closure), estimated blood loss, hemoglobin levels, postoperative hospitalization days, postoperative pain levels expressed by numeric rating scale (NRS), intra- and postoperative complications, and the types of adjuvant therapy were obtained. Clinical and pathological variables were stages (2018 FIGO stages), grade, histopathologic type, depth of myometrial invasion (as < 50% or ≥ 50%), lymph node involvement, lymph-vascular space invasion (LVSI), number of lymph nodes yielded, and survival outcomes. OS and DFS were also assessed. DFS was defined as the time between the first treatment and recurrence, death or last follow-up, whichever occurred first. OS was defined as the time interval from the day of surgery to the date of death or last follow-up.

The *Shapiro-Wilk test* was used to test normality of the data. Mean ± standard deviation was used for normal distributions and median (range) was used for non-normal distributions. Frequency distributions among categorical variables were compared using the *Chi-Squared test* or *Fisher's exact test*. Survival curves were calculated according to the *Kaplan-Meier methods* with the *log-rank test*. The *Cox proportional hazards model* was used for multivariate analysis to assess different prognostic factors. A *p*-value less than 0.05 was considered statistically significant. Statistical analysis was performed with SPSS software (Version 21.0; SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 2,298 patients were identified who had completed surgical staging for endometrial cancer during the study period. Among them, 282 patients (12.3%, 282/2,298) were confirmed with tumor invasion to the uterine cervix. Of the 282 patients, 76 patients underwent MIS (27.0%, 76/282) while 206 patients underwent laparotomy (73.0%, 206/282) for staging (**Figure 1**). In the MIS group, LAVH was the most common surgical approach (52%) followed by LRH (24%), robotic hysterectomy (12%), LARVH (8%), and TLH (4%). **Table 1** shows the baseline

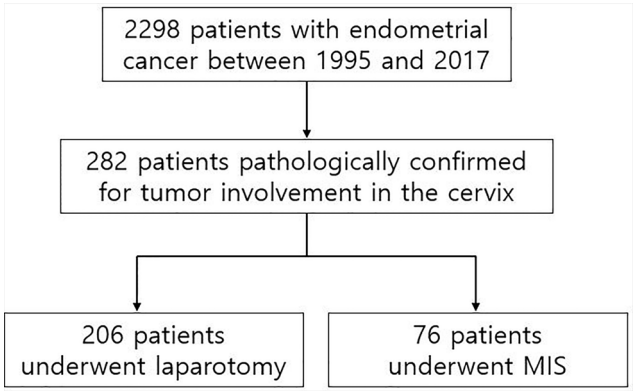

FIGURE 1 | Flowchart of the patient selection.

characteristics of the patients (**Table 1**). Compared to the patients in the MIS group, those in the laparotomy group were older (55.4 ± 11.5 *vs.* 51.3 ± 10.7, *p*-value: 0.007), had lighter body weight (58.1 ± 9.3 *vs.* 61.3 ± 12.3, *p*-value: 0.022), and had higher CA-125 levels (153.5 ± 414.8 *vs.* 13.4 ± 14.9, *p*-value: 0.005). The difference in the CA-125 levels between the two groups was presumably due to more advanced stages of the disease in the laparotomy group. Pathologic findings after the surgeries were compared between the two groups (**Table 2**). As it was reflected by the higher tumor marker levels of the laparotomy group in pre-operative evaluations, it was found that the disease status of the patients in the laparotomy group was more advanced than that of the patients in the MIS group. More patients in the laparotomy group had advanced FIGO stages, higher histology grades, deeper depth of myometrial invasion, adnexal metastasis, intraperitoneal tumor metastasis, and larger tumor size. Although differences in cellular differentiation grades were observed between the two groups as

TABLE 1 | Baseline characteristics of the patients.

	Laparotomy (N=206)	MIS† (N=76)	Total (N=282)	*p*-value
Age at diagnosis (years)	55.4 ± 11.5	51.3 ± 10.7	54.3 ± 11.5	0.007
Body weight (kg)	58.1 ± 9.3	61.3 ± 12.3	59.0 ± 10.3	0.022
Height (cm)	156.6 ± 6.3	156.1 ± 6.7	156.5 ± 6.4	0.506
BMI† (kg/m_2)	23.7 ± 3.6	25.2 ± 5.0	24.1 ± 4.0	0.005
Concurrent cancer				
Ovarian cancer	3 (1.46%)	0	3 (1.06%)	0.706
Colorectal cancer	4 (1.94%)	2 (2.63%)	6 (2.13%)	
Other gynecologic cancers	2 (0.97%)	0	2 (0.71%)	
Breast cancer	6 (2.91%)	3 (3.95%)	9 (3.19%)	
None	187 (90.78%)	69 (90.79%)	256 (90.78%)	
Menopause at diagnosis				
No	92 (44.66%)	41 (53.95%)	133 (47.16%)	0.166
Yes	114 (55.34%)	35 (46.05%)	149 (52.84%)	
Hormone replacement therapy				
Never	201 (97.57%)	76 (100%)	277 (98.22%)	0.598
Past user	3 (1.45%)	0	3 (1.06%)	
Current user	1 (0.48%)	0	1 (0.35%)	
Tamoxifen use				
No	205 (99.51%)	75 (98.68%)	280 (99.29%)	0.461
Yes	1 (0.48%)	1 (1.31%)	2 (0.70%)	
Diabetes mellitus				
No	176 (62.41%)	70 (92.10%)	246 (87.23%)	0.137
Yes	30 (10.63%)	6 (7.89%)	36 (12.76%)	
Hypertension				
No	153 (74.27%)	62 (81.57%)	215 (76.24%)	0.201
Yes	53 (25.72)	14 (18.42%)	67 (23.75%)	
Dyslipidemia				
No	204 (99.02%)	71 (93.42%)	275 (97.51%)	0.007
Yes	2 (0.97%)	5 (6.57%)	7 (2.48%)	
Endometrial hyperplasia				
No	201 (97.57%)	73 (96.05%)	274 (97.16%)	0.716
Simple without atypia	1 (0.48%)	0	1 (0.35%)	
Complex without atypia	1 (0.48%)	1 (1.31%)	2 (0.70%)	
Simple with atypia	0	0	0	
Complex with atypia	3 (1.45%)	2 (2.63%)	5 (1.77%)	
Pre-operative CA-125 (U/mL)	153.5 ± 414.8	13.4 ± 14.9	115.8 ± 359.9	0.005
Pre-operative CA 19-9 (U/mL)	150.4 ± 501.3	33.8 ± 65.8	124.3 ± 444.6	0.344
Pre-operative CEA† (ng/mL)	7.4 ± 29.6	7.3 ± 5.2	6.8 ± 5.4	0.212
Cervical involvement of cancer on pre-operative imaging	86 (41.7%)	25 (32.5%)	111 (39.2%)	0.155

†*MIS, minimally-invasive surgery; BMI, body mass index; CEA, carcinoembryonic antigen.*

seen in **Table 2**, no statistical differences were shown in terms of histology types. The Kaplan-Meier survival analysis revealed no statistical differences in DFS or OS between the two groups (**Figures 2** and **3**). The Cox proportional hazards model revealed that age at diagnosis, FIGO stage, histology grade, tumor size, and LVSI were independent prognostic markers for poor DFS while age at diagnosis, FIGO stage, histology grade, and LVSI were prognostic markers for poor OS (**Tables 3** and **4**). Types of surgical approach (MIS *vs.* laparotomy) or methods of colpotomy (intracorporeal *vs.* transvaginal) did not affect DFS or OS (**Figures 4** and **5**). We also performed subgroup analysis with those patients who were found to be FIGO stage II on their final pathology excluding the patients with other disease stages. The Cox proportional hazards models with the same variables were performed, which revealed similar results as the analysis that included all stage patients (**Supplementary Tables 1** and **2**).

Among the patients in the MIS group, 45 patients underwent surgery in the form of LAVH (including both LAVH and LARVH) while the rest received surgery in the form of TLH (including TLH, LRH, and robotic hysterectomy). The main difference between the two types of surgical approach was how to ligate the uterine arteries and perform colpotomy. Subgroup analysis was performed to rule out the possibilities that each surgical method balances advantages or disadvantages of one another. Perioperative outcomes did not reveal significant differences between the two groups (**Supplementary Table 1**) while the Cox proportional hazards model showed that the differences of surgical approach did not affect survival outcomes as seen in **Tables 3** and **4**. The two groups showed significantly different perioperative outcomes (**Table 5**). While the total operation time was significantly longer in the laparotomy group compared to the MIS group, the MIS group demonstrated less blood loss during the operations, lower rates of transfusion during or after surgery, less post-operative pain measured by NRS, and shorter duration of hospital stay. Perioperative complications did not differ between the two groups. We experienced 4 distal ureteral injuries and two bladder serosal injuries that were all found intraoperatively and repaired. One patient in the laparotomy group had vaginal vault bleeding on her post-operative day 1, which was managed by gauze compression. One patient from each group had vaginal vault dehiscence, which required re-suture. All post-operative bleeding patients received red blood cell transfusion in addition to tranexamic acid infusion but none required re-operation. Abdominal wound complications were found in three patients which included infection and dehiscence (at the level of subcutaneous tissue with intact fascia). However, it was apparent that the longer operation time of the laparotomy group was due to the advanced stages of the patients who required more surgical procedures with high complexity. Four patients in the laparotomy group went to the intensive care unit (ICU) post-operatively. The length of stay in the ICU of all patients was less than 24 hours and the main reason for the stay was for close surveillance. In the present institution, anesthesiologists often recommend post-operative ICU care for patients who underwent extensive surgical procedures even if their vital signs and hematologic parameters stay stable, which was the case for all 4 patients in the present study. The mean estimated blood loss during the operations of them was 387 mL.

DISCUSSION

In the present study, we evaluated the patients with endometrial cancer that involved the cervix on final pathology and compared the outcomes between those who received MIS *vs.* laparotomy. It was found that MIS was not associated with decreased survival outcomes. Furthermore, perioperative outcomes mainly favored MIS over laparotomy demonstrating the benefits of MIS that were also seen in numerous previous studies (10–12).

Previous studies in the literature have consistently demonstrated the non-inferiority of MIS in terms of oncologic outcomes in endometrial cancer compared to laparotomy. Among the studies, the LAP2 study by the Gynecologic Oncology Group is the largest randomized controlled trial, in which the authors compared MIS *vs*. laparotomy in 2,616 patients (7). In that study, patients with clinical stages I to IIA were randomly allocated to laparoscopy versus laparotomy. The trial demonstrated the feasibility and safety of MIS by showing almost identical 5-year overall survival at 89.8%. Other oncologic outcomes were also comparable between the two groups. Among the patients included in the study, 99 patients were found to have FIGO stage II on final pathologic evaluations (65 patients in the MIS group *vs.* 34 patients in the laparotomy group). Subgroup analysis of those patients also demonstrated no decrement of survival in the MIS group.

Another landmark randomized controlled trial evaluated 760 women with FIGO stage I endometrioid endometrial cancer (6). The results of the trial also supported the use of laparoscopic hysterectomy by showing equivalent DFS at 4.5 years and no difference in OS. Among the patients included, 72 patients were found to be FIGO stage II on final pathologic evaluation (32 patients in the MIS group *vs.* 45 patients in the laparotomy group) and there was no statistically significant difference between the MIS group *vs*. the laparotomy groups in any of subgroup analysis including FIGO stages.

Endometrial cancer is commonly confined to the uterus at diagnosis. According to the data from the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program, FIGO stage I disease was found in 73% of patients, and 10% had stage II disease among all endometrial cancer patients (13). The 26th Annual Report of the FIGO on 9,386 endometrial cancer patients also demonstrated that 83% of patients were stage I – II (14). Cervical involvement of endometrial cancer is often not detected prior to hysterectomy and superficial involvement of the cervix by tumor may not be diagnosed by frozen section analysis. Only about 40% of the patients in the present study showed the cervical involvement of tumor on pre-operative imaging. Therefore a significant portion of the patients who were initially thought to have FIGO stage I disease before surgical treatment are eventually diagnosed with FIGO stage II

TABLE 2 | Pathologic findings and adjuvant treatments.

	Laparotomy (N=206)	MIS† (N=76)	Total (N=282)	*p*-value
Treatment type				
Surgery	25 (12.13%)	10 (13.15%)	35 (12.41%)	0.810
Surgery + RT†	85 (41.26%)	33 (43.42%)	118 (41.84%)	
Surgery + CCRT†	39 (18.93%)	16 (20.05%)	55 (19.50%)	
Surgery + CT†	56 (27.19%)	16 (20.05%)	72 (25.53%)	
Types of hysterectomy				
Type I	120 (58.25%)	55 (72.36%)	175 (62.05%)	0.072
Type II	33 (16.01%)	6 (7.89%)	39 (13.82%)	
Type III	53 (25.72%)	15 (19.73%)	68 (24.16%)	
FIGO† stage				
Stage I	30 (14.56%)	19 (25.00%)	49 (17.37%)	0.011
Stage II	70 (33.98%)	23 (30.26%)	93 (32.97%)	
Stage III	62 (30.09%)	25 (32.89%)	87 (30.85%)	
Stage IV	37 (17.96%)	3 (3.94%)	40 (14.18%)	
No data	5 (2.42%)	4 (5.26%)	9 (3.19%)	
Histology				
Endometrioid	126 (61.16%)	58 (76.31%)	184 (65.24%)	0.355
Papillary serous	18 (8.73%)	7 (9.21%)	25 (8.86%)	
Mucinous	1 (0.48%)	1 (1.31%)	2 (0.70%)	
Clear cell	6 (2.91%)	0	6 (2.12%)	
Squamous cell	1 (0.48%)	0	1 (0.35%)	
MMMT†	24 (11.65%)	4 (5.26%)	28(9.92%)	
Undifferentiated	4 (1.94%)	0	4 (1.41%)	
High-grade EST†	1 (0.48%)	0	1 (0.35%)	
Leiomyosarcoma	1 (0.48%)	0	1 (0.35%)	
Adenosarcoma	1 (0.48%)	0	1(0.35%)	
Mixed	18 (8.73%)	3 (3.94%)	21 (7.44%)	
Others	5 (2.42%)	3 (3.94%)	8 (2.83%)	
Grade				
Grade 1	44 (21.35%)	27 (35.52%)	71 (25.17%)	0.003
Grade 2	53 (25.72%)	26 (34.21%)	79 (28.01%)	
Grade 3	80 (33.83%)	13 (17.10%)	93 (32.97%)	
Others	26 (12.62%)	8 (10.52%)	34 (12.05%)	
Ascites or washing cytology				
Not done	48 (24.30%)	10 (13.15%)	58 (20.56%)	0.390
Negative malignant cells	114 (55.33%)	44 (57.89%)	158 (56.02%)	
Positive atypical cells	15 (7.28%)	7 (9.21%)	22 (7.80%)	
Positive malignant cells	29 (14.07%)	14 (18.42%)	43 (15.24%)	
Oophorectomy				
Not done	10 (4.85%)	6 (7.89%)	16 (5.67%)	0.111
Unilateral	2 (0.97%)	3 (3.90%)	5 (1.77%)	
Unilateral with wedge resection on contralateral side	6 (2.90%)	0	6 (2.12%)	
Bilateral	187 (90.77%)	66 (86.84%)	253 (89.17%)	
Pelvic lymphadenectomy				
Not done	35 (16.99%)	5 (6.57%)	40 (14.18%)	0.076
Unilateral	4 (1.94%)	0	4 (1.41%)	
Bilateral	166 (80.58%)	71 (93.42%)	237(84.04%)	
Paraaortic lymphadenectomy				
Not done	120 (58.25%)	40 (52.63%)	160 56.73%)	0.124
Sampling only	6 (2.91%)	1 (1.31%)	7 (2.48%)	
Infra-IMA†	58 (28.15%)	31 (40.78%)	89 (31.56%)	
Infra-renal	22 (10.67%)	4 (5.26%)	26 (9.21%)	
Myometrial invasion				
No invasion	15 (7.28%)	8 (10.52%)	23(8.15%)	0.046
Superficial invasion	12 (5.82%)	6 (7.89%)	18 (6.38%)	
Inner half invasion	60 (29.12%)	23 (30.26%)	83 (29.43%)	
Outer half invasion	78 (37.86%)	32 (42.10%)	110 (39.00%)	
Full invasion	41 (19.9%)	7 (9.21%)	48 (17.02%)	
LVSI†				
No	102 (49.51%)	49 (64.47%)	148 (52.48%)	0.100
Yes	104 (50.48%)	30 (39.47%)	134 (47.51%)	

(Continued)

TABLE 2 | Continued

	Laparotomy (N=206)	MIS† (N=76)	Total (N=282)	*p*-value
Adnexal metastasis				
No	153 (74.27%)	69 (90.78%)	222 (78.72%)	0.003
Yes	53 (25.72%)	7 (9.21%)	60 (21.27%)	
Intraperitoneal tumor				
No	147 (71.35%)	68 (89.47%)	215 (76.24%)	0.002
Yes	59 (28.64%)	8 (10.52%)	67 (23.75%)	
Pelvic lymph nodes				
Right				
Yield	6.7 ± 5.5	7.3 ± 5.2	6.8 ± 5.4	0.365
Positive for metastasis	0.7 ± 1.9	0.4 ± 2.0	0.7 ± 2.0	0.241
Left				
Yield	5.9 ± 4.8	6.4 ± 4.0	6.0 ± 4.6	0.448
Positive for metastasis	0.5 ± 1.4	0.3 ± 1.3	0.5 ± 1.4	0.309
Paraaortic lymph nodes				
Yield	4.4 ± 7.1	4.6 ± 6.2	4.5 ± 6.9	0.808
Positive for metastasis	0.8 ± 3.7	0.5 ± 2.8	0.8 ± 3.5	0.462
Tumor size (cm)	6.4 ± 4.4	3.9 ± 2.2	5.7 ± 4.1	< 0.001
Residual tumor				
No	192 (93.02%)	76 (100%)	268 (95.03%)	0.116
Yes	10 (4.85%)	0	10 (3.54%)	
Estimated blood loss (mL)	478.3 ± 611.7	228.7 ± 189.4	412.3 ± 544.5	< 0.001
Post-operative care				
PACU†	202 (98.05%)	76 (100%)	277 (98.22%)	0.224
ICU†	4 (1.94%)	0	4(1.41%)	
Post-operative RT†				
Not done	74 (35.92%)	21 (27.63%)	95 (33.68%)	0.302
Brachytherapy	25 (12.13%)	8 (10.52%)	33 (11.70%)	
Whole pelvic RT†	96 (46.60%)	38 (50.00%)	134 (47.51%)	
Paraaortic RT†	3 (1.45%)	1 (1.31%)	4 (1.41%)	
Done at other institutions	4 (1.94%)	5 (6.57%)	9 (3.19%)	
No data	4 (1.94%)	3 (3.94%)	7 (2.48%)	

†*MIS, minimally-invasive surgery; RT, radiotherapy; CCRT, concurrent chemoradiotherapy; CT, chemotherapy; FIGO, International Federation of Gynecology and Obstetrics; MMMT, malignant mixed Müllerian tumor; EST, endometrial sinus tumor; IMA, inferior mesenteric artery; LVSI: lymph-vascular space invasion; PACU, post-anesthesia care unit; ICU, intensive care unit.*

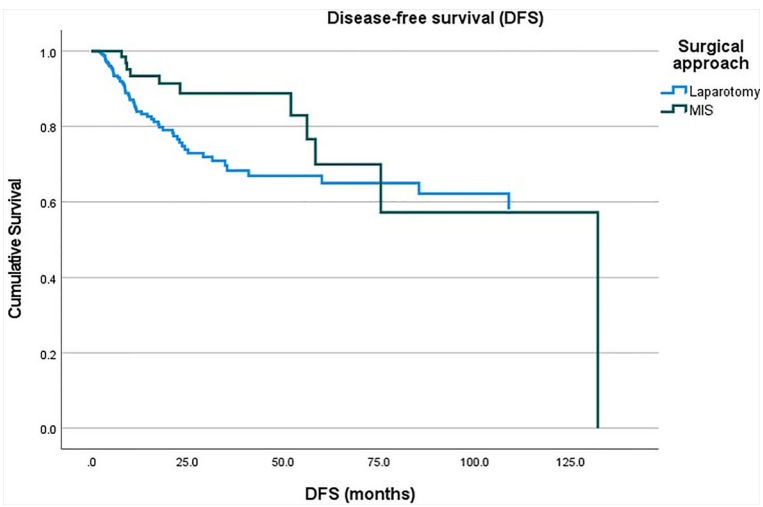

FIGURE 2 | Disease-free survival of the patients with endometrial cancer between the minimally-invasive surgery (MIS) group *vs*. laparotomy group.

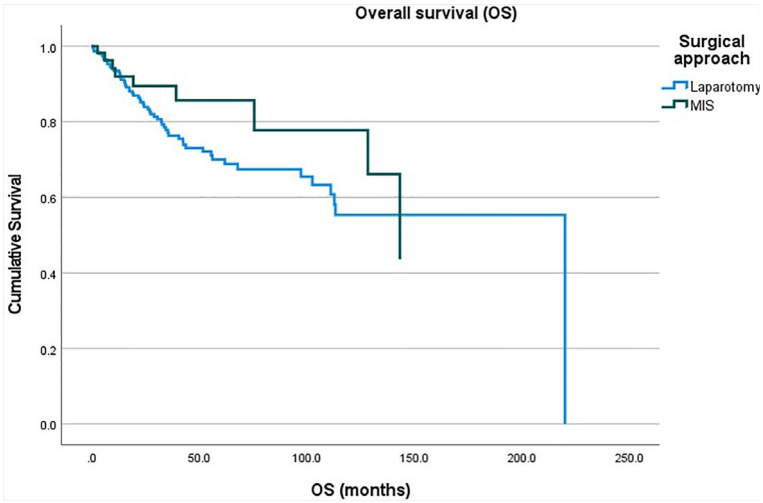

FIGURE 3 | Overall survival of the patients with endometrial cancer between the minimally-invasive surgery (MIS) group *vs*. laparotomy group.

after final pathology evaluation. This may generate aforementioned concerns for both surgeons and patients. However, the results from the present study, combined with previous findings from the literature, reassures that laparoscopic surgery can safely be performed for the patients whose tumor invades the uterine cervix. Furthermore, recent studies demonstrated that laparoscopic surgery did not impair OS in more advanced stages of endometrial cancer such as stage IIIC suggesting that the indication to MIS might be broadened to more advanced disease status, provided that the entire disease is removed (15). In other words, data are being accumulated supporting the use of MIS in endometrial cancer. Studies to date evaluating a variety of factors such as histology, grade, stage, and nodal status, did not reveal any evidence of a particular subgroup of patients that should not be treated with laparoscopy. Moreover, recent studies in robotic surgery revealed that elderly patients in particular may benefit the advantages and favorable perioperative outcomes of MIS when multidisciplinary approach is taken to provide the best management pathway (16, 17).

One of the potential explanations for the decreased survival outcomes seen in the patients who were treated laparoscopically for early cervical cancer in the LACC trial is the use of uterine

TABLE 3 | Disease free survival, Cox model.

	Hazard ratio	95% confidence interval	*p*-value
Age at diagnosis (years)	1.024	1.003 – 1.046	0.027
Pre-operative CA-125	0.999	0.999 – 1.000	0.132
Types of hysterectomy			
Type I	1		
Type II	1.758	0.994 – 3.107	0.052
Type III	0.953	0.430 – 2.112	0.905
FIGO† stage			
Stages 1-2	1		
Stages 3-4	2.228	1.228 – 4.040	0.008
Grade			
Grade 1	1		
Grade 2-3	2.646	1.260 – 5.539	0.010
Tumor size	1.070	1.015 – 1.128	0.011
Lymph-vascular space invasion	1.705	1.056 – 2.753	0.029
Types of surgery			
Laparotomy	1		
Minimally-invasive surgery	0.696	0.371 – 1.306	0.260
Types of colpotomy			
Intracorporeal	1		
Transvaginal	0.317	0.058 – 1.722	0.183

†*FIGO, International Federation of Gynecology and Obstetrics.*

TABLE 4 | Overall survival, Cox model.

	Hazard ratio	95% confidence interval	*p*-value
Age at diagnosis (years)	1.038	1.013 – 1.064	0.002
Pre-operative CA-125	1.000	0.999 – 1.001	0.750
Types of hysterectomy			
Type I	1		
Type II	2.010	1.041 – 3.883	0.038
Type III	1.448	0.611 – 3.621	0.381
FIGO† stage			
Stages 1-2	1		
Stages 3-4	1.777	1.002 – 3.152	0.047
Grade			
Grade 1	1		
Grade 2-3	2.491	1.383 – 4.485	0.030
Tumor size	1.048	0.966 – 1.136	0.259
Lymph-vascular space invasion	2.512	1.358 – 4.645	0.003
Types of surgery			
Laparotomy	1		
Minimally-invasive surgery	1.661	0.890 – 3.100	0.111
Types of colpotomy			
Intracorporeal	1		
Transvaginal	1.241	0.174 – 8.878	0.830

†*FIGO, International Federation of Gynecology and Obstetrics.*

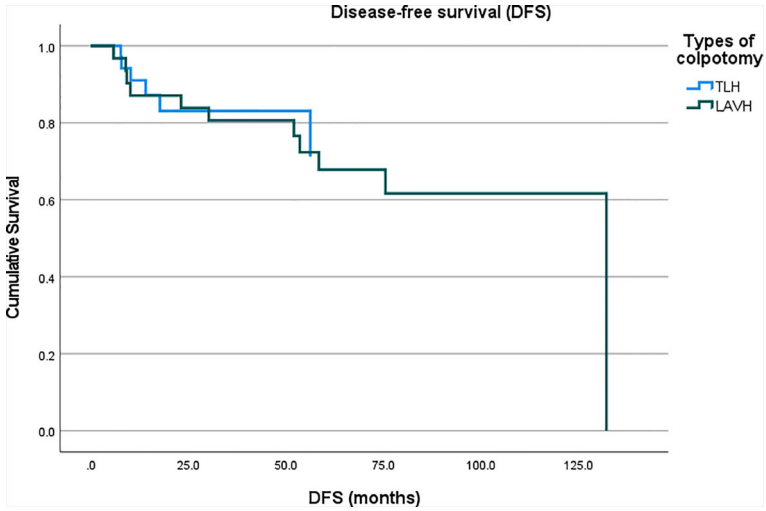

FIGURE 4 | Comparison of disease-free survival of the patients with endometrial cancer who underwent intracorporeal colpotomy (TLH, total laparoscopic hysterectomy) *vs*. transvaginal colpotomy (LAVH, laparoscopy-assisted vaginal hysterectomy).

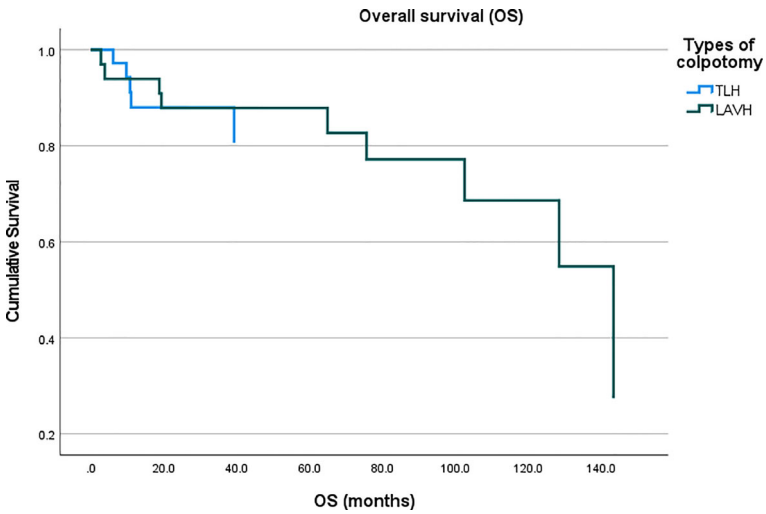

FIGURE 5 | Comparison of overall survival of the patients with endometrial cancer who underwent intracorporeal colpotomy (TLH, total laparoscopic hysterectomy) *vs*. transvaginal colpotomy (LAVH, laparoscopy-assisted vaginal hysterectomy).

manipulator, which might increase the propensity for tumor spillage. During the study period, the surgeons at the present institution also used uterine manipulators routinely. However, the device was installed only after the electrocoagulation of the isthmus of the fallopian tubes with bipolar forceps. The colpotomy was performed by either intracorporeal approach or transvaginal approach at the surgeons' discretion. The surgeons were not particularly concerned in regards to the increased likelihood of tumor recurrence in patients whose tumor invaded the cervix. The report of the LACC trial called into question whether the decreased survival of MIS would apply to endometrial cancer. The results of the present study showed the methods of colpotomy were not associated with survival outcomes. Further investigation is warranted to explain the difference in the observations of the adverse effects of uterine manipulators in the two different types of malignancies.

The present study adds valuable information to the literature in that it is the first study to compare MIS *vs*. laparotomy in patients with endometrial cancer whose tumor involves the uterine cervix. It showed comparable survival outcomes between the two groups. It also has limitations. The number of patients evaluated in the present study is still relatively small to

TABLE 5 | Perioperative outcomes.

	Laparotomy	MIS†	*p*-value
Intraoperative factors			
Anesthesia time (min)	198 (80 – 383)	259 (128 – 724)	0.029
Operation time (min)	460 (65 – 321)	222 (93 – 623)	0.008
Blood transfusion required			
RBC† transfusion during or after surgery	42 (20.39%)	4 (5.26%)	0.002
Hemoglobin drop†† on POD† #1	1.8 (-0.2 – 4.2)	1.25 (-0.2 – 3.5)	0.319
Postanesthesia care unit (PACU)			
PACU stay (min)	90 (50 – 190)	80 (48 – 130)	0.133
Perioperative complications			
Distal ureteral injury	3	1	0.935
Bladder injury	2	0	
Vaginal vault bleeding	1	0	
Vaginal vault dehiscence	1	1	
Postoperative bleeding	4	2	
Abdominal wound complications	2	1	
Postoperative floor numeric rating score (NRS)			
NRS 0 – 6 hours after surgery	5 (2 – 8)	3 (2 – 8)	0.054
NRS 12 – 24 hours after surgery	3 (2 – 6)	3 (2 – 5)	0.019
Hospital stay (days)	7.1 ± 4.7	4.4 ± 2.3	0.002

†*MIS, Minimally-invasive surgery; RBC, red blood cell; POD, postoperative day.*
††*Defined as postoperative hemoglobin levels subtracted from preoperative hemoglobin levels.*

generalize the results to all stages of endometrial cancer patients. Another limitation of the study is that, due to the retrospective design, there might have been selection bias of the patients. It is evident that the patients with advanced stages of endometrial cancer were more likely to receive laparotomy. This could not exclude that patients with more advanced FIGO stages, higher histology grades, deeper myometrial invasion, adnexal and intraperitoneal metastases, and larger tumor size underwent laparotomy, which makes them not comparable to those treated by MIS. However, already given the positive evidence of MIS from previous studies, it was ethically not feasible to randomize the patients into MIS *vs.* laparotomy. Therefore, it was our best effort to analyze this issue retrospectively with collected data from our patients. In order to minimize the potential bias, we performed the Cox proportional hazards model with other variables that are already known to affect patient survivals in endometrial cancer. Although statistical methods were implemented to control this factor, this certainly limits the interpretation of the results and remains as the main limitation of the study.

Despite the presence of the aforementioned limitations, the results of the present study along with those from other previous studies suggest that surgical staging can be performed laparoscopically in patients with endometrial cancer that involves the cervix of the uterus. Long-term survival analysis should be supported by randomized controlled studies to demonstrate that laparoscopic approach may be an acceptable alternative to laparotomy in this patient group.

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 human participants were reviewed and approved by Samsung Medical Center. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

The present study was designed, directed and coordinated by Y-YL, as the principal investigator. Y-YL provided conceptual and technical guidance for all aspects of the project. JJ and JN planned and performed the analyses of the data with CC, T-JK, and J-WL. The data were collected by CC, T-JK, J-WL, B-GK, and D-SB. The manuscript was written by JJ and JN and commented on by all authors. All the authors meet the recommendations for the conduct, reporting, editing, and publication of scholarly work in medical journals provided by the International Committee of Medical Journal Editors. All authors contributed to the article and approved the submitted version.

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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.2021.670214/full#supplementary-material

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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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ORIGINAL RESEARCH
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Laterally Extended Endopelvic Resection Versus Chemo or Targeted Therapy Alone for Pelvic Sidewall Recurrence of Cervical Cancer

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Background: Laterally extended endopelvic resection (LEER) has been introduced for treatment of pelvic sidewall recurrence of cervical cancer (PSRCC), which occurs in only 8% of patients with relapsed cervical cancer. LEER can only be performed by a proficient surgeon due to the high risk of surgical morbidity and mortality, but there is no evidence as to whether LEER is may be more effective than chemo or targeted therapy alone for PSRCC. Thus, we aimed to compare the efficacy and safety between LEER and chemo or targeted therapy alone for treatment of PSRCC.

Methods: We prospectively recruited patients with PSRCC who underwent LEER between December 2016 and December 2019. Moreover, we retrospectively collected data on patients with PSRCC who received chemo or targeted therapy alone between January 2000 and December 2019. We compared treatment-free interval (TFI), progression-free survival (PFS), treatment-free survival (TFS), overall survival (OS), tumor response, neurologic disturbance of the low extremities, and pelvic pain severity in the different patient groups.

Results: Among 1295 patients with cervical cancer, we included 28 (2.2%) and 31 (2.4%) in the prospective and retrospective cohorts, respectively. When we subdivided all patients into two groups based on the median value of prior TFI (PTFI, 9.2 months), LEER improved TFI, PFS, TRS and OS compared to chemo or targeted therapy alone (median, 2.8 *vs.* 0.9; 7.4 *vs.* 4.1; 30.1 *vs.* 16.9 months; $P \leq 0.05$) in patients with PTFI < 9.2 months despite no difference in survival in those with PTFI ≥ 9.2 months, suggesting that LEER may lead to better TFI, PFS, TRS and OS in patients with PTFI < 9.2 months (adjusted hazard ratios, 0.28, 0.27, 0.44 and 0.37; 95% confidence intervals, 0.12-0.68, 0.11-0.66, 0.18-0.83 and 0.15-0.88). Furthermore, LEER markedly reduced the number

of morphine milligram equivalents necessary to reduce pelvic pain when compared with chemo or targeted therapy alone.

Conclusion: Compared to chemo or targeted therapy alone, LEER improved survival in patients with PSRCC and PTFI < 9.2 months, and it was effective at controlling the pelvic pain associated with PSRCC.

Trial Registration: ClinicalTrials.gov, identifier NCT02986568.

Keywords: laterally extended endopelvic resection, pelvic sidewall recurrence, survival, pain, cervical cancer

INTRODUCTION

Pelvic exenteration can be attempted as a cure for central recurrence of cervical cancer, which is seen in 10.7% of patients with disease recurrence after radical treatment such as radiotherapy and radical hysterectomy. Vaginectomy provides another option for patients with isolated vaginal recurrence with acceptable postoperative complications and quality of life compared to radiotherapy or pelvic exenteration (1, 2). The five-year survival rate of such patients ranges from 30 to 60% (3). On the other hand, pelvic sidewall recurrence of cervical cancer (PSRCC) is relatively rare, occurring in 8.3% of patients with disease recurrence (4). However, tumors invading the pelvic sidewall structure are not easy to remove by pelvic exenteration, and residual tumors after pelvic exenteration are associated with poor prognosis (5, 6). Since salvage radiotherapy reportedly fails to treat loco-regional tumors in a previously irradiated field, palliative chemotherapy is mainly used to slow disease progression and control the pelvic pain caused by tumor invasion in the pelvic sidewall structure (3, 4).

Laterally extended endopelvic resection (LEER), an ultra-radical surgery that aims to remove pelvic sidewall tumors, has been used since 1999 in an effort to improve patient survival (7). Based on the ontogenetic compartment theory, LEER can provide tumor-free margins (R0) by resecting tumors that propagate through multi-compartmental borders between the pelvic floor and sidewall muscles and the internal iliac vessel system (8). However, LEER is a highly skilled surgery that can only be done by a proficient surgeon. It requires definite anatomical knowledge of pelvic sidewall structure due to the risk of massive bleeding during resection of tumors invading the major pelvic vessels, and adhesion and fibrosis in a previously debulked or irradiated pelvis can increase surgical morbidity and mortality (9, 10).

Despite these limitations, LEER reportedly produces a five-year survival rate of about 50%, and the number of studies on the feasibility of LEER for selected patients with PSRCC has gradually been increasing since 2015 (6, 9–14). However, the criteria for identification of patients for whom LEER may be beneficial remain ambiguous, and there is no evidence as to whether LEER may be more effective than palliative chemo or targeted therapy alone. This is an especially important question considering the high number of morbidities related to LEER. Thus, we performed a prospective cohort study to evaluate the efficacy and safety of LEER for patients with PSRCC and investigated the criteria for selection of patients who may benefit from LEER compared to chemo or targeted therapy alone.

MATERIALS AND METHODS

Study Design

We prospectively collected data on patients with PSRCC who underwent LEER in Seoul National University Hospital between December 2016 and December 2019. The study protocol was registered on ClinicalTrials.gov (NCT02986568) before any patients. For the prospective cohort study, we consecutively recruited patients who were aged 20 years or older; had Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; had recurrent or refractory cervical cancer; had unilateral PSRCC not involving the greater sciatic foramen with or without uncontrolled pelvic pain despite sufficient opioid usage; had PSRCC that might be cured or uncontrolled pelvic pain that might be relieved by LEER; signed the approved informed consent form; and had no other treatment options except for LEER. We excluded patients who were under 20 years of age; had ECOG performance status of 2 or more; had bilateral PSRCC; had a treatment option other than LEER; or refused to sign the approved informed consent form.

As historical controls, we retrospectively collected data on patients with PSRCC who received chemo or targeted therapy alone without LEER between January 2000 and December 2019. The inclusion and exclusion criteria for the retrospective group were the same as those for the prospective group except that informed consent was not necessary. For both cohorts, we collected data such as patient age; histologic type; size of pelvic sidewall tumors on imaging studies; disease extent according to TNM stage on radiologic imaging studies (15); topographic location and direction of pelvic sidewall tumors; types of prior treatment; tumor response to prior treatment; prior treatment-free interval (PTFI), defined as the time from completion of prior treatment to disease progression necessitating the current treatment; the current treatment line for PSRCC; regimen types and cycles of chemo or targeted therapy for the current treatment; and the duration of follow-up.

Procedures

In the prospective cohort, LEER was performed according to the surgical procedures detailed in previous reports (7, 9). In brief, a midline incision was made on the abdomen, the bilateral paracolic gutters were incised, and the peritoneum was dissected at the base of the radix mesenterii for bowel mobilization. Then, the bilateral ureters were identified and liberated. If pelvic sidewall tumors had invaded the bladder

and rectum, the bilateral paravesical and pararectal spaces and the space of Retzius were developed. The bilateral ureters were cut as close to the bladder as possible and the negative margins of the distal ureters were identified by frozen sections. Moreover, the mesosigmoid or mesorectum was skeletonized, and the blood vessels therein were ligated at a sufficient distance from the tumor. Bowel continuity was interrupted using a gastrointestinal anastomosis (GIA) staplers at the level of the proximal margin with no gross tumor.

For *en bloc* resection of pelvic sidewall tumors with negative resection margins, we first ligated the internal iliac artery just below the bifurcation of the common iliac artery and then divided the internal iliac vein at the bifurcation. The branches of the posterior division of the internal iliac vessel system, including the superior gluteal, inferior gluteal, and internal pudendal arteries and veins were transected using hemoclips or hemolock clips. Depending on the topography of PSRCC, the obturator internus muscle, the coccygeus muscle, and the levator ani muscles such as the pubococcygeus and iliococcygeus muscles were incised and separated from the pelvic sidewall with a Cobb periosteal dissector. Thereafter, the vulva was incised for removal of the urethra, lower vagina, and anus, and a dissection was carried to enter the space of Retzius and divide the pelvic floor musculature laterally and posteriorly. Recurrent pelvic sidewall tumors that were surrounded by the pelvic organs and adjacent pelvic floor muscles were removed through the inferior pelvic opening. After LEER, permanent colostomy and ileal conduit urinary diversion were carried out. In some cases, depending on the tumor location, the bladder, vagina and rectum could be preserved after checking the negative resection margin in frozen sections.

R0 resection was defined as lack of tumor invasion in the tissues of the lateral margins of the obturator internus, coccygeus, iliococcygeus and pubococcygeus muscles and the internal iliac vessel system ipsilateral to pelvic sidewall tumors on pathologic examination. If the bladder or rectum was preserved, an absence of tumor invasion in tissues surrounding the removed lesions according to multiple biopsies was considered R0 resection.

Postoperative complications were assessed by the Memorial Sloan Kettering Cancer Center criteria (16). For chemo or targeted therapy, single or combination regimens were used in both the prospective and retrospective cohorts. Moreover, targeted therapy using paclitaxel, cisplatin, and bevacizumab based on the Gynecologic Oncology Group (GOG) 240 trial was used beginning in August 2015 due to changes in insurance coverage (17, 18).

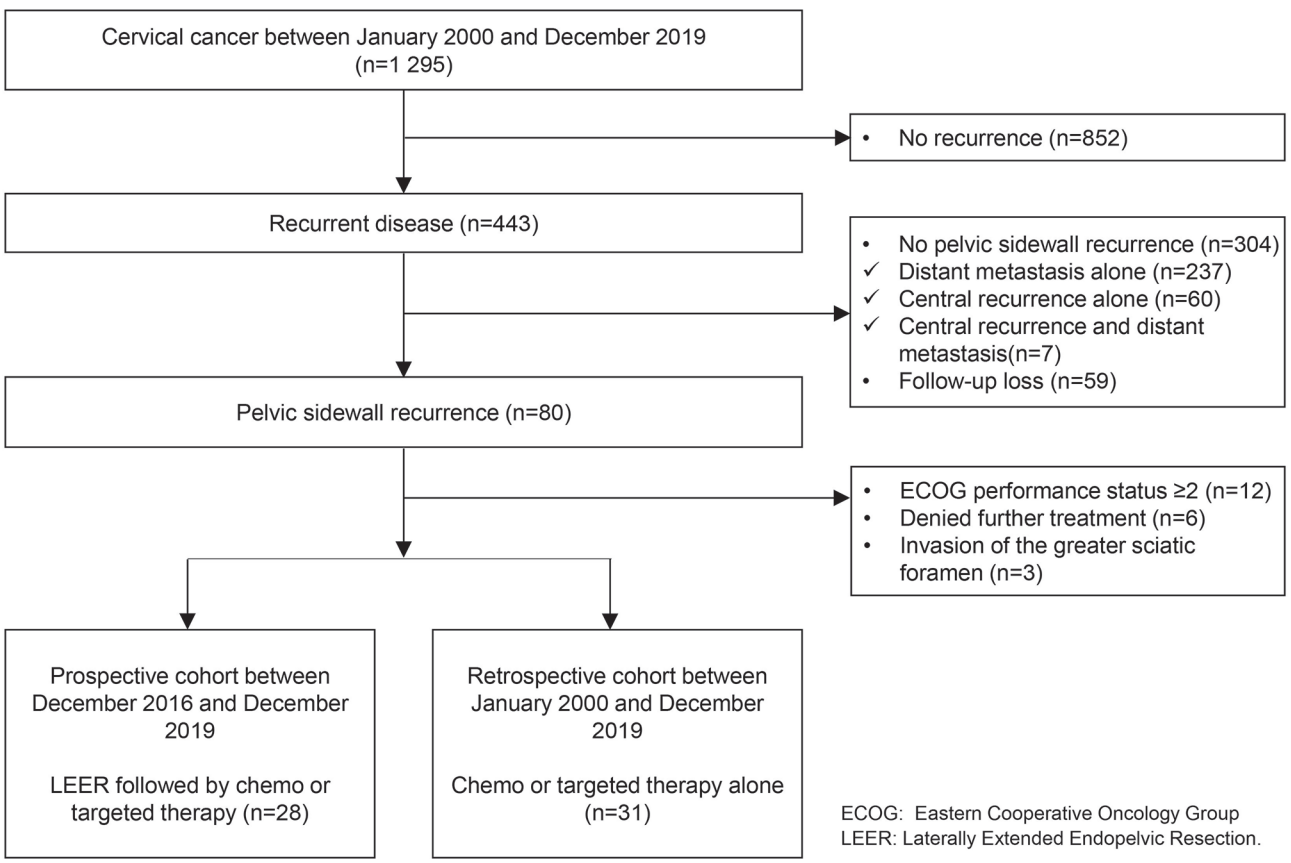

FIGURE 1 | Diagram of the study flow.

Outcomes

The primary outcomes were the differences in treatment-free interval (TFI), progression-free survival (PFS), treatment-free survival (TFS), and overall survival (OS) between the prospective and retrospective cohorts. TFI was defined as the time interval from completion of treatment for PSRCC to disease progression; PFS was defined as the time interval from the start of treatment for PSRCC to disease progression; TFS was defined as the time interval from the start of treatment for PSRCC to cancer-related death or the end of the study; and OS was defined as the time interval from the diagnosis of cervical cancer to cancer-related death or the end of the study.

The secondary outcomes were the differences in tumor response, neurologic disturbance of the lower extremities, and severity of pelvic pain between the two groups. We assessed tumor response using the revised Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (19), and neurologic disturbance of the lower extremities was evaluated by the severity of muscle weakness and neuralgia in the lower limbs according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Moreover, pelvic pain severity was evaluated using both a numerical rating scale (NRS) and the number of morphine milligram equivalents (MME), representing the total amount of various opioids prescribed to control pelvic pain (20).

Statistical Analysis

We compared non-parametric variables between the two groups with Mann-Whitney U, chi-square, and Fisher's exact tests. Moreover, we compared TFI, PFS, TRS, and OS between the two groups by Kaplan-Meier survival analysis with the log-rank and Breslow tests and identified factors affecting survival using univariate and multivariate Cox proportional hazard regression models. All statistical tests were two-sided, and $P < 0.05$ was considered statistically significant. SPSS software version 21.0 (SPSS Inc., Chicago, IL, USA) was used.

RESULTS

Study Population

Among 1,295 patients with cervical cancer, 443 (34.2%) showed disease recurrence. Of patients with disease recurrence, we excluded those with distant metastasis alone (n = 237, 18.3%), central recurrence alone (n = 60, 4.6%), and central recurrence and distant metastasis (n = 7, 0.5%); also, 59 (4.6%) were lost to follow-up. Among the remaining 80 patients with PSRCC (6.2%), we also excluded 12 (0.9%), six (0.5%), and three patients (0.2%) due to an ECOG performance status of two or more, denial of further treatment, and invasion of the greater sciatic foramen, respectively. Finally, we included 28 (2.2%) and 31 (2.4%) patients in the prospective and retrospective cohorts, respectively (**Figure 1**).

Table 1 shows the clinico-pathologic characteristics of the study subjects. There were no differences in age, histologic types,

TABLE 1 | Clinicopathologic characteristics.

Characteristics	Prospective cohort (n=28)	Retrospective cohort (n=31)	P value
Age (years)	44.5 (28-70)	47 (31-71)	0.76
Histological types			0.47
Squamous cell carcinoma	21 (75)	26 (83.9)	
Endocervical adenocarcinoma	3 (10.7)	4 (12.9)	
Mucinous adenocarcinoma	2 (7.1)	0 (0)	
Adenosquamous carcinoma	1 (3.6)	1 (3.2)	
Large cell neuroendocrine tumor	1 (3.6)	0 (0)	
FIGO stage			0.829
Stage I	15 (53.6)	14 (45.2)	
Stage II	7 (25)	7 (22.6)	
Stage III	3 (10.7)	5 (16.1)	
Stage IV	3 (10.7)	5 (16.1)	
Size of pelvic sidewall tumor on imaging studies (cm)	3.5 (1.7-7.7)	3.6 (1-9.7)	0.83
Radiologic TNM stage			
T - Tumor			0.24
rT3b	23 (82.1)	29 (93.5)	
rT4	5 (17.9)	2 (6.5)	
N – Regional lymph nodes			0.46
rN0	18 (64.3)	17 (54.8)	
rN1	10 (35.7)	14 (45.2)	
M – Distant metastasis			0.54
rM0	21 (75)	21 (67.7)	
rM1	7 (25)	19 (32.3)	
Topographic location of pelvic sidewall tumor			0.27
Infra-iliac ischiopubic	2 (7.1)	0 (0)	
Infra-iliac acetabular	14 (50)	23 (74.2)	
Peri-iliac acetabular	2 (7.1)	2 (6.5)	
Infra-iliac sacrococcygeal	9 (32.1)	5 (16.1)	
Peri-iliac iliosacral	1 (3.6)	1 (3.2)	
Direction of pelvic sidewall tumor			0.42
Right	15 (53.6)	14 (45.2)	
Left	13 (46.4)	17 (54.8)	
Types of prior treatment			0.83
CCRT	3 (10.7)	0 (0)	
Surgery and chemoradiation	5 (17.9)	7 (22.6)	
Chemoradiation and chemotherapy	3 (10.7)	10 (32.3)	
Surgery, chemoradiation and chemotherapy	17 (60.7)	14 (45.2)	
Tumor response to prior treatment			0.89
Complete response	10 (47.6)	3 (42.9)	
Partial response	4 (19)	1 (14.3)	
Progressive disease	7 (33.3)	3 (42.9)	
Prior treatment-free interval 2(months)	9.3 (0.5, 321.5)	7.5 (0.6, 158.5)	0.89
Current treatment line for pelvic sidewall tumor			0.01
1	21 (75)	31 (100)	
2	5 (17.9)	0 (0)	
3	2 (7.1)	0 (0)	
Use of bevacizumab			0.02
No	14 (50)	25 (80.6)	
Before the current treatment	12 (42.9)	2 (6.5)	
During the current treatment	2 (7.1)	3 (9.7)	
After the current treatment	0 (0)	1 (3.2)	
Duration of follow-up (months)	36.7 (14.5-331.7)	35.7 (9.4-196.2)	0.51

Data are median (range) or n (%).

Patients in the prospective cohort received laterally extended endopelvic resection followed by chemo or targeted therapy, whereas those in the retrospective cohort received chemo or targeted therapy alone for pelvic sidewall recurrence of cervical cancer.

size of pelvic sidewall tumors on imaging studies, radiologic TNM stage, topographic location and direction of pelvic sidewall tumors, types of prior treatment, tumor response to prior treatment, PTFI, or duration of follow-up between the two groups. In the prospective cohort, only 21 patients (75%) received LEER immediately after being diagnosed with PSRCC, whereas seven (25%) received second- or third-line chemo or targeted therapy prior to LEER. After LEER, three patients (10.7%) did not receive chemo or targeted therapy due to renal failure (n = 1) and rapid disease progression during management of postoperative complications (n = 2). Although there was no difference in the types of treatment regimens between the two groups, combination therapy using paclitaxel, cisplatin and bevacizumab was more common in the prospective cohort than in the retrospective cohort, and more cycles of chemo or targeted therapy were administered in the retrospective cohort than in the prospective cohort (**Table 2**).

Treatment Outcomes

In terms of surgical extents, we were able to preserve the rectum alone and both the rectum and bladder in ten (35.7%) and three patients (10.7%), respectively. Among the pelvic sidewall structures, the obturator internus, pubococcygeus, iliococcygeus, and coccygeus muscles were resected in nine (32.1%), 12 (42.9%), 16 (57.1%), and 15 patients (53.6%), respectively, and the internal iliac vessel system was removed in 27 patients (96.4%; **Table 3**).

With regard to pathologic outcomes related to LEER, the median value of the size of pelvic sidewall tumors was 4.6 cm, and we achieved R0 resection in 26 patients (92.9%). Among the pelvic sidewall structures, tumor involvement in the obturator internus, pubococcygeus, iliococcygeus, and coccygeus muscles was seen in five (17.9%), four (14.3%), six (21.4%), and four (14.3%) patients, respectively, and 14 (50%) showed tumor involvement in the internal iliac vessel system (**Table 4**).

Postoperative complications developed in 17 patients (60.7%) after LEER. Arterial or venous thrombus was the most common complication (14.2%). Moreover, grade 3 or 4 complications according to the MSKCC surgical secondary events grading system were observed in 14 patients (50%; **Table 5**). In the retrospective cohort, the recto-vaginal fistula developed in 5 patients (16.1%); of these, four patients (12.9%) received bevacizumab. The association between bevacizumab usage and fistula development was not statistically significant (p = 0.088).

TABLE 2 | Regimen type and number of cycles of chemo or targeted therapy for the current treatment.

	Prospective cohort (n = 28)	Retrospective cohort (n = 31)	P value
Types			0.13
No	3 (10.7)	0 (0)	
Paclitaxel/carboplatin	3 (10.7)	12 (38.7)	
Paclitaxel/cisplatin	2 (7.1)	0 (0)	
Topotecan/cisplatin	9 (32.1)	8 (25)	
5-fluorouracil/cisplatin	2 (7.1)	2 (6.5)	
5-fluorouracil/carboplatin	1 (3.6)	1 (3.2)	
Gemcitabine	4 (14.3)	1 (3.2)	
Cisplatin	1 (3.6)	2 (6.5)	
Topotecan	1 (3.6)	0 (0)	
Etoposide	0 (0)	1 (3.2)	
Irionotecan	0 (0)	1 (3.2)	
Paclitaxel/cisplatin/bevacizumab	2 (7.1)	3 (9.7)	
Cycles	3.5 (2 - 6)	5 (3 - 15)	<0.03

Data are median (range) or n (%).
Patients in the prospective cohort received laterally extended endopelvic resection followed by chemo or targeted therapy, whereas those in the retrospective cohort received chemo or targeted therapy alone for pelvic sidewall recurrence of cervical cancer.

TABLE 3 | Surgical extent.

	Prospective cohort (n = 28)
Preservation of the pelvic organs	
No	15 (53.6)
Rectum alone	10 (35.7)
Bladder and rectum alone	3 (10.7)
Extent of resection	
Bladder and urethra	25 (89.3)
Rectum and anus	15 (53.6)
Uterus	18 (64.3)
Vagina	20 (71.4)
Perineum	15 (53.6)
Obturator internus muscle	9 (32.1)
Pubococcygeus muscle	12 (42.9)
Iliococcygeus muscle	16 (57.1)
Coccygeus muscle	15 (53.6)
Internal iliac vessel system	27 (96.4)
Estimated blood loss (ml)	1800 (400 - 16800)
Transfusion	4 (0 - 39)
Operation time (minutes)	465 (190 - 760)
Hospitalization (days)	22 (8 - 86)

Data are median (range) or n (%).
Patients in the prospective cohort received laterally extended endopelvic resection followed by chemo or targeted therapy.

TABLE 4 | Pathologic outcomes.

	Prospective cohort (n = 28)
Size of pelvic sidewall tumors (cm)	4.6 (1 - 11)
Resection margin	
R0	26 (92.9)
R1	2 (7.1)
Extent of tumor involvement	
Bladder	17 (60.7)
Urethra	3 (10.7)
Rectum	11 (39.3)
Anus	7 (25)
Uterus	1 (3.6)
Vagina	16 (57.1)
Perineum	0 (0)
Obturator internus muscle	5 (17.9)
Pubococcygeus muscle	4 (14.3)
Iliococcygeus muscle	6 (21.4)
Coccygeus muscle	4 (14.3)
Internal iliac vessel system	14 (50)

Data are median (range) or n (%).
Patients in the prospective cohort received laterally extended endopelvic resection followed by chemo or targeted therapy.

TABLE 5 | Postoperative complications.

	Prospective cohort (n = 28)
ypes	
No	11 (39.3)
Arterial or venous thrombus	4 (14.2)
Leakage from the anastomotic site	3 (10.7)
Infected lymphocele	2 (7.1)
Inflammatory pelvic fluid collection	2 (7.1)
Acute pyelonephritis	1 (3.6)
Hydronephrosis	1 (3.6)
Ileus	1 (3.6)
Renal stone	1 (3.6)
Paralysis of low extremity	1 (3.6)
Wound dehiscence	1 (3.6)
Grade	
0	11 (39.3)
2	3 (10.7)
3	12 (42.9)
4	2 (7.1)

Data are median (range) or n (%).
Patients in the prospective cohort received laterally extended endopelvic resection followed by chemo or targeted therapy.

Survival

Survival analysis between the two groups revealed no differences in TFI, PFS, TRS, and OS between the prospective and retrospective cohorts in all patients (**Figure 2**). To go into greater detail, we also performed subgroup analyses based on the following favorable indications according to previous reports: tumor size ≤ 5 cm; PFTI > 5 months; and no distant metastasis (8, 21). As a result, we also found no difference in TFI, PFS, TRS, and OS between the prospective and retrospective cohorts based on the favorable indications (**Figure 3**). Furthermore, we conducted subgroup analyses based on the median value of PTFI, 9.2 months. In the 30 patients with PTFI ≥ 9.2 months, there were no differences in TFI, PFS, TRS and OS between the two groups, whereas LEER followed by chemo or targeted therapy was associated with improved TFI, PFS, and OS compared to chemo or targeted therapy alone (median values, 2.8 *vs.* 0.9 months; 7.4 *vs.* 4.1 months; 30.1 *vs.* 16.9 months; P ≤ 0.05) in the 29 patients with PTFI < 9.2 months (**Figure 4**).

Next, we conducted univariate and multivariate analyses to identify factors affecting survival (**Supplementary Tables 1**–**3**).

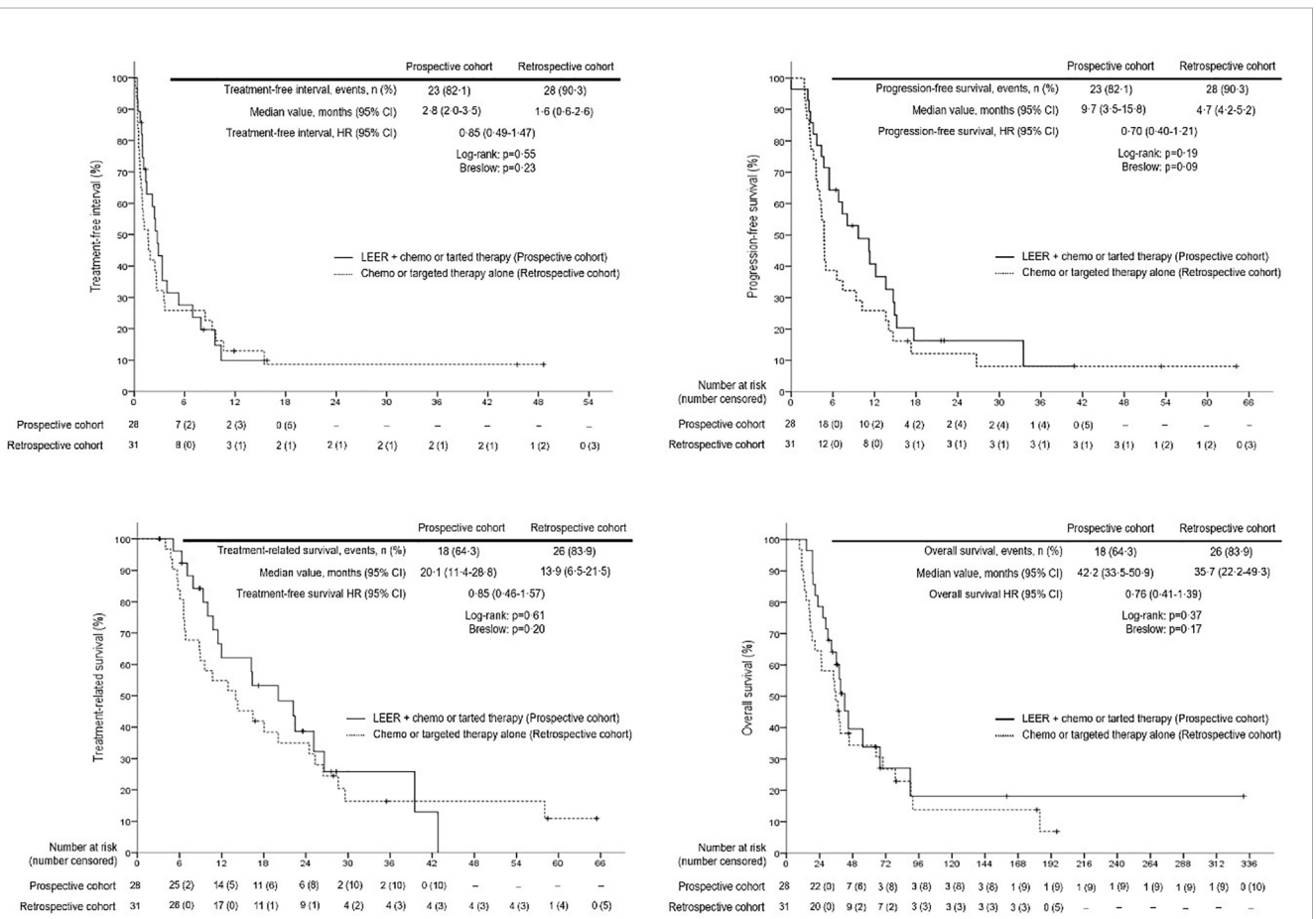

FIGURE 2 | Comparison of treatment-free interval, progression-free survival, treatment-related survival and overall survival between the prospective and retrospective cohorts in all patients.

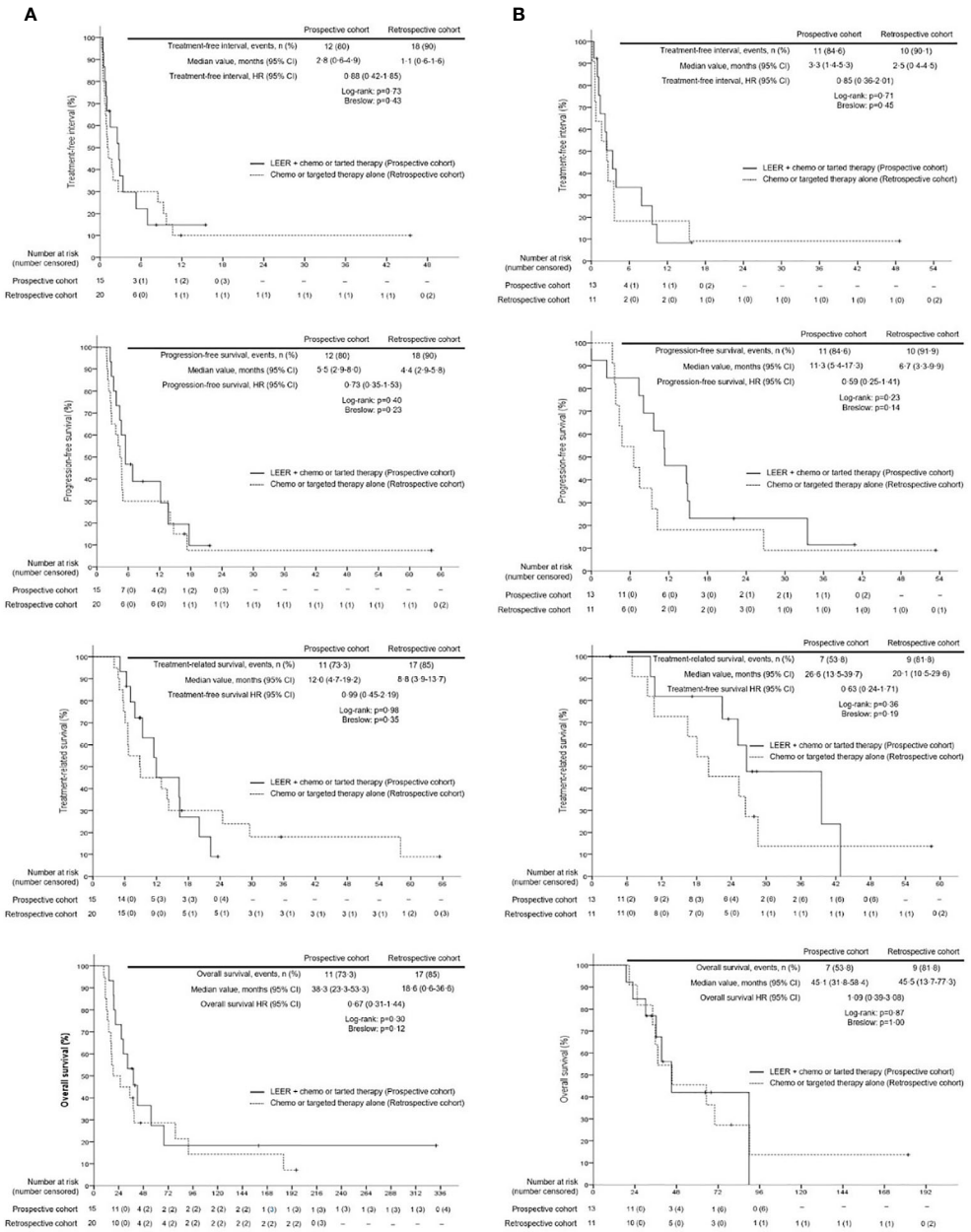

FIGURE 3 | Comparison of treatment-free interval, progression-free survival, treatment-related survival and overall survival between the prospective and retrospective cohorts according to the favorable indication (tumor size ≤5 cm, prior treatment-free interval >5 months, and no distant metastasis) for laterally extended endopelvic resection; **(A)** unfavorable indication; **(B)** favorable indication.

The results showed that PTFI ≥ 9.2 months and LEER followed by chemo or targeted therapy were associated with improved TFI, PFS, TRS, and OS in all patients. Moreover, first-line treatment for PSRCC improved TFI and TRS, and rT3b was related to better TRS and OS. However, previous use of bevacizumab was related to worse TRS. In the subgroup analyses based on median PTFI, rT3b and current use of bevacizumab were factors associated with improved TFI, PFS, TSR, and OS in the 30 patients with PTFI ≥ 9.2 months. Furthermore, first-line treatment for PSRCC improved TRS and OS, and squamous cell carcinoma was associated with better OS. Although LEER was related to better TRS, previous use of bevacizumab was associated with reduced TRS. In the 29 patients with PTFI < 9.2 months, LEER was associated with improved TFI, PFS, TRS, and OS. Moreover, first-line treatment for PSRCC was associated with improved TFI and tumor size < 4.2 cm on imaging studies was related to better TRS and OS (**Table 6**).

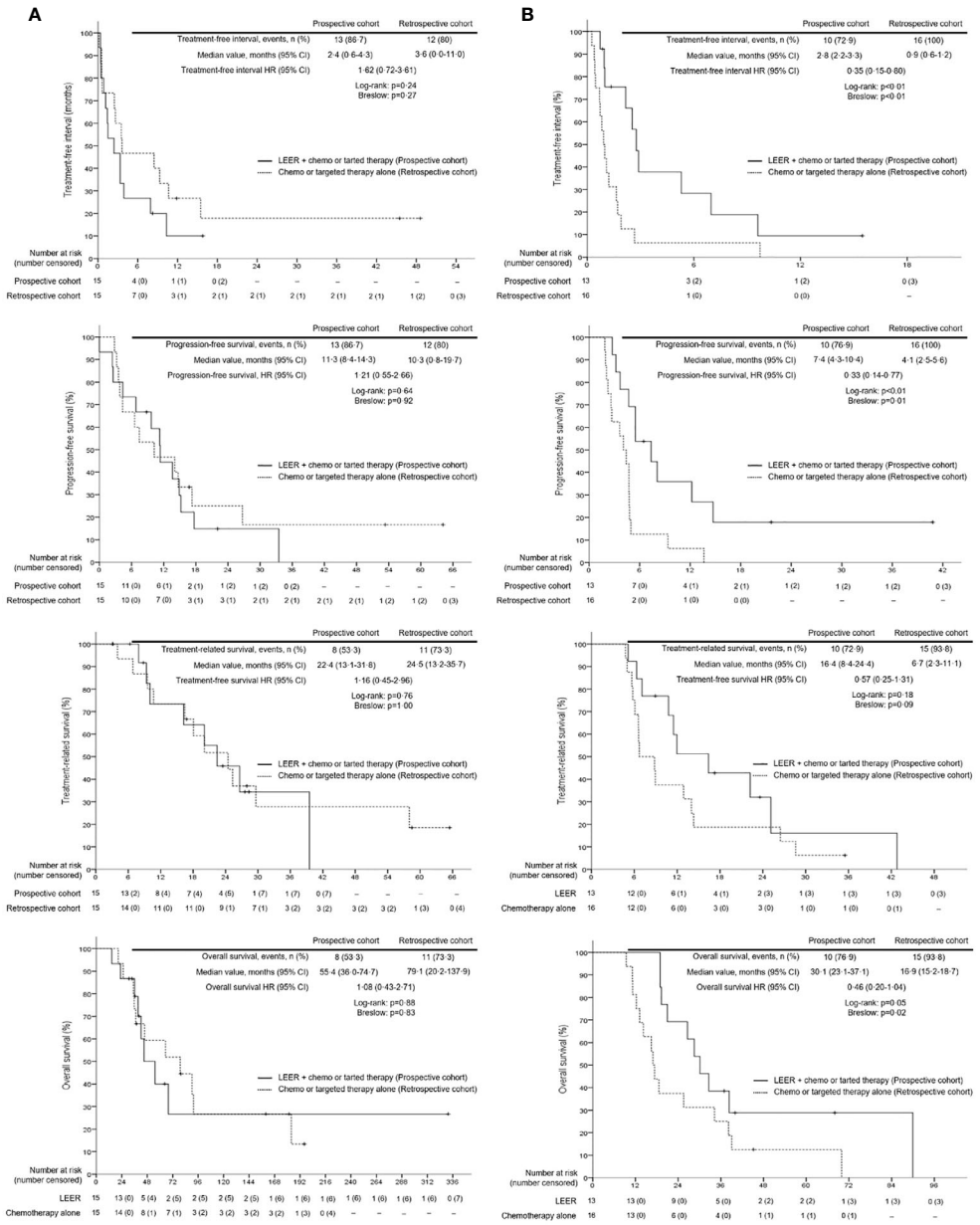

FIGURE 4 | Comparison of treatment-free interval, progression-free survival, treatment-related survival and overall survival between patients treated with laterally extended endopelvic resection (LEER) followed by chemo or targeted therapy (prospective cohort) and those treated with chemo or targeted therapy alone (retrospective cohort) according to prior treatment-free interval: **(A)** ≥ 9.2 months and **(B)** < 9.2 months.

Tumor Response, Neurologic Disturbance and Pelvic Pain Severity

In terms of tumor response, complete response was more common in the prospective cohort than in the retrospective cohort (55.6 *vs.* 19.4%; P < 0.01). Despite the lack of differences in disease recurrence and death between the two groups, the prospective cohort showed a lower rate of PSRCC (25 *vs.* 67.7%; P = 0.01) and a higher rate of distant metastasis (53.6 *vs.* 6.5%; P = 0.01) than the retrospective cohort. Although the incidence of muscle weakness after treatment did not differ between the two groups, neuralgia was more common in the prospective cohort than in the retrospective cohort (50 *vs.* 12.9%; P < 0.01). However, there was no difference in grade 3 neuralgia between the two groups (3.6 *vs.* 0%; P = 0.48). Regarding pelvic pain severity, the lowest and highest NRS did not differ before and after treatment between the two groups. Although there was also no difference in the MME required to control pelvic pain before treatment between the two groups, the MME required to control pelvic

TABLE 6 | Factors affecting survival.

	All (n = 59)	PTFI ≥ 9.2 months (n = 30)	PTFI < 9.2 months (n = 29)
Treatment-free interval	–	–	–
rT3b	–	0.04 (0.01 - 0.57)	–
PTFI ≥ 9.2 months	0.42 (0.23 - 0.78)	–	–
First-line treatment for PSRCC	0.28 (0.09 - 0.80)	–	0.18 (0.03 - 0.98)
Use of bevacizumab			
Current	–	0.13 (0.02 - 0.65)	–
LEER followed by chemo or targeted therapy	0.54 (0.28 - 0.98)	–	0.28 (0.12 - 0.68)
Progression-free survival			
rT3b	–	0.18 (0.03 - 0.97)	–
PTFI ≥ 9.2 months	0.47 (0.26 - 0.85)	–	–
Use of bevacizumab			
Current	–	0.26 (0.06 - 0.82)	–
LEER followed by chemo or targeted therapy	0.60 (0.33 - 0.83)	–	0.27 (0.11 - 0.66)
Treatment-related survival			
Tumor size < 4.2 cm on imaging studies	–	–	0.41 (0.17 - 0.96)
rT3b	0.22 (0.08 - 0.57)	0.03 (0.02 - 0.58)	–
PTFI ≥ 9.2 months	0.51 (0.27 - 0.98)	–	–
First-line treatment for PSRCC	0.29 (0.10 - 0.88)	0.10 (0.01 - 0.76)	–
Use of bevacizumab			
Previous	3.28 (1.21 - 8.86)	5.48 (1.12 - 34.01)	–
Current	–	0.02 (0.01 - 0.36)	–
LEER followed by chemo or targeted therapy	0.25 (0.09 - 0.68)	0.15 (0.02 - 0.84)	0.44 (0.18 - 0.83)
Overall survival			
Squamous cell carcinoma	–	0.09 (0.01 - 0.58)	–
Tumor size < 4.2 cm on imaging studies	–	–	0.38 (0.16 - 0.89)
rT3b	0.24 (0.09 - 0.61)	0.23 (0.01 - 0.32)	–
PTFI ≥ 9.2 months	0.28 (0.14 - 0.55)	–	–
First-line treatment for PSRCC	–	0.06 (0.01 - 0.69)	–
Use of bevacizumab			–
Current	–	0.12 (0.02-0.79)	–
LEER followed by chemo or targeted therapy	0.50 (0.09 - 061)		0.37 (0.15 - 0.88)

Data are adjusted hazard ratio (95% confidence interval).

LEER, laterally extended endopelvic resection; PSRCC, pelvic sidewall recurrence of cervical cancer; PTFI, prior treatment-free interval.

pain after treatment was less in the prospective cohort than in the retrospective cohort (median, 0 *vs.* 15; P < 0.01; **Table 7**).

DISCUSSION

LEER has long been used to remove pelvic tumors within ontogenetic cancer fields and sustain loco-regional tumor control (22–24). LEER is done by resecting the pelvic floor and sidewall muscles and the internal iliac vessel system surrounding pelvic sidewall tumors. R0 resection is more common in patients treated with LEER than in those who undergo pelvic exenteration. Given these treatment options, the rates of five-year PFS and OS have been reported to reach 65% and 75%, respectively, in patients with relapsed pelvic malignancies (25). A recent multicenter study showed that achieving R0 resection during laterally extended pelvic resection is the most important prognostic factor for gynecologic malignancies involving pelvic sidewall (22). Previous research excluded recurrent gynecologic cancer patients who achieved a disease-free interval of less than 6 months, but there are no relevant published studies to evaluate the favorable indications for LEER (23, 26). Therefore, there is no evidence by which to judge the efficacy and safety of LEER compared to chemo or targeted therapy alone, which is a major limitation in generalizing the application of LEER for patients with PSRCC.

Although a previous study showed that the five-year OS and PFS rates were 46% and 35%, respectively, in patients with PSRCC without a therapeutic alternative to LEER (8), the current study demonstrated that the prognosis for such patients is relatively poor, with a two-year PFS rate of 16.3% and a similar five-year OS rate of 33.9%. This poor PFS after LEER is most likely because ten patients (35.7%) with regional lymph node metastasis and seven (25%) with distant metastasis were included in the prospective cohort. By contrast, in the previous study, only 22.2% of patients had regional lymph node metastasis without distant metastasis.

Furthermore, the favorable indications for LEER (tumor size ≤5 cm; PFTI >5 months; no distant metastasis) were not related to improved survival, and the prognosis of patients with these indications remained relatively poor, with a two-year PFS rate of 23.1% despite a similar OS rate of 42.1%. This poor prognosis may be related to the high potential for distant metastasis seen in PSRCC. Although pelvic sidewall tumors can infiltrate the remaining lymphatic vessels connected to lymph node basins in the pelvic viscero-parietal compartments (27, 28), complete resection of these compartments outside the scope of LEER is difficult because of severe fibrosis or adhesion due to previous surgery or radiotherapy, which can cause distant metastasis if tumor cells are present in these compartments.

TABLE 7 | Treatment outcomes.

Characteristics	Prospective cohort (n = 28)	Retrospective cohort (n = 31)	P value
Tumor response			<0.01
Complete response	15 (55.6)	6 (19.4)	
Partial response	0 (0)	4 (12.9)	
Progressive disease	12 (44.4)	21 (67.7)	
Disease recurrence	23 (82.1)	28 (90.3)	0.46
Recurrent sites			0.01
Central	1 (3.6)	4 (12.9)	
Pelvic sidewall	7 (25)	21 (67.7)	
Ipsilateral	6 (21.4)	21 (67.7)	
Contralateral	1 (3.6)	0 (0)	
Distant	15 (53.6)	2 (6.5)	
Death	18 (64.3)	26 (83.9)	0.08
Neurologic disturbance of low extremity			
Muscle weakness			0.06
No	22 (78.6)	31 (100)	
Grade 1	1 (3.6)	0 (0)	
Grade 2	2 (7.1)	0 (0)	
Grade 3	3 (10.7)	0 (0)	
Neuralgia			0.01
No	14 (50)	27 (87.1)	
Grade 1	8 (28.6)	1 (3.2)	
Grade 2	5 (17.9)	3 (9.7)	
Grade 3	1 (3.6)	0 (0)	
Pelvic pain severity			
Pre-treatment NRS			
Lowest	2 (0 - 4)	2 (0 - 5)	0.86
Highest	3 (0 - 9)	3 (0 - 10)	0.90
Post-treatment NRS			
Lowest	0 (0 - 4)	0 (0 - 5)	0.35
Highest	3 (0 - 6)	3 (0 - 9)	0.37
Pre-treatment MME/day	0 (0 - 312)	0 (0 - 210)	0.40
Post-treatment MME/day	0 (0 - 60)	15 (0 – 219)	<0.01

Data are median (range) or n (%).
Patients in the prospective cohort received laterally extended endopelvic resection followed by chemo or targeted therapy, while those in the retrospective cohort received chemo or targeted therapy alone for pelvic sidewall recurrence of cervical cancer.
MME, morphine milligrams equivalents; NRS, numeral rating scale.

The above explanation is supported by the finding that PSRCC was associated with a similar prognosis as distant metastasis in a previous study (4), and distant metastasis was found in 25% of patients with PSRCC in this study. On the other hand, if LEER was improperly implemented in this study, its use may be related to poor prognosis. However, the finding that 53.6% of patients with relapse after LEER showed distant metastasis supports the surgical suitability of LEER with appropriate loco-regional control.

The most important finding of this study is that LEER may be beneficial for the treatment of PSRCC in patients with PTFI <9.2 months. Patients with PTFI ≥9.2 months may have platinum sensitivity (29–31), which can increase tumor response to chemotherapy such that it matches the surgical effect of LEER. Since this study showed that targeted therapy using bevacizumab increased survival, as in the GOG 240 trial (18), combined chemotherapy with bevacizumab can be considered as a first-line treatment for PSRCC because its use avoids surgical complications and its efficacy is similar to that of LEER in patients with PTFI ≥ 9.2 months.

Importantly, LEER may be effective at reducing the MME required to control pelvic pain. Although opioid-based analgesic treatment can relieve pelvic pain in more than 70% of patients, many patients still suffer due to underutilization of opioids and the adverse effects of opioids (32). Since sciatica occurs when one or more nerve roots from L4 to S3 are compressed by pelvic sidewall tumors, tumor removal through LEER can relieve the pressure on nerve roots and markedly reduce the associated pain (33), which means that LEER can be considered as a palliative surgery for relief of uncontrolled sciatica caused by PSRCC (12).

This study has some limitations. First, the small number of enrolled patients and the heterogeneity of both cohorts due to the rarity of PSRCC may have introduced bias. Second, little relevant data was available with which to design this study and to calculate the appropriate sample size. Third, only the combination therapy using paclitaxel, cisplatin, and bevacizumab has been approved for recurrent cervical cancer since March 2015, whereas the use of bevacizumab monotherapy is not currently approved in our country. Thus, the rate of bevacizumab usage was low this study. Fourth, the comparison of pain severity should be interpreted carefully, considering the two different study designs. Fifth, we did not include bilateral pelvic sidewall recurrence, because bilateral LEER is insufficiently safe. Thus, it is essential to more clearly evaluate the efficacy and safety of LEER compared to chemo or targeted therapy alone through a multicenter study based on our results.

To the best of our knowledge, this is the first comparative study between LEER and chemo or targeted therapy alone for PSRCC. Compared to chemo or targeted therapy alone, LEER may improve survival, with increased tumor response in patients with PSRCC and PTFI < 9.2 months. Moreover, LEER may be an effective means of controlling the pelvic pain caused by PSRCC.

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 human participants were reviewed and approved by Seoul National University Hospital. The patients/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

SP: methodology, validation, formal analysis, investigation, data curation, writing-original draft, and visualization. JM: validation, investigation, and writing-review & editing.

SL:validation, investigation, and writing-review & editing. YL: validation, investigation, and writing-review & editing. HC: methodology, validation, investigation, and supervision. J-WK: methodology, investigation, and writing-review & editing. NP: methodology, validation, investigation, and supervision. YS: methodology, validation, investigation, and supervision. HK: conceptualization, methodology, formal analysis, resources, writing - review & editing, supervision, project administration, and funding acquisition. All authors contributed to the article and approved the submitted version.

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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.2021.683441/full#supplementary-material

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Laparoscopic Radical Hysterectomy Results in Higher Recurrence Rate Versus Open Abdominal Surgery for Stage IB1 Cervical Cancer Patients With Tumor Size Less Than 2 Centimeter: A Retrospective Propensity Score-Matched Study

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Objective: To compare the oncologic outcomes between laparoscopic and open radical hysterectomy in patients with stage IB1 cervical cancer lesion less than 2 cm.

Methods: Patients diagnosed FIGO (2009) stage IB1 (tumor diameter <2 cm) and underwent radical hysterectomy in our hospital between March 2008 and November 2018 were studied. A propensity-matched comparison (1:2) was conducted to minimize selection biases. Demographic and baseline oncologic characteristics were balanced between groups. Overall survival (OS) and disease-free survival (DFS) were assessed using the Kaplan–Meier model, along with univariable and multivariable regression analysis.

Results: A total of 261 patients were enrolled in this study after propensity-matching, with 174 in the open group and 87 in the laparoscopic group. Disease relapsed in seven patients in laparoscopy group, and the recurrence rate was 8.0% (7/87). There were eight patients underwent abdominal radical hysterectomy experienced recurrence, and the recurrence rate was 4.6% (8/174). The multivariate analysis model revealed that laparoscopic operation was associated with higher risk of recurrence than abdominal radical hysterectomy (HR, 3.789; 95% CI, 1.143–12.559; p = 0.029). There were five patients or 2.9% (5/174) died in open surgery group and the corresponding percentage in laparoscopy group was 2.3% (2/87). No difference was found in OS between the two groups (HR, 1.823; 95% CI, 0.2673–12.44; log-rank p = 0.5398). All the recurrence occurred within two years after operation in the laparoscopy group, among which pelvic recurrence (85.7%) was dominant.

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Conclusion: Traditional laparotomy radical hysterectomy has a lower recurrence rate when compared with laparoscopic operation in those cervical cancer patients with a foci diameter less than 2 cm. However, no detrimental effect on survival was found in minimal invasive operation group. Further multi-center prospective trials are needed to confirm our results on a large scale.

Keywords: laparotomy, prognosis, radical hysterectomy, survival, cervical cancer, laparoscopy

INTRODUCTION

Cervical cancer is a disease that is curable with early diagnosis and intervention, yet it remains the fourth leading cause of cancer-related death worldwide (1). Radical hysterectomy (RH) with bilateral pelvic lymph node dissection represents the first-line treatment for early-stage cervical cancer (2). The advantages and disadvantages of laparoscopic RH are controversial since the first case of a laparoscopic RH and paraaortic and pelvic lymphadenectomy was performed to treat a stage IA2 carcinoma of the cervix (3). Laparoscopic RH has gradually emerged as an alternative procedure for cervical cancer treatment in the last decade in China due to the improved laparoscopic equipment and accumulated experience and expertise of oncologists. More importantly, previous studies showed that patients could benefit from laparoscopic surgery with similar survival outcomes (4–8) as those, who underwent laparotomy, but had better life quality after the operation (9, 10).

The published result of Laparoscopic Approach to Carcinoma of Cervix (LACC) trial challenged the oncologic safety of minimally invasive radical hysterectomy and endorsed open surgery. The phase 3 trial indicated that minimally invasive radical hysterectomy had lower disease-free survival as well as higher local recurrence rate than open surgery (11). Meanwhile the postoperative life quality was similar between the two groups (12). The NCCN Clinical Practice Guidelines thus regarded open surgery as the standard approach for radical hysterectomy since 2019 (13).

However, LACC trial has its limitations. It cannot be generalized to patients with tumor size <2 cm, as it was not powered to evaluate the oncologic outcomes of the two surgical approaches in this context (11). So far, few articles directly explored the benefits of laparoscopic RH in cervical cancer with a foci diameter less than 2 cm (14–17).

The primary purpose of this propensity-matched retrospective observational analysis is to evaluate the oncologic outcome between laparoscopic RH and open surgery in cervical cancer patients with a lesion <2 cm. The highlight of this study is that all lesions were assessed by preoperative imaging exam, which were more practical in clinical practice. The findings of this study contribute to the growing body of evidence against the use of minimally invasive surgery for cervical cancer.

MATERIALS AND METHODS

Sample Collection

This is a retrospective observational study. Cervical cancer patients, who were diagnosed and treated at the Division of Gynecology of The First Affiliated Hospital of Wenzhou Medical University between March 2008 and November 2018, were considered for our study. The criteria of choosing patients to be included in this study were as follows (1): histological diagnosis of adenocarcinoma, squamous cell carcinoma, or adenosquamous carcinoma of the cervix (2), age between 18 and 70 years old (3), International Federation of Gynecology and Obstetrics (FIGO) 2009 clinical stage IB1 with tumor size <2 cm and limited to the cervix (4), normal renal, hepatic, and cardiac function, and (5) signed informed consent and compliance to follow-up. The exclusion criteria were as follows (1): patients underwent vaginal radical hysterectomy or fertility-sparing procedures, and (2) synchronous malignancies in 5 years. The study was approved by the First Affiliated Hospital of Wenzhou Medical University Institutional Ethics Committee for Non-Interventional Research.

Surgical and Perioperative Management

Primary preoperative evaluation consisted of a complete medical history, physical examination, laboratory examinations, electrocardiogram, pelvic ultrasonography, chest X-ray, pelvic computed tomography (CT)/magnetic resonance imaging (MRI) or positron emission tomography- computed tomography (PET-CT). Preoperative imaging assessment confirmed that there were no extrauterine or lymph node metastasis. Prior to surgery, all patients underwent mechanical bowel preparation and antibiotic prophylaxis. Deep venous thrombosis prophylaxis with low-molecular-weight heparin were performed according to Caprini Risk Assessment Scale for high risk of thromboembolism (12 h before surgery and postoperatively for 4 weeks). According to the NCCN guideline, all patients underwent type C radical hysterectomy with bilateral pelvic lymphadenectomy (18, 19). All procedures were performed by skilled surgeons. The uterine manipulator used in laparoscopic RH was a modified metal uterine manipulator. There were no significant differences in the facilities available for patient care. Adjuvant treatment was recommended, according to the National Comprehensive Cancer Network (NCCN) guidelines. Adjuvant radiation therapy was suggested according to Sedlis criteria, while chemo and radiation therapy was suggested in case of positive nodes, parametrial involvement, or positive surgical margins.

Data Collection

All medical records were reviewed simultaneously by three trained residents, and independently checked by two experts to ensure the accuracy.

Patients were followed up 1 month and then every 3 months during the first 2 years after surgery, and twice a year afterwards.

At each scheduled follow-up visit, pelvic examination and squamous cell carcinoma antigen (SCC) (for squamous and adenosquamous cancer) or carbohydrate antigen 125 (CA125) (for adenocarcinoma and adenosquamous cancer) were performed. Pelvic and chest CT were tested once a year. The median follow-up time was calculated from the date of surgery. A secretary made periodic phone call to patients before scheduled outpatient follow-up visit to reduce omitted follow-ups. Dates and sites of recurrence were recorded.

Staging system and architectural grade were reported according to the FIGO statements. The World Health Organization (WHO) taxonomy was used to classify histologic subtypes. Tumor size was defined as the largest diameter of the lesion in preoperative imaging evaluation according to pelvic MRI or CT. DFS was defined as the interval from the operation to the first finding of any recurrence or last follow-up visit. OS was defined as the interval from the operation to the cervical cancer related death or last follow-up visit.

Statistical Analysis

Patients underwent laparoscopic radical hysterectomy (LRH) were matched 1:2 to those underwent open abdominal radical hysterectomy (ARH). Six baseline characteristics (age, histology, parametrial involvement, lymphovascular space invasion, pelvic lymph nodes, surgical margin, and depth of cervical stromal invasion) were selected as covariates in propensity score match model, and the match tolerance was set to 0.01 (**Supplementary Figure 1**) (20). Two-independent samples t-test and the χ^2 test were used to analyze the clinicopathologic characteristics between the LRH and ARH. DFS and OS after surgery were estimated using the Kaplan–Meier method, and a p-value < 0.05 was considered statistically significant. The log-rank test was used to compare the risk of developing recurrence and the risk of death between the two groups over the time (21). Cox proportional risk regression models were used to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) for the effect of surgical approaches on the OS and DFS (22). Statistical analysis was performed using GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA) and IBM-Microsoft SPSS version 22.0 (IBM Corporation, Armonk, NY, USA).

RESULTS

Patient Characteristics Before and After Propensity-Matching

Over the study period, 335 patients met our inclusion and exclusion criteria. Among them, 247 patients underwent laparotomy and 88 underwent laparoscopy surgery (**Figure 1**). Patients in the laparoscopy group were propensity-matched 1:2 with those in the open RH group. After propensity score matching, 261 patients (87 in the laparoscopic group and 174 in the open procedure) were included in the following analysis, and no significant differences between two groups were observed in baseline characteristics. The clinicopathologic characteristics of the two groups before and after propensity-matching are presented in **Table 1**. Those patients who underwent ARH were more likely to have deeper depth of cervical stromal invasion (p = 0.047) and poorer differentiation (p = 0.002).

Recurrence and Survival in Propensity-Matched Cohort

The median follow-up time was 42 months (range from 12 to 138 months). In the propensity matching cohort, there were eight

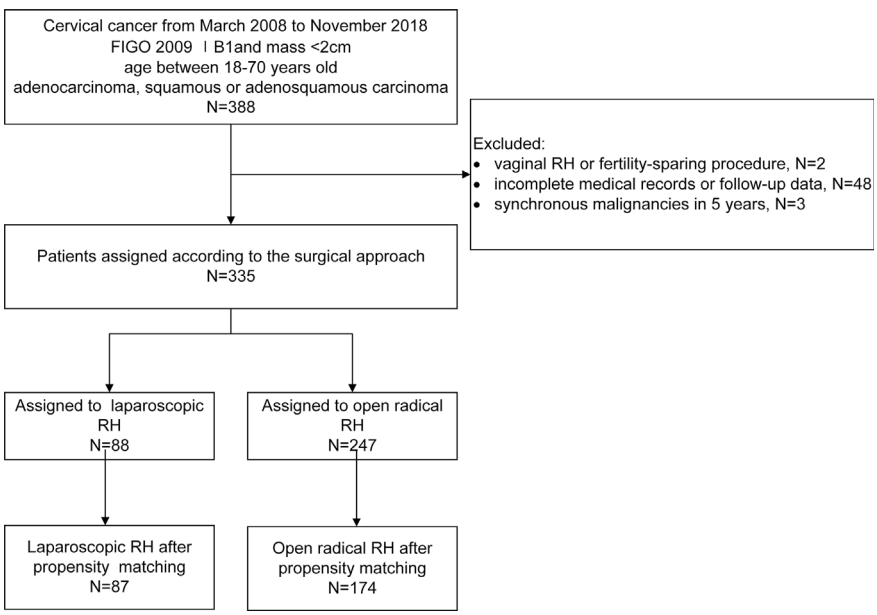

FIGURE 1 | Flow diagram of recruitment and exclusions.

TABLE 1 | Clinicopathologic characteristics before and after propensity score matching.

Variables	Before propensity score matching			After propensity score matching		
	ARH 247	LRH 88	*p* value	ARH 174	LRH 87	*p* value
Age (year, Mean ± SD)	51.53 ± 10.31	49.35 ± 8.81	0.079	48.00 ± 14.00	49.00 ± 12.00	0.979
Histology (%)			0.153			0.388
Squamous	205 (82.99)	66 (75.00)		140 (80.46)	66 (75.86)	
Adenocarcinoma	32 (12.96)	19 (21.59)		27 (15.52)	18 (20.69)	
Adenosquamous	10 (4.05)	3 (3.41)		7 (4.02)	3 (3.45)	
Differentiation (%)			0.002*			0.676
G1/G2	92 (37.25)	34 (38.64)		58 (33.33)	30 (34.48)	
G3	110 (44.53)	24 (27.27)		77 (44.25)	34 (39.08)	
Unknown/missing	45 (18.20)	30 (34.10)		39 (22.40)	23 (26.44)	
Surgical margin (%)			1.000			1.000
Negative	245 (99.19)	87 (98.86)		173 (99.43)	86 (98.85)	
Positive	2 (0.81)	1 (1.14)		1 (0.57)	1 (1.15)	
Pelvic lymph nodes (%)			0.340			1.000
Negative	227 (91.90)	84 (95.45)		166 (95.40)	83 (95.40)	
Positive	20 (8.10)	4 (4.55)		8 (4.60)	4 (4.60)	
LVSI (%)			0.638			0.856
Negative	199 (80.57)	74 (83.15)		148 (85.06)	73 (83.91)	
Positive	48 (19.43)	15 (16.85)		26 (14.94)	14 (16.09)	
DCSI (%)			0.047*			0.974
Inner 1/3	128 (51.82)	59 (67.05)		118 (67.82)	58 (66.67)	
Medium 1/3	72 (29.15)	17 (19.32)		32 (18.39)	17 (19.54)	
Outer 1/3	47 (19.03)	12 (13.64)		24 (13.79)	12 (13.79)	
Parametrial involvement (%)			0.570			
No	244 (98.79)	88 (100.00)		174 (100.00)	87 (100.00)	
Yes	3 (1.21)	0 (0.00)				
Adjuvant treatment given (%)			0.370			0.605
No	150 (60.73)	55 (62.50)		117 (67.24)	54 (62.07)	
Radiotherapy	30 (12.15)	5 (5.68)		14 (8.05)	5 (5.75)	
Chemotherapy	35 (14.17)	15 (17.05)		23 (13.22)	15 (17.24)	
Concomitant chemoradiotherapy	32 (12.96)	13 (14.77)		20 (11.49)	13 (14.94)	
SCC before surgery (Mean ± SD)			0.406			0.846
	1.00 ± 0.70	0.90 ± 1.00		1.00 ± 0.50	0.90 ± 1.00	

*ARH, abdominal radical hysterectomy; LRH, laparoscopic radical hysterectomy; LVSI, lymphovascular space invasion, DCSI, depth of cervical stromal invasion; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated; LVSI, lymph vascular space invasion. Values are presented as mean ± standard deviation or number (%). *p < 0.05, statistically significant.*

patients underwent ARH experienced recurrence, which gives a recurrence rate of 4.6% (8/174). Disease relapsed in seven patients in laparoscopy group, for which the recurrence rate was 8.0% (7/87). Two-year and 5-year DFS was 97.1% versus 92.0% (p = 0.060) and 95.4% versus 92.0% (p = 0.260) for the open versus laparoscopic groups, respectively. Interestingly, Kaplan–Meier analysis indicated that patients in the LRH group showed tendency to suffer recurrence (HR, 2.838; 95% CI, 0.888–9.032; log-rank p = 0.078), even though there was no statistics difference between the two groups. Kaplan–Meier plot of DFS after PSM are presented in **Figure 2**.

There were five patients or 2.9% (5/174) died in open surgery group and the corresponding percentage in laparoscopy group was 2.3% (2/87). Two-year and 5-year OS was 99.4% versus 97.7% (p = 0.218) and 97.1% versus 97.7% (p = 0.787) for the open versus laparoscopic groups, respectively. Kaplan–Meier analysis showed no difference in OS between the two groups in propensity score weighting cohort (HR, 1.823; 95% CI, 0.267–12.44; log-rank p = 0.540). Kaplan–Meier plot of OS after PSM are presented in **Figure 3**.

Univariable and Multivariable Regression Analysis for Prognostic Factors

We performed univariate and multivariate Cox analysis to investigate the comprehensive prognostic factors for RFS (**Table 2**) and OS (**Table 3**). In univariable regression analysis of the matched cohort histology subtype adenosquamous (HR, 9.619; 95% CI, 2.545–36.353; p = 0.001) and positive pelvic lymph node (HR, 5.593; 95% CI, 1.577–19.835; p = 0.008) were potentially predictive factors of prognosis for RFS. The multivariate analysis model revealed histology subtype adenosquamous (HR, 8.919; 95% CI, 1.978–40.227; p = 0.004), positive pelvic lymph node (HR, 5.593; 95% CI, 1.577–19.835; p = .008) as well as laparoscopic operation procedure (HR, 3.789; 95% CI, 1.143–12.559; p = 0.029) were potentially predictive factors of DFS. Univariate Cox proportional hazard regression analysis revealed that positive pelvic lymph node (HR, 8.439; 95% CI, 1.637–43.504; p = 0.011), histology subtype adenosquamous (HR, 13.132; 95% CI, 1.187–145.267; p = 0.036) and adenocarcinoma (HR, 11.074; 95% CI, 2.019–60.733; p = 0.006) were predictors of OS. Furthermore, the multivariate survival analysis model revealed that the adenosquamous (HR, 17.662; 95% CI,

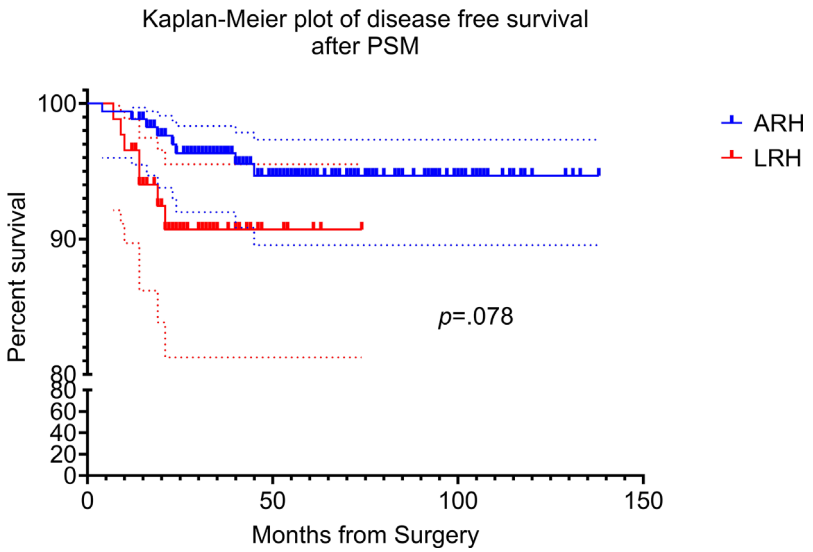

FIGURE 2 | Kaplan–Meier disease free survival curves for laparoscopic radical hysterectomy and abdominal radical hysterectomy. The disease-free survival (DFS) rate of ARH and LRH after propensity score matching.

1.837–169.853; p = 0.013), adenocarcinoma (HR, 20.647; 95% CI, 1.234–345.376; p = 0.035) and positive pelvic lymph node (HR, 11.372; 95% CI, 1.890–68.440; p = 0.008) were potentially predictive factors of OS.

The Pattern of Recurrence

All the recurrence occurred within two years after operation in the laparoscopy group, while in the open surgery group, five cases relapsed within 2 years and the other three cases recured within 5 years. When it comes to the recurrence type, most of the cases in the laparoscopic group suffered pelvic recurrence (6/7, 85.7%), and one case suffered vaginal stump recurrence. In the laparotomy group, two cases experienced vaginal stump recurrence, four cases experienced hematogenous recurrences (one case liver and lung recurrences, one case liver and two cases lung), and two cases suffered pelvic recurrence.

DISCUSSION

Although the safety of minimal invasive surgery in cervical cancer was questioned since the published result of LACC trial

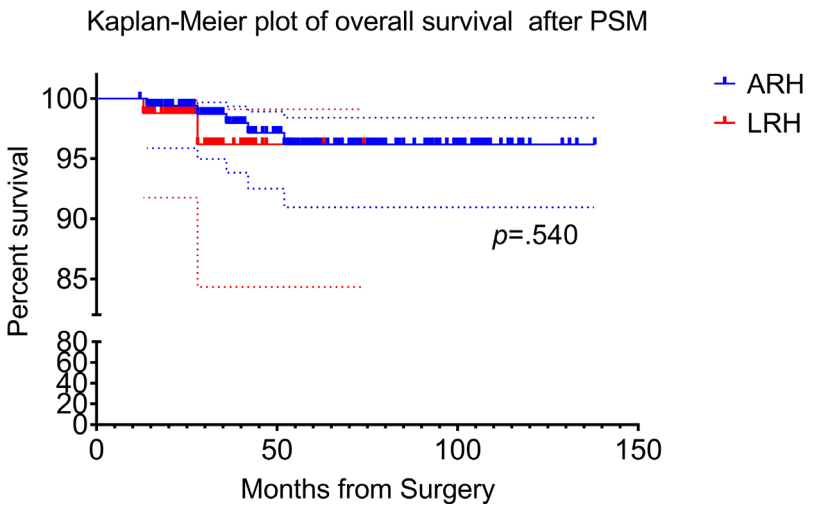

FIGURE 3 | Kaplan–Meier survival curves for laparoscopic radical hysterectomy and abdominal radical hysterectomy. The overall survival (OS) rate of ARH and LRH after propensity score matching.

TABLE 2 | Factors Associated with Recurrence-Free Survival.

Characteristics	Univariate		Multivariate	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Histology				
Squamous	Reference		Reference	
Adenocarcinoma	2.457 (0.739–8.171)	0.143	2.536 (.618–10.404)	0.196
Adenosquamous	9.619 (2.545–36.353)	0.001*	8.919 (1.978–40.227)	0.004*
Surgery Approach				
Open	Reference		Reference	
Laparoscope	1.405 (0.143–3.145)	0.088	3.789 (1.143–12.559)	0.029*
Parametrial Involvement				
No	Reference			
Yes	5.169 (0.699–38.231)	0.108		
Pelvic lymph node				
Negative	Reference		Reference	
Positive	5.593(1.577–19.835)	0.008*	4.716 (1.067–20.8430)	0.041*
Surgical Margin				
Negative	Reference			
Positive	0.049 (0.000–400413)	0.814		
LVSI				
Negative	Reference			
Positive	0.739 (0.208–2.619)	0.639		
DCSI				
Inner 1/3	Reference			
Medium 1/3	0.569 (.151–2.147)	0.406		
Outer 1/3	1.056 (.236–4.718)	0.944		
Differentiation				
G1/G2	Reference			
G3	1.168 (0.515–2.649)	0.709		
Unknown/missing	0.525 (0.146–1.881)	0.322		
Adjuvant Therapy				
No	Reference			
Radiotherapy	0.819 (0.177–3.793)	0.799		
Chemotherapy	2.795 (0.995–7.857)	0.984		
Chemoradiotherapy	1.612 (0.295–8.806)	0.581		

LVSI, lymphovascular space invasion, DCSI, depth of cervical stromal invasion; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated.
**P < 0.05.*

in 2018 (23), its advantages are undeniable. These advantages include less intraoperative blood loss, a shorter length of hospital stay, faster bowel and bladder function recovery, and a lower risk of postoperative complications (24, 25). Gynecological oncologists are trying to select patients with specific characteristics, who might benefit from minimal invasive surgery (26). Tumor dimension is one of the most studied specific characteristics. A consensus has been reached that there was no distinct advantage of LRH over ARH in tumors diameter >2 cm (11). However, the exact effect of surgical approach on oncological outcomes in patients with tumor diameter <2 cm is still controversial, and the related studies are limited.

Some studies found similar hazards of recurrence and death in both subgroups. Kim et al. reported that minimal invasive surgery did not influence PFS of stage IB1 patients with cervical mass ≤2 cm on pre-operative MRI (14). No difference in DFS was noted between robotic and open RH in cervical cancer tumor size ≤2 cm in a Sweden cohort (17). These results were supported by a multicenter retrospective study published by Chinese researchers (15). Recently, several studies reached the conclusion that minimally invasive RH had inferior DFS even for tumors that have size less than 2 cm. A multi-institutional retrospective study performed in the United States found that patients with tumor size ≤2 cm on final pathology had a higher recurrence rate in the minimally invasive approach (27). A Korean Gynecologic Oncology Group Study reached conclusion that LRH was associated with inferior DFS among patients with IB–IIA and tumor size <2 cm (16). There has been no widely accepted conclusion on the exact effect of surgical approach on oncological outcomes in tumor diameter <2 cm so far. On the other hand, there is a lack of uniformity in definition between different studies (i.e., tumor size based on MRI, clinical examination, or pathology; lesion diameter <2 cm or ≤2 cm; Da Vinci Robotic Surgery included or excluded; 3D laparoscopy or not; FIGO 2009 or 2018). In this study, we analyzed the clinical data from our center to explore the safety of LRH in FIGO 2009 stage IB1 cervical cancer patients with tumor diameter <2 cm in preoperative imaging exam. We concluded that LRH was associated with higher risk of recurrence than ARH and there is no difference between two groups in OS.

The multivariate analysis revealed that histology subtype adenosquamous, positive pelvic lymph node as well as laparoscopic operation procedure were potentially predictive factors of DFS. Adenosquamous, adenocarcinoma, and positive pelvic lymph node were potentially predictive factors for OS. We included those variables that were reported as risk factors for

TABLE 3 | Factors Associated with Overall Survival.

Characteristics	Univariate		Multivariate	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Histology				
Squamous	Reference		Reference	
Adenocarcinoma	11.074 (2.019–60.733)	0.006*	20.647 (1.234–345.376)	0.013*
Adenosquamous	13.132 (1.187–145.267)	0.036*	17.662 (1.837–169.853)	0.035*
Surgery Approach				
Open	Reference			
Laparoscope	1.694 (0.309–9.296)	0.544		
Parametrial Involvement				
No	Reference			
Yes	0.848 (0.000–43)	0.767		
Pelvic lymph node				
Negative	Reference			
Positive	8.439 (1.637–43.504)	0.011*	11.372 (1.890–68.440)	0.008*
Surgical Margin				
Negative	Reference			
Positive	.049 (0.000–5.48E15)	0.880		
LVSI				
Negative	Reference			
Positive	1.083 (0.130–9.001)	0.941		
DCSI				
Inner 1/3	Reference			
Medium 1/3	2.325 (0.388–13.916)	0.355		
Outer 1/3	2.979 (0.497–17.838)	0.232		
Differentiation				
G1/G2	Reference			
G3	0.932 (0.178–2.247)	0.478		
Unknown/missing	0.343 (0.041–2.850)	0.322		
Adjuvant Therapy				
No	Reference			
Radiotherapy	0.529 (0.055–5.089)	0.582		
Chemotherapy	0.000 (.000)	0.986		
Chemoradiotherapy	2.471 (.257–23.772)	0.433		
Relapse				
No	Reference			
Yes	1.000 (.024–42.036)	1.000		

*LVSI, lymphovascular space invasion, DCSI, depth of cervical stromal invasion; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated. *P < 0.05.*

recurrence in the multivariate analyses (28, 29). Several variables showed no association with survival in univariate analysis in our study, including LVSI, depth of invasion, tumor grade, surgical margins, etc., which are typically associated worse outcomes. This situation might be explained by the small sample size and/or the uneven distribution between subgroups. Meanwhile, it is undeniable that these factors might, to a certain extent, influence the route of surgery. Doctors are more likely to perform open surgery on those patients with poor differentiation, deeper invasion and LVSI. There might be inter-operator variation in surgical treatment of cervical cancer between different surgeons.

The diameter of the tumor was measured *via* preoperative pelvic imaging evaluation according to MRI or CT in our study. Preoperative imaging assessment was more valuable and practical in clinical practice, and it is an important factor for surgeons to decide the route of surgery. There are still some differences between the preoperative imaging (CT or MRI) and the pathologic report. Although postoperative pathology could be interfered by preoperative conization and specimen treatment, it is still the gold standard of final diagnosis and stage. We had encountered the patients who had been underestimated by preoperative imaging assessment. How to accurately predict the tumor size and stage before surgery is a valuable research field.

Our results indicated that cervical cancer patients with a lesion less than 2 cm, who underwent LRH, were more likely to experience recurrence than those underwent ARH. In our study, all the recurrence in the laparoscopic group occurred within 2 years after surgery, and most of the recurrence occurred in pelvic. Our result was similar with the results from a study in South Korea (14). There are several potential reasons contributing to the higher recurrence for LRH. The uterine manipulator and the exposure of cervical cancer to circulating CO2 might increase tumor spillage (23). Besides, the prolonged Trendelenburg position (30) might also influence the relapse of cancer. A constructive manner to limit the use of invasive uterine manipulator and the time interval of opening the vagina should be taken into consideration. Intraperitoneal exposure during minimally invasive surgery had a significantly worse prognosis than no intraperitoneal exposure. Intraperitoneal tumor exposure was an independent prognostic factor for worse survival (31). A novel fluorescence imaging-based tool for feasible and direct visualization of peritoneal contamination

during colpotomy might serve as a quality assessment tool for surgeons and surgical techniques (32). Specific measures were adopted by some surgeons to prevent tumor spillage during LRH, such as creation of a vaginal cuff, minimized handling of the uterine cervix, and bagging the specimen (33). Recently, a multicenter retrospective observational cohort study concluded that conization before radical hysterectomy was associated with improved DFS in FIGO 2009 stage IB1 cervical cancer, and no conization before radical hysterectomy was an independent factor for higher risk of recurrence (34). However, whether conization before surgery would influence the oncologic outcomes between laparoscopic and open radical hysterectomy is still unknown and is an interesting direction for further study.

The current study had several limitations. First, this is a retrospective study. The heterogeneity differences between treatment groups still existed, even though propensity score matching was performed. Second, there might be inter-operator variation in surgical treatment of cervical cancer between different surgeons. Third, the sample size is small and the distribution of subgroup is uneven. Prospective multicenter studies are still needed to confirm our findings. Fourth, there might be some difference between the preoperative imaging modality (CT or MRI) and the actual pathologic tumor size. Pathological tumor size should be taken into consideration in future study.

In conclusion, we observed that cervical cancer patients with a lesion less than 2 cm might be more likely to have recurrence in LRH group than those taken ARH. Further randomized controlled perspective trials are needed to explore the advantages and disadvantages of the adoption of minimally invasive techniques in the treatment of cervical cancer patients with a lesion less than 2 cm.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

XC performed data analysis, reviewed the literature and drafted the article. JY collected clinical data. HYZ and YH designed the study and finalized the paper. HQZ and YH provided suggestions to improve it. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.683231/full#supplementary-material

Supplementary Figure 1 | Diagram of propensity score matching.

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Loop-Mediated Isothermal Amplification Assay for Detecting Tumor Markers and Human Papillomavirus: Accuracy and Supplemental Diagnostic Value to Endovaginal MRI in Cervical Cancer

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Objective: To establish the sensitivity and specificity of a human papillomavirus (HPV) and tumor marker DNA/mRNA assay for detecting cervical cancer that is transferrable to a Lab-on-a-chip platform and determine its diagnostic benefit in early stage disease when used in conjunction with high-resolution endovaginal magnetic resonance imaging (MRI).

Methods: Forty-one patients (27 with Stage1 cervical cancer [Group1] and 14 non-cancer HPV negative controls [Group2]) had DNA and RNA extracted from cervical cytology swab samples. HPV16, HPV18, hTERT, TERC/GAPDH and MYC/GAPDH concentration was established using a loop mediated isothermal amplification (LAMP) assay. Thresholds for tumor marker detection for Group1 were set from Group2 analysis (any hTERT, TERC/GAPDH 3.12, MYC/GAPDH 0.155). Group 1 participants underwent endovaginal MRI. Sensitivity and specificity for cancer detection by LAMP and MRI individually and combined was documented by comparison to pathology.

Results: Sensitivity and specificity for cancer detection was 68.8% and 77.8% if any tumor marker was positive regardless of HPV status (scenario1), and 93.8% and 55.8% if tumor marker or HPV were positive (scenario 2). Adding endovaginal MRI improved specificity to 88.9% in scenario 1 (sensitivity 68.8%) and to 77.8%% in scenario2 (sensitivity 93.8%).

Conclusion: Specificity for cervical cancer detection using a LAMP assay is superior with tumor markers; low sensitivity is improved by HPV detection. Accuracy for early stage

cervical cancer detection is optimal using a spatially multiplexed tumor marker/HPV LAMP assay together with endovaginal MRI.

Keywords: cervical cancer, loop mediated isothermal amplification, human papilloma virus, tumor markers, magnetic resonance imaging

INTRODUCTION

Treatment of cervical intraepithelial neoplasia at colposcopy with cold knife cone (CKC) biopsy or large loop excision of the transformation zone (LLETZ) can sometime excise a small volume cervical cancer. Defining the presence and extent of any residual disease crucially determines subsequent surgical management (1). As women being treated for cervical precancer or early cancer are of similar age to women having their first child, fertility and reproductive effects of local excision of disease are important. The risk of post-surgical complications such as primary and secondary haemorrhage and cervical stenosis that may require further intervention, particularly where excisions are radical or repeated (2, 3), should be kept to a minimum. Increasing evidence suggests that the amount of cervical tissue excised or destroyed, measured as the cone length in excisional techniques, is a predictor for subsequent obstetric risk (4, 5). Moreover, a larger amount of residual cervical tissue detected on scan after treatment for both dysplasia and cancer is associated with improved obstetric outcomes (6–8). It is therefore critical to balance risk related to oncological *versus* reproductive outcomes by enabling the optimal local excisional treatment for these women.

Optimal surgical management may be achieved by assessing surgical margins on initial CKC or LLETZ biopsy supplemented by high-resolution endovaginal magnetic resonance imaging (MRI) (9). The latter offers a sensitivity of >90% for small tumors, albeit with a specificity around 70% for tumors $<1.7cm^3$ because of confounding appearances from scarring and fibrosis after CKC biopsy or LLETZ (10). In these cases, detection of the human papillomavirus (HPV) genome (a key event in cervical carcinogenesis)and genetic tumor markers in cellular samples potentially provides an additional means of improving specificity of cancer detection prior to planning surgical management.

Of the 14 high-risk HPV types carcinogenic to humans, HPV-16 is consistently the most prevalent, detected in 60% of cases of cervical cancer (11). HPV-16 is detected more often in squamous cell carcinoma (62%) than in adenocarcinoma (50%), while HPV18 and HPV45 are detected more often in adenocarcinoma (32% and 12%, respectively) than in squamous cell carcinoma (8% and 5%, respectively). More than 50% of HPV16-positive and almost all HPV18-positive cases are associated with integration of virus genomes into cervical epithelial DNA (12, 13). Hybrid capture 2 (HC2; Digene Corporation, Gainthersburg, MD, USA) assays and polymerase chain reactions (PCR) for HPV detection amplify a broad spectrum of HPV genotypes and focus on the L1 gene but risk a false negative result because in cervical cancer this is lost in 10% of integrated HPV genomes (14). In these cases, detection of E6/E7 mRNA transcripts with PCR may be of higher prognostic value compared with HPV DNA testing (15).

As an alternative to PCR, loop-mediated isothermal amplification (LAMP) methods (16) incorporated into a lab-on-a-chip (LOC) allow rapid amplification of nucleic acids at a single temperature, typically between 63-65^{0}C and have been used for the detection of infectious diseases such as malaria, dengue fever, bacterial and viral infections, notably SARS-CoV-2 (17–20). The lack of thermocycling makes this technique ideal for point-of-care testing. LAMP based assays have been successfully developed for a multitude of purposes, including genotyping HPV from cervical cytology samples (21, 22) but have not previously been combined with tumor markers associated with cervical cancer such as human telomerase reverse transcriptase (hTERT), which is significantly overexpressed in cervical lesions with low to nil expression in normal tissue and detectable in at least 90% of cervical cancers (23, 24), TERC and c-MYC, which are widely recognised tumor markers (25–28) for early detection of cancer. The aim of this work, therefore, was to establish the sensitivity and specificity of a HPV and tumor marker DNA/mRNA LAMP assay for detecting cervical cancer that is transferrable to a LOC platform and determine its diagnostic benefit in early stage disease when used in conjunction with high-resolution endovaginal MRI. HPV readouts from a conventional PCR platform (PCR) and cervical cytology/histology provided ground truth.

METHODS

Study Design

A prospective pilot study (Molecular Diagnostics Using a novel Lab-on-a-chip and MRI for detecting cervical cancer, MODULAR, NCT03380741) was conducted in accordance with the Declaration of Helsinki (1964), and local research ethics committee and Health Research Authority (HRA) approval. Patients were studied in 2 groups: 1) new diagnosis of cervical cancer 2) non-cancer HPV negative controls. Written informed consent was obtained from each patient. In this hypothesis testing pilot study, where a biomarker could be positive or negative, and assuming the prevalence of a biomarker positive value (tumor DNA or HPV 16 or 18 DNA) among cancer patients is ~75% in women aged 30-39 years (29), we estimated that 24 patients with suspected cancer would establish the ability of the LAMP assay to detect cancer with a power of >0.9 at an alpha of <0.05, warranting a larger trial.

Participants

Between August 2018 and May 2019, all patients with Stage 1 cervical cancer (squamous or adenocarcinoma on histology) referred for MRI to a tertiary oncology centre prior to being

considered for curative surgery were invited to participate, so they formed a consecutive series of cases. Women with neuroendocrine tumors or unusual histological subtypes, or those unable to have MRI because of ferromagnetic implants or claustrophobia were excluded. A control group taken from women attending a separate local colposcopy clinic for follow up of either conservatively managed or previously treated cervical dysplasia, who were judged to have a normal cervix on colposcopic examination was recruited to establish threshold values for tumor marker positivity and confirm validity of a negative HPV result. As part of the routine management of the patients attending the colposcopy clinic for follow up, tests for both HPV 16 and 18 DNA (real time PCR using the GenoID assay kit) and HPV E6/7 mRNA (PreTect HPV-Proofer, Norchip) was obtained through The Doctors Laboratory (TDL). GenoID is a PCR based assay for the HPV L1 gene, followed by an ELISA based 96 well hybridisation assay to a cocktail of probes for the type-specific detection of high-risk HPV from 20 HPV phage types (30). PreTect Proofer is a real-time multiplex nucleic acid sequence based amplification assay for isothermal amplification of E6/E7 mRNA expressed by five high-risk HPV types (16, 18, 31, 33 and 45) using proprietary primer sets (31). These commercially available tests were considered the gold-standard (GenoID CE marked), and were performed in all 27 patients with cancer. HPV positivity on these tests therefore resulted in their exclusion from the control group. Patients who were positive for Type 45 and 31 on TDL HPV typing were not included in this comparative analysis.

Cervical Swab Sampling

A cervical swab was taken either at an out-patient visit or prior to an examination under anesthesia (EUA) in all study subjects with cancer. In those patients with a normal cervix at colposcopy, the sample was taken as part of the colposcopic examination. Following insertion of a speculum, the cervix was swabbed with a standard cervical smear brush and the exfoliated cells deposited in PreservCyt transport medium. A separate, sequential cervical swab sample was examined conventionally to assess cytology and HPV DNA and RNA typing as per standard institutional clinical practice. Cytological sampling was adequate in all cases, so that inadequate sampling did not lead to withdrawal from the study in any instance.

Sample Preparation

The exfoliated cells were pelleted and PreservCyt solution discarded. The remaining pellet was washed with phosphate buffered saline (PBS) solution and pelleted again. The PBS solution was discarded. DNA and RNA were extracted from the pellet using Qiagen AllPrep kit (Qiagen, Manchester, UK) according to the manufacturer's instructions. Total DNA and RNA yield was determined using a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, USA). Only those samples which yielded both DNA and RNA were selected for analysis.

Loop-Mediated Isothermal Amplification (LAMP) Assay

A LAMP assay that is transferrable to a lab-on-a-chip was utilised using a conventional qPCR platform for readout. LAMP methods initially designed using 4 primers targeting 6 regions (16) and where the reaction proceeds without thermocycling is an ideal method for point-of-care testing. It relies on auto-cycling strand displacement DNA synthesis conducted by a DNA polymerase with high strand displacement activity. Subsequently, the LAMP method was extended to six primers targeting 8 regions (17) to accelerate the reaction. The six primers are Forward-Inner (FIP), Backward-Inner (BIP), Forward Outer (F3), Backward Outer (B3), Forward Loop (LF) and Backward Loop (LB). A stem-loop structure is constructed, in which the sequences of both DNA ends are derived from the inner primer. Subsequently, an exponential generation of inverted repeats is constructed as the inner primers anneal and cause amplification from the loops in the original structure (**Figure 1**). The addition of the loop primers LF and LB allow hybridisation of the available stem-loops that are not hybridised by the inner primers (FIP/BIP) and markedly accelerates the reaction from 1 hour to 10-15 minutes depending upon the concentration of the starting material. The primer sequences are given in **Table 1A**. The GenBank Accession numbers for the primers used are given in **Table 1B**.

Due to the small volume of DNA and RNA available following extraction of samples, only a single assay procedure was performed for each sample which required a final volume of 5 μL per reaction. Each mix obtained from a mastermix contained the following: 0.50 μL of 10 × isothermal pH-based buffer (pH 8.5–9), 0.30 μL of $MgSO_4$ (100 mM stock), 0.28 μL of dNTPs (25 mM stock), 0.30 μL of BSA (20 mg/mL stock), 0.13 μL of NaOH (200 mM stock), 0.80 μL of Betaine (5M stock), 0.13 μL of Syto9 Green (20 μM stock), 0.02 μL of Bst 2.0 DNA polymerase (120,000 U/mL stock), 0.13 μL of avian myelobastosis virus (AMV, 25 U/μL stock, Promega), 0.05 μL of Rnase inhibitor (20 U/μL stock, ThermoFisher Scientific), 1 μL of extracted RNA or DNA and 0.50 μL of 10 × LAMP primer mixture (20 μM of BIP/FIP, 10 μM of LF/LB, and 2.5 μM B3/F3), and enough nuclease-free water (ThermoFisher Scientific) to bring the volume to 5 μL. In experiments targeting DNA, AMV and Rnase inhibitor were replaced by water in the reaction mix. This LAMP recipe has been previously published (18, 19). Reactions were performed at 63°C for 45 min. One melting cycle was performed at 0.1°C/s from 65°C up to 97°C for validation of the specificity of the products. Reactions were plated in 96-well plates and loaded into a LightCycler 96 real-time PCR system (LC96) (Roche Diagnostics). Following the LAMP assay a standard PCR assay was undertaken in duplicate for validation.

An hTERT mRNA result was considered positive if detection occurred within 30 min. Both the TERC DNA relative copy number and MYC mRNA expression relative to GAPDH DNA and mRNA respectively were calculated *via* the relative fold gene expression2^-(ddCt) method: a mean delta Ct (threshold cycle) was calculated from the patients in the control group, and used to calculate the relative fold change in the cancer patients. Any detection of HPV 16 or 18 DNA or RNA was considered positive. The results of the reference GenoID and Norchip tests were not available to the reader of the LAMP assay at the time of interpretation.

Imaging

All scans were performed on a 3.0 Tesla Philips Achieva (Best, The Netherlands) with a dedicated in-house developed 37 mm ring-

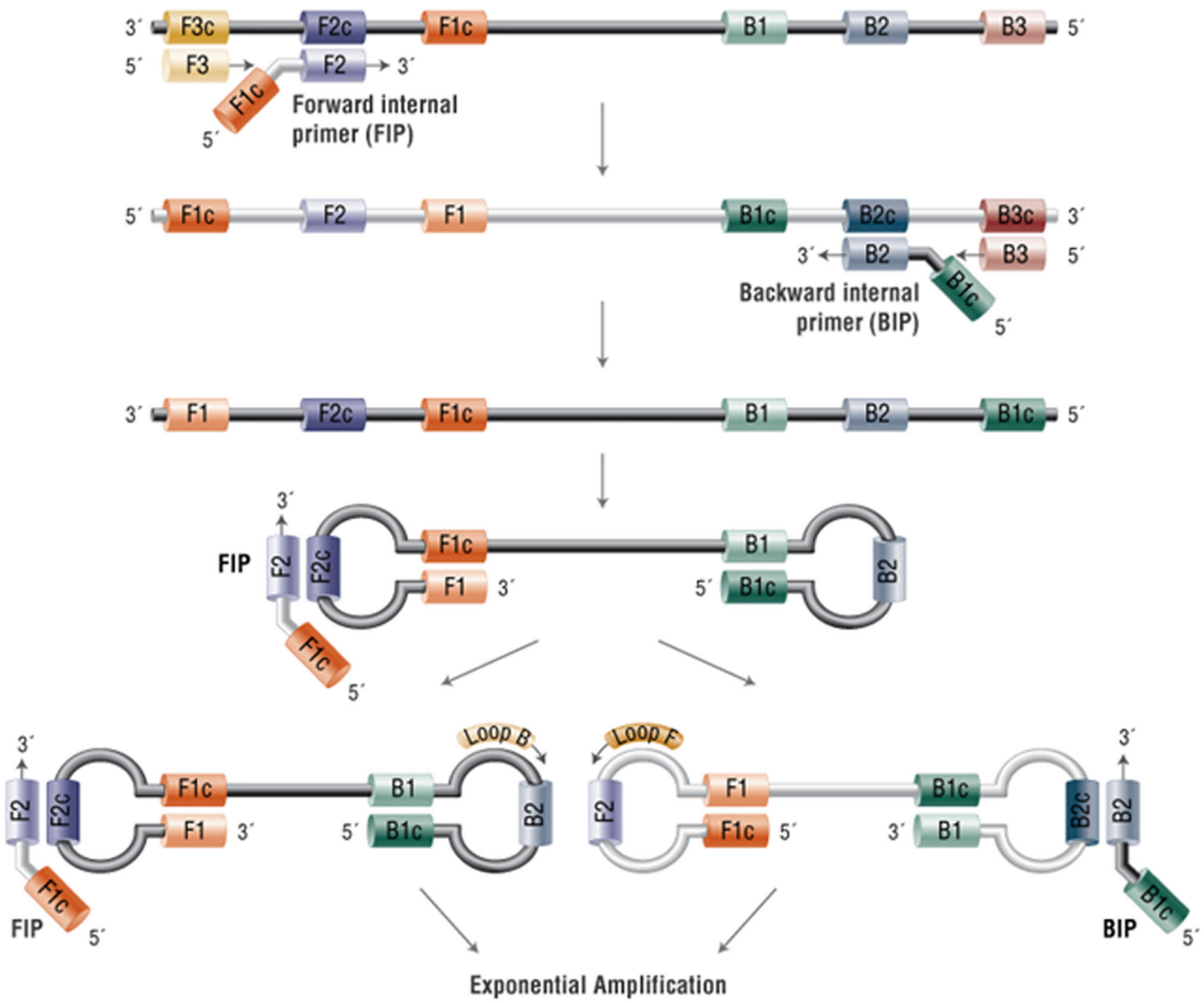

FIGURE 1 | Mechanism of Loop-mediated isothermal amplification (LAMP). In the LAMP method, 4-6 primers are used to recognise 6-8 distinct regions of target DNA. A strand-displacing DNA polymerase initiates synthesis and 2 of the primers form loop structures to facilitate subsequent rounds of amplification. Adapted from New England Biolabs https://www.neb-online.de/en/pcr-and-dna-amplification/isothermal-amplification/.

design solenoidal receiver coil that has been previously described (9, 32, 33). Cervical position was determined at vaginal examination, after which the coil was inserted and placed around the cervix. Image distortion from susceptibility artefacts were reduced by aspiration of vaginal air *via* a 4 mm diameter tube (Ryles; Pennine Healthcare, London, England).The intramuscular administration of Hyoscine butyl bromide (Buscopan) 20 mg decreased artefacts from bowel peristalsis.

T2-W images were obtained in three planes orthogonal to the cervix together with matched coronal Zonal Oblique Multislice (ZOOM) diffusion-weighted images (DWI). Sequence details are given in **Table 2**. ADC maps were automatically generated by the scanner software using a monoexponential fit of the data. These were compared with T2-W images to identify the presence and extent of a tumor within the cervix. Mass-lesions disrupting the normal cervical epithelial architecture that were intermediate signal-intensity on T2-W images with corresponding restriction on the ADC maps were recognized as tumor. Imaging reports were not available to the reader of the LAMP assay at the time of interpretation, nor was the radiologist aware of the results of the LAMP assay at the time of reporting.

Histopathology Analysis

Following definitive surgical excision, formalin fixed tissue specimens were sectioned at three to four millimeter intervals, embedded in paraffin and 2-3 micron sections mounted on glass slides. Hematoxylin and eosin (H&E) stained sections were reviewed by a specialist gynecological-oncology histopathologist and the presence or absence of residual tumor was recorded.

TABLE 1A | pH-LAMP primer sequences.

Name	Sequence
F3_TERT	GCCTGAGCTGTACTTTGTCA
B3_TERT	GGTGAGCCACGAACTGTC
FIP_TERT	TGGGGTTTGATGATGCTGGCGA-GGGCGCGTACGACACCATCC
BIP_TERT	GGTCCAGAAGGCCGCCCAT-GCTGGAGGTCTGTCAAGGTA
LF_TERT	ACCTCCGTGAGCCTGTCCTG
LB_TERT	CACGTCCGCAAGGCCTTCA
F3_MYC	CCATGAGGAGACACCGCC
B3_MYC	TGCTGATGTGTGGAGACGT
FIP_MYC	AGCCTGCCTCTTTTCCACAGAA-CACCACCAGCAGCGAC
BIP_MYC	CTGGATCACCTTCTGCTGGAGG-GGCACCTCTTGAGGACCA
LF_MYC	TCATCTTCTTGTTCCTCCTCAGA
LB_MYC	CAGCAAACCTCCTCACAGCC
F3_TERC	TGTGAGCCGAGTCCTGG
B3_TERC	TCTCCGGAGGCACCCA
FIP_TERC	AGGAAGAGGAACGGAGCGAGTC-GTGCACGTCCCACAGCT
BIP_TERC	GAAAGGCCTGAACCTCGCCC-TGCCACCGCGAAGAGT
LB_TERC	AGAGACCCGCGGCTGACA
LF_TERC	CGGCGCGATTCCCTGA
F3_GAPRNA	GATGCTGGCGCTGAGTAC
B3_GAPRNA	GCTAAGCAGTTGGTGGTGC
FIP_GAPRNA	CTTTTGGCTCCCCCCTGCAAATGGAGTCCACTGGCGTCTT
BIP_GAPRNA	TCTGCTGATGCCCCCATGTTCGGAGGCATTGCTGATGATCT
LF_GAPRNA	AGCCTTCTCCATGGTGGTG
LB_GAPRNA	GTCATGGGTGTGAACCATGAG
F3_GAPDNA	ACCCCCATAGGCGAGATC
B3_GAPDNA	TGATGACCCTTTTGGCTCC
FIP_GAPDNA	CTCCATGGTGGTGAAGACGCC-CAAAATCAAGTGGGGCGATG
BIP_GAPDNA	CGGGAGGGGAAGCTGACTCA-ACAGCAGAGAAGCAGACAGT
LF_GAPDNA	TCCACGACGTACTCAGCG
LB_GAPDNA	GCAGGACCCGGGTTCAT

(FIP, forward inner primer; BIP, backward inner primer; LF, loop F; LB, loop B, ***Figure 1****)*

TABLE 1B | GenBank Accession numbers for primers used.

Primer	GenBank Accession number
hTERT	NG_009265.1, NM_198253.2, NM_001193376.1,NR_149162.1, R_149163.1
TERC	NG_016363.1
MYC	NG_007161.2, NM_002467.5, NM_001354870.1
GAPDH	NG_007073.2, NM_002046, NM_001256799,NM_001289745, NM_001289746 and NM_001357943
HPV16	K02718.1
HPV18	AY262282.1

Statistical Analysis

Statistical analysis used commercially-available software GraphPad Prism for Windows, (v8.3, GraphPad Software Inc., San Diego, CA, USA) and utilized primarily sensitivity and specificity analyses with 95% confidence limits (Wilson/Brown method) for comparison of the LAMP assay with the gold standard (qPCR or histology), the endovaginal MRI with histology and a combination of LAMP assay and endovaginal MRI with histology. Accuracy as defined by [(true positive) + (true negative)]/[(true positive) + (true negative) + (false positive) + (false negative)] were calculated. These analyses represent the clinical performance of the tests. As we did not perform repeat experiments due to low amount of starting material it was not possible to estimate the precision (degree to which the measurements were repeatable under the same conditions) of the experiments.

RESULTS

Participants

Between August 2018 and June 2019, 27 patients with newly diagnosed Stage 1 cervical cancer (4 = 1a1, 9 = 1a2, 12 = 1b1, 2 = 1b2 by FIGO 2009 staging, Group 1) and 14 non-cancer HPV negative (normal) controls (Group 2) were prospectively recruited. Mean age and BMI were 34.7 years (range 25-51 years) and 23.7 (range 16.9-35.5) respectively. In Group 1, initial diagnosis was made with a LLETZ in 20 patients (where the tumor may have been removed in part or in entirety leaving no residual) and with punch biopsy in 7. Seventeen patients had squamous cell carcinoma (SCC), 10 had adenocarcinoma, with grade of disease distributed between well, moderate and poor differentiated in 5, 14 and 4 cases respectively (4 ungraded on histology). Lymphovascular space invasion was present in 7 cases, absent in 17 cases and not mentioned in 3. In Group 1, 26 of 27 patients had high-resolution MRI (I declined) and cervical swabs for LAMP assay analysis; patients in Group 2 had cervical swabs for LAMP assay analysis only.

In Group 1, 23 underwent primary surgery within 4 weeks of diagnosis (8 radical hysterectomy, 9 radical trachelectomy, 6 cold knife cone biopsy). On final histology, 12 of these patients had residual tumor. 2 further patients had radical trachelectomy after neoadjuvant chemotherapy and 2 had chemoradiotherapy following adverse findings at examination under anesthesia. These 4 patients

TABLE 2 | Scan parameters for endovaginal MRI.

Parameter	T2-W	ZOOM-DWI
TR (ms)	2500	6500
TE (ms)	80	54
FOV (mm x mm)	100 x 100	100 x 100
Slice thickness/gap (mm)	2.0/0.1	2.0/0.1
Voxel size (acquired) (mm^3)	0.42 x 0.42 x 2.0	1.25 x 1.25 x 2.0
Voxel size (reconstructed) (mm^3)	0.35 x 0.35 x 2.0	0.45 x 0.45 x 2.0
b-values (s/mm^2)	N/A	0, 100, 300, 500, 800
No. slices	24 (coronal, axial); 22 (sagittal)	24 coronal
NSA	2	1

N/A, not applicable.

were considered positive for tumor as swabs and diagnostic biopsies confirming tumor presence had been taken prior to treatment. Overall, 25 of 27 patients in Group 1 had endovaginal MRI, available histology and sufficient DNA/mRNA extracted on swabbing for inclusion in the analysis. DNA/mRNA extraction was sufficient for analysis in all patients from Group 2 (**Figure 2**).

DNA/RNA Marker Yield

In Group 1, following DNA/RNA extraction the mean yield of DNA and RNA was 31.79ng/μL and 57.37ng/μL respectively. Nucleic acid purity (assessed by ratio of light absorbance at 260nm and 280nm) gave a mean of 1.62 for DNA (values >1.8 show high purity) and 2.01 for RNA (values >2 show high purity) indicating some contamination in the DNA samples, but that pure RNA was successfully extracted. The extracted nucleic acid yield was insufficient in 1 patient (negative on histology), so they were excluded from further analysis. In Group 2 mean yield of DNA and RNA was 43.28ng/μL and 56.07ng/μL respectively with a mean 260/280 ratio of 1.28 and 1.98 respectively, suggesting contaminants remained in the DNA samples but that RNA extraction was successful.

Comparison of HPV-16 and HPV-18LAMP Assay With GenoID and Norchip Tests

In Group 1, 15 of 25 patients were positive for HPV 16 or 18 DNA and/or RNA on the gold standard DNA (GenoID) or E6/7 mRNA (Norchip) tests. Eleven were Type 16 and 4 were Type 18. Nine patients were negative for these HPV types, and in 1 case the results of the test were missing. Of these, 14 were positive on LAMP assay and 10 were negative, time to positive of clinical samples and the limits of detection for the synthetic HPV16 and 18 primers used are given in **Table 3**. All patients in Group 2 (14 HPV negative controls) were negative for HPV-16 and 18 DNA and RNA on the GenoID and Norchip tests. In this group, there were 2 false positives for the LAMP HPV-16 assay and 7 false positives with the LAMP HPV-18 DNA assay because of primer-dimer formation with the HPV-18 DNA LAMP primers in PCR negative cases. The LAMP HPV-18 mRNA assay was more reliable and detected 4 Type 18 mRNA detected by the Norchip test with 1 false positive. Overall sensitivities, specificities, positive and negative predictive values by HPV type are given in **Table 4**.

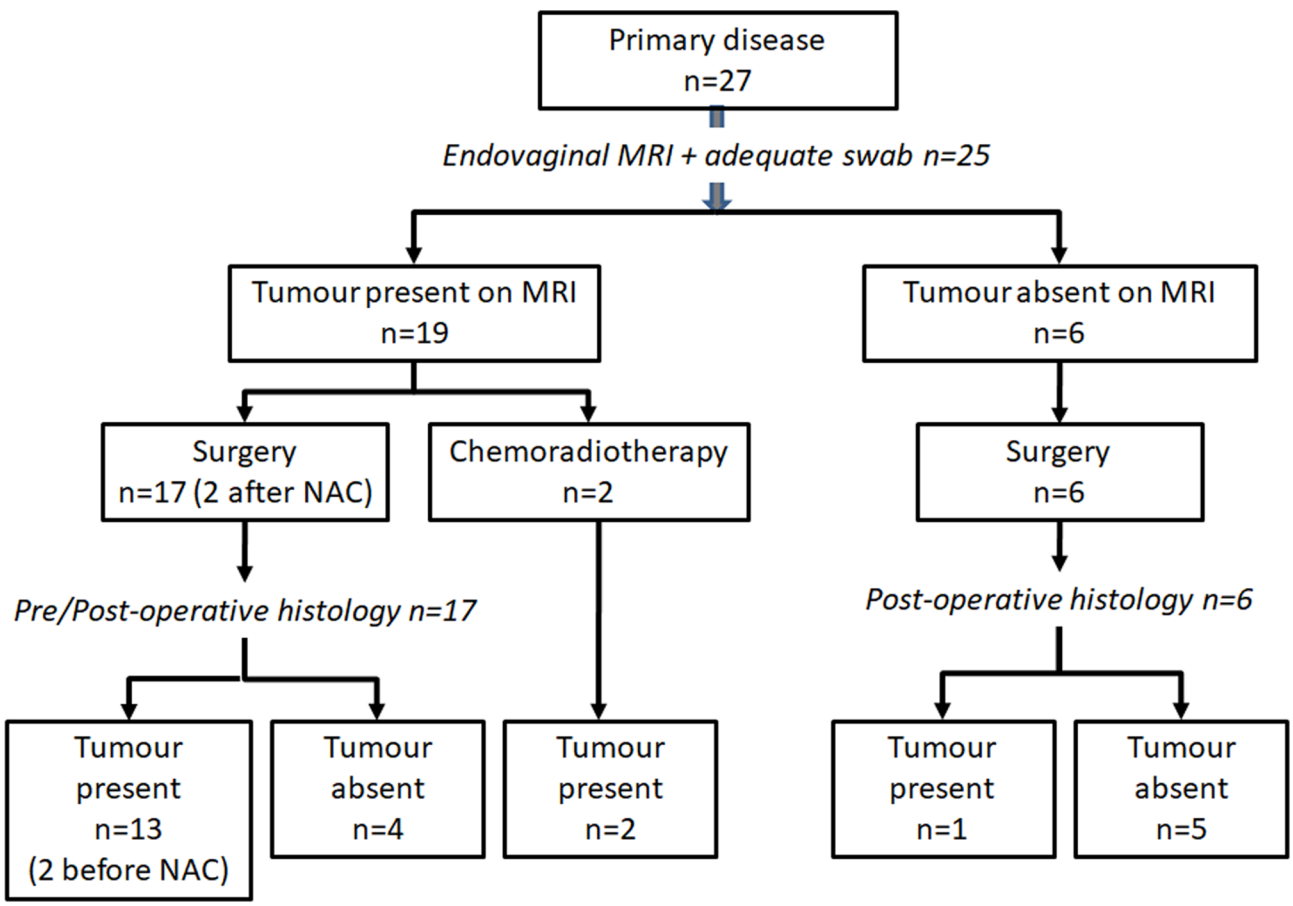

FIGURE 2 | Patient cohort studied. Inclusion criteria and management in patients with newly diagnosed cervical cancer recruited to the MODULAR study.

TABLE 3 | Limits of detection and time to positive for synthetic sequences and clinical samples in Groups 1 and 2 of tumor markers and HPV 16 and 18.

Tumour marker	Limit of detection (copies/reaction)	Time to positive of synthetic sequence Minutes (Mean ± SD)	Time to positive of Group 1 (n = 25) Minutes (Mean ± SD)	Time to positive of Group 2 (n = 14) Minutes (Mean ± SD)
hTERT	10^3	12.91 ± 0.44	37.4 ± 8.3 (in 13 positive cases)	42.9 ± 4.3 (in 7 positive cases)
MYC	10^1	14.98 ± 1.95	18.3 ± 7.9 (in 23 positive cases)	18.0 ± 3.3 (in 11 positive cases)
GAPDH RNA	10^3	9.35 ± 0.17	11.2 ± 1.0	11.2 ± 1.3
TERC DNA	10^1	11.95 ± 0.15	15.0 ± 1.4 (in 23 positive cases)	15.8 ± 1.9 (in 10 positive cases)
GAPDH DNA	10^0	13.62 ± 0.86	16.2 ± 2.6 (in 23 positive cases)	18.3 ± 4.6 (in 11 positive cases)
HPV 16 DNA	cf. primers as in Luo et al. (34)		18.0 ± 6.1 (in 11 positive cases)	21.8 (in 1 positive case)
HPV 16 mRNA	10^2	15.27 ± 1.10	25.6 ± 7.2 (in 7 positive cases)	48.6 (in 1 positive case)
HPV 18 DNA	cf. primers as in Luo et al. (34)		28.8 ± 13.2 (in 16 positive cases)	42.9 ± 4.5 (in 7 positive cases)
HPV 18 mRNA	10^4	17.06 ± 1.04	21.1 ± 4.6 (in 5 positive cases)	No positive cases

TABLE 4 | Sensitivity, specificity, positive and negative predictive values of LAMP assays for detection of small volume Stage 1 cervical cancer alone and together with endovaginal MRI.

Assay for cancer detection	n	Reference standard	Sensitivity [%] (lower and upper 95%CI)	Specificity [%] (lower and upper 95%CI)	PPV [%] (lower and upper 95%CI)	NPV [%] (lower and upper 95%CI)
LAMP-HPV 16 DNA and/or mRNA	38	GenoID and Norchip test	90.9 (62.3, 99.5)	88.9 (71.9, 96.1)	76.9 (41.7, 91.8)	96.0 (80.5, 99.8)
LAMP-HPV 18 DNA and/or mRNA	38	GenoID and Norchip test	100.0 (80.6, 100)	22.2 (7.3, 38.5)	47.1 (31.5, 63.3)	100.0 (51.0, 100)
LAMP-hTERT	25	Histopathology	31.3 (14.2, 55.6)	77.8 (45.3, 96.1)	71.4 (35.9, 94.9)	38.9 (20.3, 61.4)
LAMP-TERC	24	Histopathology	40 (19.8, 64.3)	100 (70.1, 100)	100.0 (61.0, 100)	50.0 (29.0, 71.0)
LAMP-cMYC	25	Histopathology	43.8 (23.1, 66.8)	100 (70.1, 100)	100.0 (64.6, 100)	50.0 (29.0, 71.0)
LAMP-Scenario 1 (tumor marker positive regardless of HPV status	25	Histopathology	66.8 (44.4, 85.8)	77.8 (45.3, 96.1)	84.6 (57.8, 97.3)	77.8 (45.3, 96.1)
LAMP-Scenario 2 (tumor marker or HPV positive)	25	Histopathology	93.8 (71.7, 99.7)	55.8 (26.7, 81.1)	78.9 (56.7, 91.5)	83.3 (43.7, 99.1)
Endovaginal MRI	25	Histopathology	93.8 (71.7, 99.7)	44.4 (18.9, 73.3)	75.0 (53.1, 88.8)	80.0 (37.6, 99.0)
Endovaginal MRI+ Scenario 1	25	Histopathology	68.8 (44.4, 85.8)	88.9 (56.5, 99.4)	91.6 (64.6, 99.6)	61.5 (35.5, 82.3)
Endovaginal MRI+ Scenario 2	25	Histopathology	93.8 (71.7, 99.7)	77.8 (45.3, 96.1)	88.2 (65.7, 97.9)	87.5 (52.9, 99.4)

Comparison of LAMP Tumor Marker Assay With Standard qPCR

In the non-cancer controls (Group 2), hTERT was positive at the outer limit of detection in 7 cases with a mean Ct of 42.9 minutes. Therefore, this was used as a Ct threshold for a positive result for the presence of cervical cancer. The positivity of TERC and MYC on LAMP assay was assessed by relative expression to GAPDH. Based on the 2^-(ddCt) for TERC/GAPDH and MYC/GAPDH from the controls in Group 2, the threshold of cancer detection for these markers was set at 3.12 and 0.155 respectively. Limits of detection and time to positive for the synthetic primers designed for hTERT, TERC, MYC, GAPDH and HPV mRNA and of clinical samples are given in **Table 3**. The relative expression of TERC DNA to GAPDH DNA and MYC mRNA to GAPDH mRNA in those without and with residual tumour in Group 1 is illustrated in **Figure 3**.

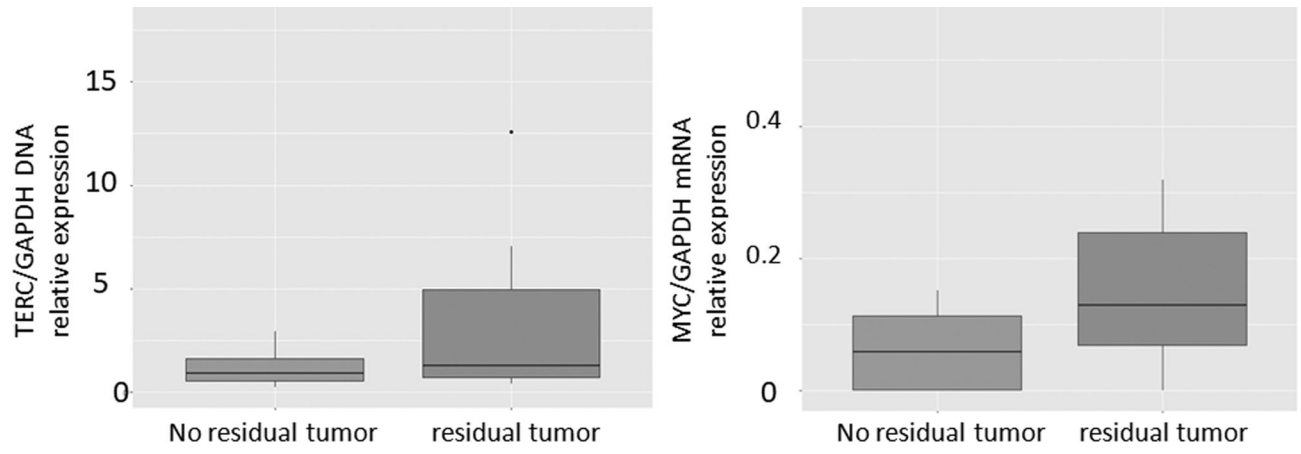

FIGURE 3 | Boxplots of TERC/GAPDH and MYC/GAPDH in patients with cytology positive for cancer compared with negative controls.

The hTERT LAMP assay results agreed with the PCR test for the presence or absence of cancer in 31 of 39 samples (sensitivity 57.1%, specificity 84.4%). The TERC/GAPDH DNA copy number PCR test was only successfully recorded in 26 of 39 samples; of these the standard deviation of the Ct for GAPDH was >0.25 in 14 cases and the standard deviation of the Ct for TERC was >0.25 in a further 4 cases, making the TERC/GAPDH replicable in only 8 cases. Similarly, with the MYC PCR assay a result was recorded in 25 of 39 samples; of these the standard deviation of the Ct for GAPDH was >0.25 in 10 cases and the standard deviation of the Ct for MYC was >0.25 in a further 10 cases, making the MYC/GAPDH replicable in only 5 cases. Comparison of LAMP assay with PCR was therefore not possible for TERC and MYC due to variability of the PCR results.

Sensitivity and Specificity of Combined HPV and Tumor Marker LAMP Assay for Cancer Detection

Sensitivity and specificity for cancer detection was documented by comparison to the gold-standard of pathology. At the threshold set for hTERT, there was a sensitivity of 31.3% and specificity of 77.8%, accuracy 48% for tumor detection. Relative TERC/GAPDH DNA copy number was successfully recorded in 24 cases on LAMP assay and achieved a sensitivity of 40% and specificity of 100%, accuracy 62.5% at the threshold relative expression of 3.12. MYC/GAPDH relative expression was recorded in all 25 patients on LAMP assay and achieved a sensitivity of 43.8% and a specificity of 100%, accuracy 64% at a threshold relative expression of 0.155.

To evaluate the performance of the combined markers within the LAMP assay in detecting residual disease two scenarios were applied. In the first, tumor was considered present if any tumor marker (hTERT n=7; TERC/GAPDH>3.12 n=6, MYC/GAPDH>0.155 n=7) was present with or without HPV positivity and tumor absent if all tumor markers were absent regardless of the presence or not of HPV. Using these criteria, gave the LAMP a sensitivity of 68.8% (5 false negatives), specificity of 77.8% (2 false positives), positive predictive value of 84.6% and a negative predictive value of 77.8%, accuracy 72%. In the second scenario, tumor was considered present if any tumor marker or HPV was present, and tumor was considered absent if all tumor markers and HPV were absent. Using these criteria, the LAMP had a sensitivity of 93.8% (1 false negative), specificity of 55.8% (4 false positives), positive predictive value of 78.9% and a negative predictive value of 83.3%, accuracy 80% (**Table 3**).

Sensitivity and Specificity of High-Resolution MRI

Of the 25 patients scanned, 20 had tumor present on MRI and 15 of these were confirmed at histology (11 at surgery, 4 on biopsy prior to chemoradiotherapy). In the 5 patients who were negative for tumor on MRI, 4 cases had no residual disease on histology and 1 was positive for tumor (**Figure 4**). Sensitivity and specificity of MRI was therefore 93.8% and 44.4% respectively, accuracy 76%, PPV 75.0%, NPV 80.0%.

Value of LAMP Assay Testing Combined With High Resolution MRI

If patients in Group 1 were considered positive only if they were positive on MRI and LAMP tumor markers (scenario 1), sensitivity was 68.8% but specificity improved to 88.9%, accuracy 76%; for scenario 2 sensitivity was 93.8%specificity 87.5% and accuracy 88% (**Table 4**).

DISCUSSION

This data indicates the feasibility of performing HPV and tumor marker testing using LAMP technology and indicates the assays current accuracy in comparison to standard PCR systems. The ability to perform multiple marker testing at point-of-care indicates the potential added value of this type of molecular testing in the diagnostic pathway of patients with early stage, small volume

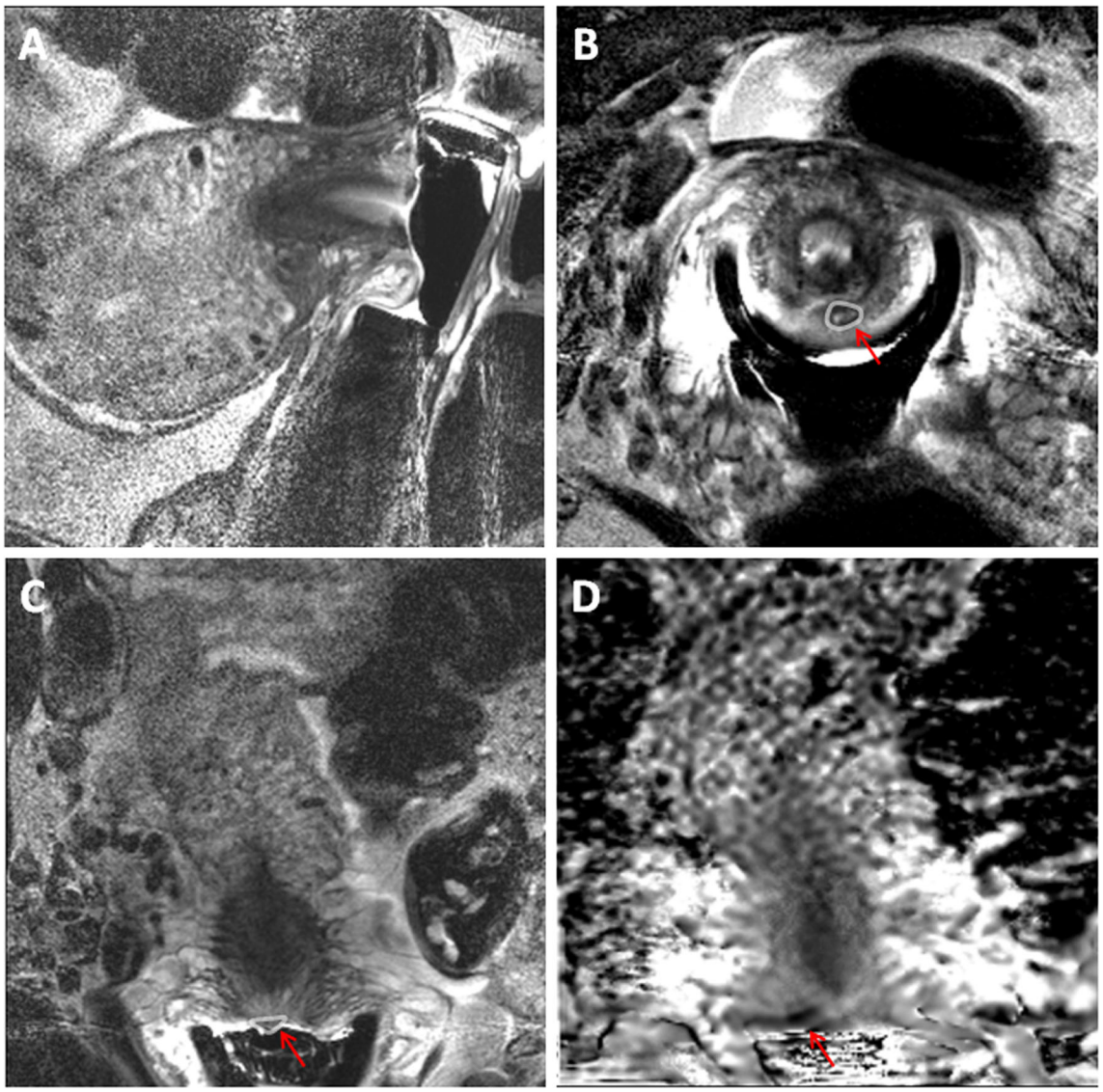

FIGURE 4 | 32-year old female with an endovaginal MRI that was a false positive for cervical cancer. T2-weighted sagittal **(A)**, axial **(B)** and coronal **(C)** MRI scans obtained using an endovaginal coil with corresponding ADC map in the coronal plane **(D)**. A small nodule on the posterior ectocervix in C (arrow) with focal diffusion restriction in D was considered positive for residual tumour. On LAMP assay from a cervical cytology swab, the cells were negative for all tumour markers and for HPV E6/7 mRNA indicating that the MRI result was likely a false positive. This was confirmed at histology from a subsequent repeat cone biopsy.

cervical cancers following cone biopsy or LLETZ, who undergo MRI for determining the presence and extent of residual disease prior to definitive surgery. The increase in accuracy is critical because the evidence for increased obstetric risk following CKC or LLETZ is substantial: although no difference in first-trimester miscarriage rates was reported in a meta-analysis (35) and subsequent Cochrane review (36), a population based study suggested an almost four-fold increase in the risk of mid-trimester loss in women post-conization (n=15 108) compared to untreated individuals (n=2 164 006; 1.5% *versus* 0.4%; RR 4.0 and 95% CI 3.3–4.8) (37). A metanalysis of 20 studies showed that the frequency and severity of these complications increased with methods that are known to remove large amounts of cervical tissue (38) and was confirmed by a later meta-analysis (39) and a Cochrane review (40). The combination of MRI and HPV and tumour marker testing thus enables decision making for optimal surgical approach in patients wishing to preserve fertility. The increased accuracy of the LAMP assay also comes with major advantages: the time to positive (TTP) of less than 25 minutes for all tests demonstrates the true point-of-care potential of this assay to deliver rapid, accurate results when utilised on a portable lab-on-a-chip platform.

Validation of the methodology against conventional PCR showed largely equivalent results for both the DNA and RNA tests in the HPV and hTERT primers. The PCR primers for MYC

and TERC/GAPDH performed poorly in the experiments, perhaps indicating alternative PCR primers should be used as their technical sensitivity was below expectation in the clinical samples. Similarly, validation of the HPV-16 and the tumor markers against standard PCR was also equivalent. The pH-LAMP HPV Type 18 DNA marker, as developed by Luo et al. (34), did not perform as expected and resulted in a large number of false positives through unexpected primer-dimer formation in clinical samples. This could be ameliorated by setting a very short time to positive threshold but alternative HPV 18 DNA LAMP primer sets would be available to test which may provide more reliable real-world results. The development of a robust, sensitive set of HPV DNA type specific pH-LAMP primers would be a prerequisite prior to the platform being successful as a screening platform.

Approaches combining HPV DNA testing with cytology have been previously tried (41) to optimise the sensitivity and specificity of cancer detection at the time of colposcopy in patients referred because of abnormal smears. HPV DNA testing is very sensitive (~95%) but lacks the specificity (30-50%) required for cervical cancer detection (42). There are a wide range of commercially available HPV detection assays which are based on different techniques such as target amplification (mainly PCR), signal amplification, and probe amplification (43). Food and Drug Administration (FDA) approved assays for HPV DNA are aimed at a panel of 13 or14 high-risk HPVs. Nevertheless, none of these is used routinely in a screening setting, and their low specificity makes them unsuitable for screening. HPV E6/E7 mRNA tests (as described here) have superior specificity to HPV DNA tests (42, 43). Overexpression of HPV E6 and E7 mRNAs has been evaluated as a marker for the transition from a productive infection to an abortive infection that eventually promotes cell transformation. Thus, the advantage of our spatially multiplexed LAMP assay system is that it also allows utilization of HPV mRNA which can substantially improve the specificity for cancer detection in patients at high risk of invasive disease.

The three pH-LAMP tests (hTERT mRNA, MYC mRNA and TERC DNA) each had poor sensitivity but excellent specificity for predicting the presence of residual tumor. The need for three pH-LAMP nucleic tests is warranted to cover a range of possible scenarios; comparison between these showed that all 3 were positive in 1 case, 2 were positive in 6 cases and 1 was positive in 7 cases. The poor sensitivity of the tumor marker tests is counteracted by having the HPV markers included as part of the assay, as their sensitivity for detecting cancer was high. Unfortunately, the HPV 18 DNA LAMP assay designed by Luo et al. (34) in synthetic sequence testing did not reveal false positive tests but was unreliable in our clinical samples as false positive results were in abundance due to primer-dimer formation. However, the newly designed HPV 18 mRNA assay included evaluating primer-dimer formation using NuPack assessment and was highly sensitive and specific. Our analysis however, considered a sample to be HPV positive if either DNA or RNA was positive, so that the HPV DNA data reduced the overall specificity of the result. Jointly utilising HPV and tumor marker testing and interpreting the tumor marker status on those with positive HPV results would help differentiate the true positives from those with an indeterminate result that require further investigation. Conversely, it is also true that the few false negative cases seen with HPV DNA testing may be successfully detected by a positive tumor marker status.

Other markers could be considered for inclusion on spatially multiplexed chip technology in future. Because the expression of HPV viral E7 leads to an increase of cyclin-dependent kinase inhibitor p16 (p16INK4a), p16 would be a possible candidate. However, as p16 overexpression, fundamentally is a marker of HPV infection, it was not selected for the current study. It provides a similar sensitivity and specificity profile to the HPV markers. A meta-analysis of seventeen studies showed a pooled sensitivity and specificity of p16INK4a to detect CIN2 or worse in patients with squamous intraepithelial lesions was 83.8% and 65.7% respectively (44). DNA methylation of several human genes has been shown to be also a relevant event for cervical carcinoma development. The use of differential methylation hybridization using a pilot methylation array allowed the identification of SOX1, NKX6-1, PAX1, WT1, and LMX1A as frequently methylated genes in cervical cancer and precursor lesions (45). In future, optimal marker selection and methods to identify DNA methylation may substantially improve the sensitivity of the tumor marker testing.

Nucleic acid based tests have yet to be evaluated at a population screening level: the change to HPV DNA primary screening has only recently been adopted (46), especially in the UK. The introduction of the HPV vaccine has reduced the number of CIN2/3 diagnoses in Scotland (47), so the economic benefit of testing for HPV within a screening programme remains debatable, especially where high quality cytology services are available. In areas with limited cervical screening programmes and without the high quality, well-resourced colposcopic service seen in developed countries, however, the benefit of a rapid, low-cost, point-of-care approach to cervical screening could potentially be transformational. It would provide the opportunity for developing countries to skip over several hurdles which developed countries have encountered in establishing their screening programmes.

LOC technology is versatile to a wide range of targets including bacterial and viral transcripts (20) and sample types when coupled with a sample preparation module. Additionally, its use of standard electronic components promotes scalability and portability which ideally match the requirements of portable diagnostics and allow for future pathogen multiplexing capabilities. Other reported commercial isothermal assays for COVID-19 detection such as Lucira's COVID-19 All-In-One Test Kit is a good example of a molecular *in vitro* diagnostic test that generates results in 30 minutes with analytical sensitivity comparable to the RT-PCR assays. However, it is limited to COVID-19 hence does not allow multi-pathogen detection, and the sensitivity is expected to be reduced due to the all-in-one test kit approach when compared to the full sample extraction methodology. We optimised our isothermal methods to enable the compatibility to our microchip technology as an alternative to fluorescence and time-consuming incubation. This approach has been shown to hold significant potential for the development

of a cheap, portable and quantitative diagnostic tool (48) using an external thermal controller in conjunction with a desktop computer. Moreover, or recent work has demonstrated a fully portable LOC platform which has integrated thermal management within the diagnostic platform and uses a smartphone application (Android OS) for data acquisition, visualization and cloud connectivity and has been used to detect breast cancer mutations (49), genes related to antimicrobial resistance (18) and COVID-19 (19).

There are several limitations to our study. Firstly, we did not include any HPV positive non-cancer controls in this study. This would be the ideal if testing HPV alone as a biomarker for the presence of tumor, however, as the aim was to develop a spatially multiplexed assay with HPV and tumor markers, we felt that a control group that was negative for cancer and HPV would be more definitive in the first instance in this pilot study. Secondly, validation of the LAMP tumor marker assay was limited by the variability of the standard PCR results. This was partly because of the limited volume of extracted RNA available from these cytology samples for the multiple LAMP and PCR assays; uncertainty around the concentrations of RNA and DNA available from the cytology swabs also meant that we may have used a larger sample volume than necessary for each LAMP experiment and compromised the number of successful repeat experiments, particularly for the PCR validation. Moreover, the purity of the DNA samples was low so that contaminants and inhibitors within biological samples may have affected the performance of the PCR assay (50) This is likely to have been more pronounced from cytology samples where cellular content is low. Nevertheless, a key benefit of the LAMP method is robust detection even in crude samples (51) which lends itself to point-of-care testing possibly even on direct cervical brush samples. Developing a new methodology to better extract DNA/RNA from the tested samples would be of value but was outside the scope of this work. Other intrinsic limitations were lack of repeated testing due to insufficient starting materials which prevented us estimating the precision of our results. Therefore, reproducibility of the LAMP assay for cervical cancer biopsies remains to be established. Reduction of the cellular material for the PCR validation also may well have reduced the repeatability of the tumor marker PCR assay (52) and prevented validation of our LAMP assay for TERC/GAPDH and MYC GAPDH. The experiments will also need to be repeated on a larger sample size. Nevertheless, translation of a LAMP assay technique for spatially multiplexed tumor markers and HPV to a lab-on-a-chip is achievable, but the low sensitivity of the tumor markers and low specificity of the HPV markers mean that these markers are best tested for together to be clinically useful. It will require integration of sample preparation and nucleic acid extraction with the LAMP assay to achieve a deliverable test at point-of-care.

In summary, this work has demonstrated the feasibility of a LAMP assay comprising HPV 16 and 18 DNA/RNA and tumor markers hTERT, TERC and MYC for early detection of cervical cancer using prospectively collected cytology samples from patients with newly diagnosed cervical cancer and in normal controls. While the specificity for cancer detection was superior with the tumor markers, sensitivity was relatively low; the reverse was true for HPV detection. In patients with small cervical tumors suitable for fertility-sparing surgery, use of a spatially multiplexed LAMP assay in conjunction with high resolution endovaginal resulted in improved specificity for cancer detection.

DATA AVAILABILITY STATEMENT

The imaging data from this study are available via the Institute of Cancer Research's XNAT imaging data repository. Access requests will be granted depending on appropriate regulatory and institutional approvals upon contacting the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by London-Surrey Research Ethics Committee Ref: REC18/LO/0865. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

NdS, TI, and PG devised the study. BW, NM, IP, JR-M, and NdS contributed to the data acquisition/collection. KV and AA performed the histopathological analysis. BW, IP, JR-M, and NdS analyzed the data. All authors contributed to the article and approved the submitted version.

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Preoperative Evaluation of Perineural Invasion in Cervical Cancer: Development and Independent Validation of a Novel Predictive Nomogram

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Background: Perineural invasion (PNI) is associated with a poor prognosis for cervical cancer and influences surgical strategies. However, a preoperative evaluation that can determine PNI in cervical cancer patients is lacking.

Methods: After 1:1 propensity score matching, 162 cervical cancer patients with PNI and 162 cervical cancer patients without PNI were included in the training set. Forty-nine eligible patients were enrolled in the validation set. The PNI-positive and PNI-negative groups were compared. Multivariate logistic regression was performed to build the PNI prediction nomogram.

Results: Age [odds ratio (OR), 1.028; 95% confidence interval (CI), 0.999–1.058], adenocarcinoma (OR, 1.169; 95% CI, 0.675–2.028), tumor size (OR, 1.216; 95% CI, 0.927–1.607), neoadjuvant chemotherapy (OR, 0.544; 95% CI, 0.269–1.083), lymph node enlargement (OR, 1.953; 95% CI, 1.086–3.550), deep stromal invasion (OR, 1.639; 95% CI, 0.977–2.742), and full-layer invasion (OR, 5.119; 95% CI, 2.788–9.799) were integrated in the PNI prediction nomogram based on multivariate logistic regression. The PNI prediction nomogram exhibited satisfactory performance, with areas under the curve of 0.763 (95% CI, 0.712–0.815) for the training set and 0.860 (95% CI, 0.758–0.961) for the validation set. Moreover, after reviewing the pathological slides of patients in the validation set, four patients initially diagnosed as PNI-negative were recognized as PNI-positive. All these four patients with false-negative PNI were correctly predicted to be PNI-positive (predicted $p > 0.5$) by the nomogram, which improved the PNI detection rate.

Conclusion: The nomogram has potential to assist clinicians when evaluating the PNI status, reduce misdiagnosis, and optimize surgical strategies for patients with cervical cancer.

Keywords: perineural invasion, cervical cancer, predictive model, nomogram, biomarker

INTRODUCTION

Cervical cancer is the fourth most common cancer among women worldwide (1). Radical hysterectomy (RH) is a conventional treatment for early-stage cervical cancer that has the advantages of maintaining both ovarian function and sexual function compared with radiotherapy (2, 3). However, extensive parametrial resection during surgery has been proven to cause postoperative pelvic problems, including bladder, sexual, and colorectal dysfunction, which negatively influence quality of life (4). Nerve-sparing radical hysterectomy (NSRH), which was also known as Type C1 radical hysterectomy according to Querleu-Morrow classification to avoid these adverse effects by preserving the pelvic autonomic nerves, has been applied maturely (5). However, controversy still exists regarding the preoperative indications for NSRH. Recent studies have found that dissemination along nerves is considered an independent route for cancer spread (6, 7). NSRH may preserve not only the nerves but also the cancer cells invading the nerves. Perineural invasion (PNI) is reportedly associated with multiple high-risk factors (8, 9) and poor outcomes during early-stage cervical cancer (10, 11). PNI is relatively common in cervical cancer and may be underestimated. Pathological examinations have shown that 7.1%–35.1% of patients with early-stage cervical cancer have PNI (8–14). Therefore, preoperative diagnosis of PNI could help identify populations who would benefit from NSRH.

Unfortunately, it is not easy to identify signs of PNI before surgery. Although it has been reported that some patients with cervical cancer and PNI have different degrees of pelvic pain, this symptom was rare and not sufficiently typical (15). Researchers have examined PNI diagnosis in other types of cancer, such as colon, prostate, and pancreatic cancers, to distinguish PNI with magnetic resonance imaging (MRI) or positron emission tomography and computed tomography (CT) (16, 17). Nevertheless, few studies have investigated preoperative detection of PNI in cervical cancer.

In this study, we aimed to explore the relative clinical and radiological factors of PNI in cervical cancer and develop a predictive nomogram for PNI using preoperative clinical and radiological data.

MATERIALS AND METHODS

Participants

We screened 1836 patients diagnosed with FIGO stage IB1–IIB cervical cancer at Sun Yat-sen University Cancer Center who were admitted between January 1, 2012, and June 1, 2017, and underwent standard RH during hospitalization. Patients were excluded if they had any of the following conditions: cervical stump cancer; histological types except squamous carcinoma, adenocarcinoma, or adenosquamous carcinoma; cervical conization or radiotherapy before RH, and a history of other malignant tumors. Patients who had cervical conization before RH were excluded because it was difficult to get all the conization pathological slices to evaluate the PNI status if the conization was done in other hospitals. Additionally, neoadjuvant chemotherapy could be performed only for patients with FIGO stage IB3/IIA2/IIB. A total of 162 cervical cancer patients with PNI (PNI-positive) and 1674 cervical cancer patients without PNI (PNI-negative) were included in the training set. To avoid underestimation of the real incidence of PNI, all pathological slides of 1836 patients were to be re-read by pathologists, but this task was too difficult to complete. Therefore, we applied 1:1 propensity score matching using SPSS (version 23.0) to balance the following important patient characteristics: tumor size, histological type, FIGO stage, differentiation, and preoperative treatment (matching tolerance = 0.01) (18). Eventually, 162 matched pairs of PNI-positive and PNI-negative patients were included in the training set. The validation set comprised 49 eligible patients who were randomly enrolled using the same inclusion and exclusion criteria and who were admitted between January 1, 2020, and June 1, 2020. The study design is illustrated in **Figure 1**.

Data Collection

In our published data, we found PNI in cervical cancer was associated with deep stromal of cervical canal invasion, Lymph node invasion, and positive margin (18). This result inspired us that the occurrence of PNI should be associated with risk factors for cervical cancer. Also, we considered factors proven to be associated with PNI in previous studies (8, 19, 20). Therefore, in this study, we collected preoperative clinical and radiological data from the electronic health records accordingly. Clinical data included age, FIGO stage, histological type (determined using thinprep cytology test or cervical biopsy results), degree of differentiation (determined using cervical biopsy results), and neoadjuvant chemotherapy (NACT). Radiological data included tumor size, lower uterine segment invasion, deep stromal invasion (DSI), full-layer invasion (FLI), and lymph node enlargement (LNE), all of which were indicated by radiology before all anti-tumor treatment. Senior radiologists in gynecological oncology subspeciality from the radiology department of Sun Yat-sen University Cancer Center confirmed the quality and reports of MRI or CT for every patient. Trained researchers entered and double-checked the data independently.

To diagnose PNI, surgical specimens were fixed with 10% neutral formaldehyde fixation solution, embedded in paraffin, cut into 4-μm-thick sections, and stained with hematoxylin and eosin. Patients were classified as PNI-positive if the microscopic examination found that cancer cells infiltrated any layer of nerve fibers (including the epineurium, perineurium, and endoneurium) or surrounded more than 33% of the outer diameter of nerves. If hematoxylin and eosin staining could not verify PNI, then immunohistochemical staining of the nerve bundle S-100 was used to identify the nerves (21). The FIGO staging of all patients was performed according to the 2018 FIGO Staging guidelines (22). The histological type was obtained from the cervical biopsy results

Abbreviations: PNI, perineural invasion; NACT, neoadjuvant chemotherapy; LNE, lymph node enlargement; DSI, deep stromal invasion; FLI, full-layer invasion; RH, radical hysterectomy; NSRH, nerve-sparing radical hysterectomy; MRI: magnetic resonance imaging; LVSI, lymph vascular space invasion; LUSI, lower uterine segment invasion; ROC curve, receiver-operating characteristics curve; AUC, area under the ROC curve; PPV, positive predictive value; NPV, negative predictive value; OR, odds ratio; 95% CI, 95% confidence interval; IQR, interquartile range; CT, computed tomography.

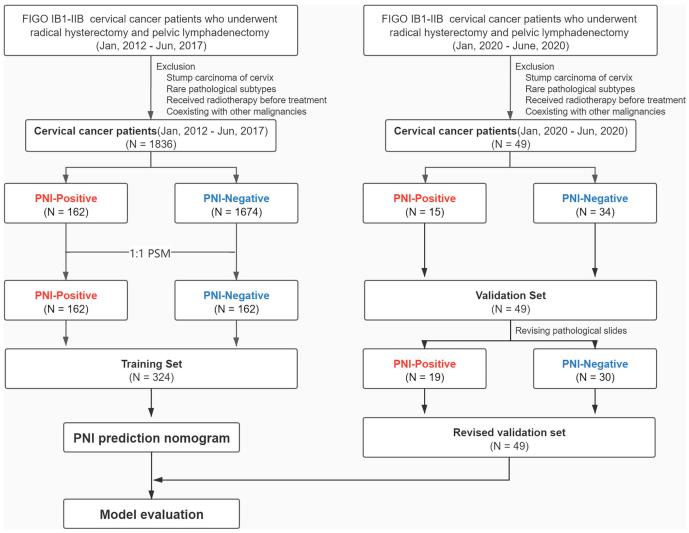

FIGURE 1 | Flowchart of the study design.

and categorized according to whether the adenocarcinoma component was present. The degree of differentiation was also determined using cervical biopsy results and classified as good, moderate, or poor. Lymph vascular space invasion (LVSI) was defined as the presence of tumor cells in a space lined by endothelial cells outside the immediate invasive border on postoperative pathological examination; therefore, we collected its data and used it as a baseline characteristic to observe but not as a predictive variable. Lower uterine segment invasion was defined as a cervical tumor extending above the uterine isthmus on preoperative CT or MRI. DSI was defined as a cervical tumor invading more than half of the cervical canal from the external cervical orifice to the cervical isthmus and more than half the thickness of the cervical transverse muscle. FLI was defined as cervical mass invasion into the epigastric layer of the cervix. LNE was defined as the pelvic lymph nodes with the short axis diameter ≥5 and ≤15 mm on CT or MRI (23–25) (i.e., lymph node metastasis that was suspected but not confirmed was included in the study). Para-aortic lymph nodes were not evaluated here because patients with suspicious enlarged para-aortic lymph nodes didn't receive RH in our center, which were considered as distant metastasis of cervical cancer previously (26, 27).

Model Development and Evaluation

Nomogram Development

First, a logistic regression model was constructed using the Stats package of R language (Version 4. 0. 1, Vienna, Austria) and variables were screened using stepwise regression with the CAR package. We included variables in the logistic regression analysis based on previous studies and clinical consensus (28). Then, we constructed the PNI prediction nomogram based on the logistic regression model with the regplot package. Each variable was given a score based on the point scale of the nomogram according to the coefficients in the logistic regression equation. By summing the total scores, we were able to estimate the probability of PNI for cervical cancer patients before surgery. Probability less than 50% was considered low risk for PNI, whereas probability more than 50% was considered high risk for PNI. The higher the total score, the higher the risk of PNI.

Evaluation of the Model

The nomogram was validated internally for the training group and externally for the validation group. We evaluated the predictive performance of the nomogram using the receiver-operating characteristics (ROC) curve, calibration curve, and performance metrics including the area under the ROC curve (AUC), accuracy, sensitivity, specificity, positive predictive value, negative predictive value, F1 score, and Cohen's kappa coefficient (kappa) using R packages pROC, RMS, and caret.

Statistical Analysis

The median value (interquartile range) and frequency (%) were used to express continuous and categorical variables, respectively. All continuous variables were compared between groups using the Mann–Whitney U test. All categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. The odds ratio (OR) and corresponding 95% confidence interval (95% CI) from the logistic regression were calculated to assess the strength of association between clinical or radiological factors and the occurrence of PNI using R package stats. The significance level (p) was set at <0.05 (two-sided p value).

Ethical Consideration

This study was approved by the Ethics Committee and Institutional Review Board of the Sun Yat-sen University Cancer Center (Guangzhou, China). All case data were anonymized, and the Institutional Review Board waived the requirement for written informed consent because it did not involve breach of patient privacy.

RESULTS

Table 1 lists the clinical characteristics of the training set. Patients who were older [PNI-positive vs. PNI-negative: 51.5 years (interquartile range, 45.25–57) vs. 49 years (interquartile range, 41.25–55); $p = 0.006$], had LNE (35.2% vs. 17.3%; $p < 0.001$), had DSI (66.0% vs. 39.5%]; $p < 0.001$), or had FLI (45.1% vs. 10.5%; $p < 0.001$) were significantly more likely to have PNI. In addition to matched factors, LVSI (33.3% vs. 31.5%; $p = 0.812$) and lower uterine segment invasion (23.5% vs. 16.0%; $p = 0.125$) were not significantly different between the PNI-positive and PNI-negative groups.

Next, we conducted a multivariate logistic regression analysis to predict the PNI status of cervical cancer patients. The pathological diagnosis of PNI was identified as an outcome variable. Backward stepwise selection with the Akaike information criterion was performed for predictor variable screening to build the final multivariate logistic regression model. In particular, we included adenocarcinoma, tumor size, and NACT as predictive variables in the final model because prior studies have shown that these variables are associated with PNI (10, 29). Finally, seven predictor variables were integrated into the multivariate logistic regression model for PNI prediction (**Figure 2**). According to the model parameters, FLI (OR, 5.119; 95% CI, 2.788–9.799; $p < 0.001$) and LNE (OR, 1.953; 95% CI, 1.086–3.550; $p = 0.026$) were significantly associated with an increased risk of PNI for cervical cancer patients. Age (OR, 1.028; 95% CI, 0.999–1.058; $p = 0.058$) and DSI (OR, 1.639; 95% CI, 0.977–2.742; $p = 0.060$) were also associated with the higher risk of PNI (p values were near the significance threshold of 0.05).

The nomogram was established based on the final logistic regression model (**Figure 3**). The score assignment of the predictor variables is shown in **Table S1**. The nomogram achieved an AUC of 0.763 (95% CI, 0.712–0.815) for the training set and 0.860 (95% CI, 0.758–0.961) for the validation set (**Figures 4A, B**). The performance matrix, including sensitivity, specificity, positive predictive value, negative predictive value, accuracy, F1 scores, and kappa values, of the two sets is shown in **Table 2**. The calibration curves of the model for the two sets (**Figures S1**, **S2**) indicated that the PNI prediction model displayed mean absolute scores of 0.021 for the training set and 0.12 for the validation set, which meaning that the prediction probability of this model is close to the actual probability.

Moreover, we invited experienced pathology specialists on gynecological oncology to review the pathological slides of patients in the validation set. Four patients who had been initially diagnosed as PNI-negative were recognized as PNI-positive, whereas the PNI diagnoses of the other patients were consistent with the original diagnoses. The baseline characteristics of the original and revised validation sets are shown in **Table 3**. After revision, the performance of the model for the validation set markedly improved (**Figure 4C**, **Table 2** and **Figure S3**). The AUC of the revised validation set was 0.915 (95% CI, 0.832–0.998) (**Figure 4C** and **Table 2**). The specificity of the revised validation set (73.3%; 95% CI, 54.1%–87.7%) increased compared with that of the original validation set

TABLE 1 | Baseline characteristics of the individuals in the training set.

		PNI-negative n=162	PNI-positive n=162	*p value*
Age (years)		49 [41.25, 55]	51.5 [45.25, 57]	0.006
FIGO stage (%)	IB1	38 (23.5%)	41 (25.3%)	0.996
	IB2	14 (8.6%)	13 (8.0%)	
	IIA1	60 (37.0%)	59 (36.4%)	
	IIA2	27 (16.7%)	26 (16.0%)	
	IIB	23 (14.2%)	23 (14.2%)	
Adenocarcinoma (%)	No	118 (72.8%)	116 (71.6%)	0.901
	Yes	44 (27.2%)	46 (28.4%)	
Differentiation (%)	Good	6 (3.7%)	8 (4.9%)	0.858
	Moderate	63 (38.9%)	63 (38.9%)	
	Poor	93 (57.4%)	91 (56.2%)	
LVSI (%)	No	111 (68.5%)	108 (66.7%)	0.812
	Yes	51 (31.5%)	54 (33.3%)	
Tumor size (cm)		4.0 [3.0, 4.5]	3.9 [3.0, 4.65]	0.929
LNE (%)	No	134 (82.7%)	105 (64.8%)	<0.001
	Yes	28 (17.3%)	57 (35.2%)	
LUSI (%)	No	136 (84.0%)	124 (76.5%)	0.125
	Yes	26 (16.0%)	38 (23.5%)	
DSI (%)	No	98 (60.5%)	55 (34.0%)	<0.001
	Yes	64 (39.5%)	107 (66.0%)	
FLI (%)	No	145 (89.5%)	89 (54.9%)	<0.001
	Yes	17 (10.5%)	73 (45.1%)	
NACT (%)	No	98 (60.5%)	111 (68.5%)	0.164
	Yes	64 (39.5%)	51 (31.5%)	

Continuous variables are presented as median (interquartile ranges [IQR]) while categorical variables as counts and percentages (%). PNI, perineural invasion; FIGO stage, International Federation of Gynecology and Obstetrics stage; LVSI, lymph vascular space invasion; LNE, lymph node enlargement; LUSI, lower uterine segment invasion; DSI, deep stromal invasion; FLI, full-layer invasion; NACT, neoadjuvant chemotherapy.

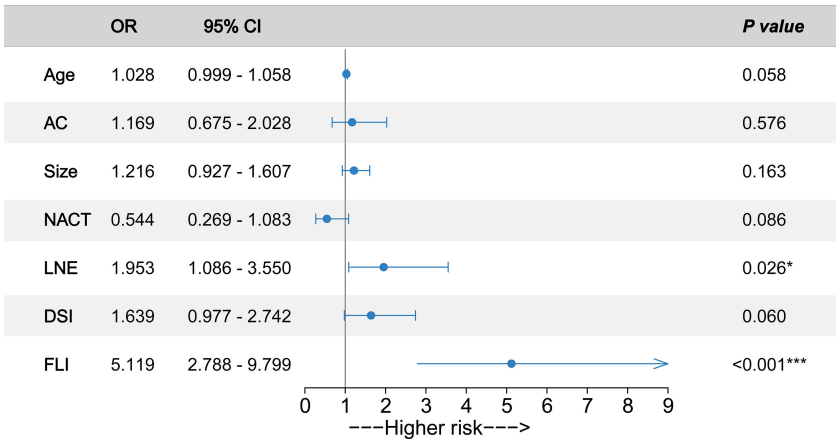

FIGURE 2 | Odds ratios (ORs) of predictors associated with perineural invasion (PNI) occurrence. Forrest plot with ORs and 95% confidence intervals (CIs) according to the multivariate logistic regression analysis. The circles represent the ORs of the predictors. Whiskers represent 95% CI. AC, adenocarcinoma; Size, tumor size; NACT, neoadjuvant chemotherapy; LNE, lymph node enlargement; DSI, deep stromal invasion; FLI, full-layer invasion. ***$p < 0.001$. *$p < 0.05$.

(64.7%; 95% CI, 46.5%–80.3%), and the sensitivity remained 100% after revision (**Table 2**). Additionally, the calibration curve showed better agreement after revision; the mean absolute score improved from 0.12 to 0.095 (**Figure S3**), indicating that the PNI prediction model could help reduce the diagnosis of false-negative PNI for cervical cancer patients. The predicted probability of PNI for PNI-positive patients was significantly greater than that for PNI-negative patients, thereby showing good discriminability (**Figure 5**).

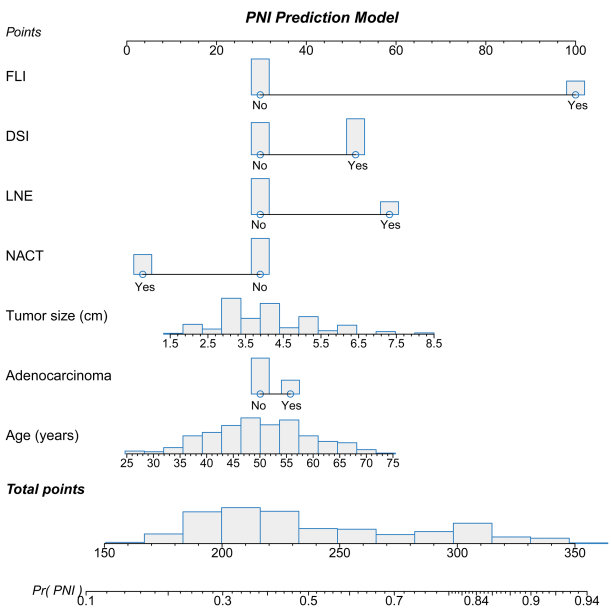

FIGURE 3 | Nomogram used to predict the probability of perineural invasion (PNI) in cervical cancer patients based on multivariate logistic regression. Each variable was given a point on the scale that was correlated with the odds ratio. After summing all points of the variables, we obtained the total point score for each patient. The total point score was used to determine the probability of PNI for cervical cancer patients. The distribution of each variable is presented as a bar graph. The point assignments are presented in **Table S1**.

DISCUSSION

We conducted a large-scale retrospective study in China to explore preoperative clinical and radiological factors associated with PNI in cervical cancer patients and to establish a PNI prediction nomogram for cervical cancer based on a multivariate logistic regression analysis including training and validation sets. Our study expands the literature regarding PNI-associated clinical characteristics and provides a feasible model for the preoperative evaluation of PNI.

In this study, we analyzed ten clinical and radiological factors according to previous researches. Seven were finally included in the final prediction nomogram. Based on the consensus, FLI and DSI indicate more locally invasive cancer (30). During this study, FLI and DSI were important predictors of PNI. Therefore, it is reasonable to hypothesize that the complex interactions among neurogenic molecules, cancer cells, and the cancer microenvironment contribute to the local spread of cancer. Adenocarcinoma, LNE, and tumor size not only were risk factors for cancer progression in cervical cancer (19) but also were associated with the occurrence of PNI in previous studies (20). NACT could kill cancer cells in the body and reduce the detection rate of PNI in later surgical specimens. The inclusion of these factors increased interpretability of the prediction model. Intriguingly, no significant difference in LVSI was found between the PNI-positive and PNI-negative groups (**Table 1**). This provided a glimpse of neural invasion as a potential independent metastasis pathway different from lymphatic metastasis, suggesting that more attention should be focused on PNI during the comprehensive evaluation of cervical cancer.

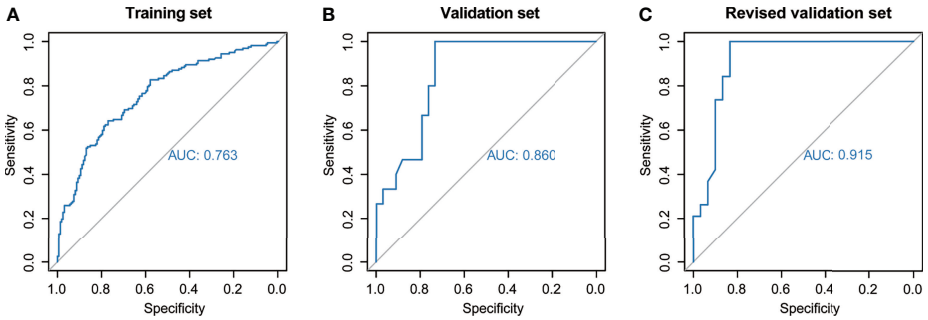

FIGURE 4 | Predictive performance of the model across different sets. The area under the receiver-operating characteristic curve was used to assess perineural invasion prediction using the nomogram for the **(A)** training set, **(B)** validation set, and **(C)** revised validation set.

The preoperative prediction of PNI in cervical cancer has important clinical implications. PNI is a sign of tumor metastasis and invasion (31). PNI in cervical cancer is significantly correlated with high risk and a poorer prognosis (8, 9, 11, 32, 33). A recent study suggested that microenvironment remodulation has an important role in PNI occurrence. Cross-talk among neural cells, supporting cells, and malignant tumor cells gradually leads to changes in and migration of the perineural matrix (31, 34). Therefore, PNI prediction can contribute to blocking cancer progression and improving patient survival (35, 36).

PNI may help optimize preoperative treatment decisions for cervical cancer patients. NSRH has been a treatment choice for patients with early-stage cervical cancer resulting in a higher quality of life than conventional RH. However, the population in which it is applicable remains controversial because of concerns regarding the safety of conserving invaded nerves. The removal of peripheral nerves has been shown to inhibit tumor invasion and metastasis associated with other malignancies. The formation of autonomic nerve fibers in the prostate has been reported to modulate the development and spread of prostate cancer in a mouse model, and the densities of sympathetic and parasympathetic nerve fibers in the tumor and surrounding normal tissues were correlated with adverse clinical outcomes during a retrospective blind analysis of prostate adenocarcinoma samples (37). Surgical denervation and drug denervation can significantly reduce the incidence and progression of tumors in animal models of gastric cancer (38). In a very large series from Europe, the rate of postoperative adjuvant therapy was 48% after radical hysterectomy for early-stage cervical cancer (5), but the adverse prognosis caused by PNI may not be completely eliminated by adjuvant therapy. A systematic review of cervical cancer found that more deaths were observed in the NSRH group than in the RH group (two in the NSRH group vs. zero in the RH group); however, all included patients had received standard postoperative adjuvant therapy (39). Since the presence of PNI was associated with the optimal resection of tumors during NSRH, preoperative PNI prediction might help to identify which populations could obtain maximum benefits from NSRH without compromising oncologic safety.

Recently, some studies have focused on preoperatively predicting PNI. Liu et al. constructed a nomogram including carcinoembryonic antigen levels, tumor size, Lauren classification, radiological stage, and lymph node metastasis to predict the PNI status with advanced gastric cancer (AUC of 0.935 for the internal validation set and AUC of 0.828 for the external validation set) (40). PNI prediction models with clinical factors have also been reported for colorectal cancer, head and neck squamous cancer, oral cancer, and pancreatic cancer (41–45). These findings suggest that using clinical pathological features to build a PNI prediction model is feasible. However, few researchers have investigated the prediction of PNI in

TABLE 2 | Performance of the nomogram in predicting PNI in different groups.

	Training Set		Validation Set		Revised Validation Set	
	Value	**95% CI**	**Value**	**95% CI**	**Value**	**95% CI**
AUC	0.763	0.712 - 0.815	0.860	0.758 - 0.961	0.915	0.832 - 0.998
Sensitivity	59.9%	51.9% - 67.5%	100%	78.2% - 100%	100%	82.4% - 100%
Specificity	79.6%	72.6% - 85.5%	64.7%	46.5% - 80.3%	73.3%	54.1% - 87.7%
PPV	74.6%	67.9% - 80.3%	55.6%	44.2% - 66.3%	70.4%	56.8% - 81.1%
NPV	66.5%	61.8% - 70.9%	100%	NA	100%	NA
Accuracy	69.8%	64.4% - 74.7%	75.5%	61.1% - 86.7%	83.7%	70.3% - 92.7%
F1	0.664		0.714		0.826	
Kappa	0.395		0.529		0.681	

AUC, area under the receiver operating characteristics curve; PPV, positive predictive value; NPV, negative predictive value; 95% CI, 95% confidence interval; NA, not available.

TABLE 3 | The baseline characteristics of the original and revised validation sets.

		Validation Set			Revised Validation Set		
		PNI-Negative n=34	**PNI-Positive n=15**	***p value***	**PNI-Negative n=30**	**PNI-Positive n=19**	***p value***
Age (years)		53 [46.5, 59.5]	51 [41, 62.5]	0.983	52.5 [46, 58]	53 [44, 62]	0.572
FIGO stage (%)	IB1	18 (52.9)	6 (40.0)	0.599	18 (60.0%)	6 (31.6%)	0.100
	IB2	16 (47.1)	9 (60.0)		12 (40.0%)	13 (68.4%)	
	IIA1	0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	
	IIA2	0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	
	IIB	0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	
Adenocarcinoma (%)	No	23 (67.6)	10 (66.7)	1.000	19 (63.3%)	14 (73.7%)	0.660
	Yes	11 (32.4)	5 (33.3)		11 (36.7%)	5 (26.3%)	
Tumor size (cm)		3.5 [2.55, 4.5]	4.0 [3.5, 4.75]	0.256	3.5 [2.5, 4.42]	4.2 [3.5, 5.0]	0.041
LNE (%)	No	29 (85.3)	3 (20.0)	<0.001	25 (83.3%)	7 (36.8%)	0.003
	Yes	5 (14.7)	12 (80.0)		5 (16.7%)	12 (63.2%)	
LUSI (%)	No	29 (85.3)	4 (26.7)	<0.001	26 (86.7%)	7 (36.8%)	0.001
	Yes	5 (14.7)	11 (73.3)		4 (13.3%)	12 (63.2%)	
DSI (%)	No	29 (85.3)	1 (6.7)	<0.001	26 (86.7%)	4 (21.1%)	<0.001
	Yes	5 (14.7)	14 (93.3)		4 (13.3%)	15 (78.9%)	
FLI (%)	No	26 (76.5)	7 (46.7)	0.085	26 (86.7)	7 (36.8)	0.001
	Yes	8 (23.5)	8 (53.3)		4 (13.3)	12 (63.2)	
NACT (%)	No	34 (100.0)	15 (100.0)	1.000	30 (100.0%)	19 (100.0%)	1.000
	Yes	0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	

Continuous variables are presented as median [interquartile ranges (IQR)] while categorical variables as counts and percentages (%). PNI, perineural invasion; FIGO stage, International Federation of Gynecology and Obstetrics stage; LNE, lymph node enlargement; LUSI, lower uterine segment invasion; DSI, deep stromal invasion; FLI, full-layer invasion; NACT, neoadjuvant chemotherapy.

cervical cancer. During this study, we built an effective PNI prediction nomogram for cervical cancer based on preoperative clinical and radiological factors. The AUC, sensitivity, and specificity were 0.763, 59.9%, and 79.6%, respectively, for the training set and 0.915, 100%, and 73.3%, respectively, for the revised validation set, thereby indicating its satisfactory prediction performance. We found that this prediction model could help identify patients with false-negative PNI, which is valuable to improving the diagnosis rate of PNI and helping unexperienced pathologists at smaller hospitals.

The two prominent strengths of this study are the large volume of PNI-positive cervical cancer patients and the comparable

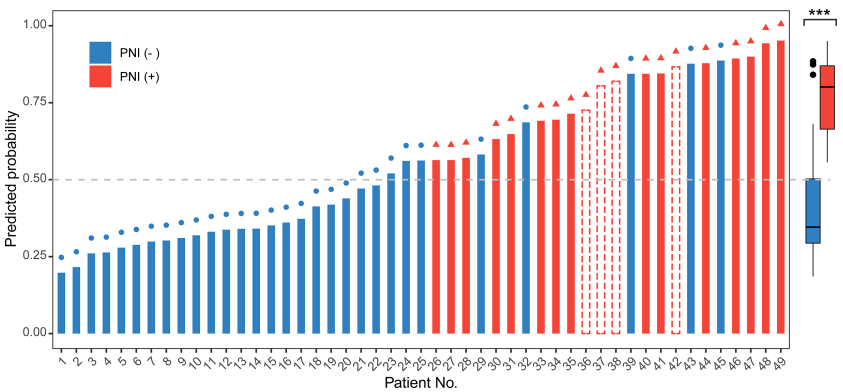

FIGURE 5 | Use of the nomogram to predict the probability of perineural invasion (PNI) occurrence for all 49 patients in the validation set. The predicted probability of PNI more than 0.5 (gray dashed line) was regarded as PNI-positive. In the left graph, the color of the bar represents the real status of PNI according to the pathological examination. The red bar represents PNI-positive, the blue bar represents PNI-negative, and the bar with the red dashed border represents PNI-positive patients who were misdiagnosed as PNI-negative before revision but were correctly predicted to be PNI-positive by the nomogram. The symbols on the top of each bar indicate the final pathological diagnosis of the PNI status after revision. A red triangle at the end of a line indicates that the patient had PNI. A blue circle indicates that the patient did not have PNI. The right box plot shows the distribution of the predicted probability of PNI for PNI-positive and PNI-negative patients included in the revised validation set. The center line represents the median probability of PNI in the different groups. Box limits represent the upper and lower quartiles. Whiskers represent a 1.5-times interquartile range. The black points represent the outliers. The Wilcoxon test was performed for the univariate comparison between groups. A two-tailed *p*-value of <0.05 was considered statistically significant. ***$p < 0.001$.

population with a different PNI status after propensity score matching, which allowed for a comprehensive analysis of multiple clinical and radiological factors. However, our study has some limitations. First, it is a single-center retrospective study; therefore, only variables already captured could be used for analysis. Second, we did not adjust for all possible confounders. Lastly, the generalizability of the nomogram is limited to the size of our external validation set. Larger-scale, multicenter investigations should be performed at different hospitals and in different regions to verify the findings of this study before our nomogram can be applied in practice.

CONCLUSIONS

This study explored factors correlated with the occurrence of PNI in cervical cancer. We constructed a feasible nomogram to predict PNI occurrence. This nomogram has the potential to assist clinicians when evaluating the PNI status and reduce the misdiagnosis of PNI preoperatively, thus optimizing treatment decisions for cervical cancer patients.

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 human participants were reviewed and approved by Ethics Committee and Institutional Review Board of the Sun Yat-sen University Cancer Center (Guangzhou, China). The ethics committee waived the requirement of written informed consent for participation.

AUTHOR CONTRIBUTIONS

JL and TW designed the study. TW and GC performed the analysis, interpreted the data, and wrote the paper. SG collected patient samples and clinical data. YF and HH helped analyze the data. JL advised on the conception and design of the study. All authors vouch for the respective data and analysis, approved the final version, and agreed to publish the manuscript.

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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.2021.774459/full#supplementary-material

Supplementary Figure 1 | Calibration curves of the model for the training set.

Supplementary Figure 2 | Calibration curves of the model for the validation set.

Supplementary Figure 3 | Calibration curves of the model for the revised validation set.

Supplementary Table 1 | The point assignments of the nomogram.

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Predicting 3D Structure, Cross Talks, and Prognostic Significance of *KLF9* in Cervical Cancer

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Our study aimed to identify the new blood-based biomarkers for the diagnosis and prognosis of cervical cancer. Moreover, the three-dimensional (3D) structure of Kruppel-like factor 9 (*KLF9*) was also determined in order to better understand its function, and a signaling pathway was constructed to identity its upstream and downstream targets. In the current study, the co-expressions of tumor protein D52 (*TPD52*), *KLF9*, microRNA 223 (miR-223), and protein kinase C epsilon (*PKCϵ*) were evaluated in cervical cancer patients and a possible relation with disease outcome was revealed. The expressions of *TPD52*, *KLF9*, miR-223, and *PKCϵ* were studied in the blood of 100 cervical cancer patients and 100 healthy controls using real-time PCR. The 3D structure of *KLF9* was determined through homology modeling *via* the SWISS-MODEL and assessed using the Ramachandran plot. The predicted 3D structure of *KLF9* had a similarity index of 62% with its template (*KLF4*) with no bad bonds in it. In order to construct a genetic pathway, depicting the crosstalk between understudied genes, STRING analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG), and DAVID software were used. The constructed genetic pathway showed that all the understudied genes are linked to each other and involved in the PI3K/Akt signaling pathway. There was a 23-fold increase in *TPD52* expression, a 2-fold increase in miR-223 expression, a 0.14-fold decrease in *KLF9* expression, and a 0.05-fold decrease of *PKCϵ* expression in cervical cancer. In the present study, we observed an association of the expressions of *TPD52*, *KLF9*, miR-223, and *PKCϵ* with tumor stage, metastasis, and treatment status of cervical cancer patients. Elevated expressions of *TPD52* and miR-223 and reduced expressions of *KLF9* and *PKCϵ* in peripheral blood of cervical cancer patients may serve as predictors of disease diagnosis and prognosis. Nevertheless, further *in vitro* and tissue-level studies are required to strengthen their role as potential diagnostic and prognostic biomarkers.

Keywords: cervical cancer, microRNA 223, *PKCϵ*, PI3K/Akt signaling pathway, Ramachandran plots, *KLF9*

INTRODUCTION

Cervical cancer arises from the cervix in women. It is the fourth most prevalent and the fourth most frequent cause of cancer mortality, with approximately 604,000 new cases and 342,000 causalities all over the world in 2020 (1). Various studies have confirmed the association between genital human papillomavirus (HPV) and cervical cancer. Sexual contact is the key risk factor associated with HPV acquisition. HPV has been recommended as solely the "necessary cause" of cervical cancer (2). Pap smear has been the most widely used cervical cytology screening technique for the past 50 years. However, the Pap smear is far from perfect, and its foremost shortcoming is the possibility of a false-negative result (3). No significant improvements in the Pap test have been made, due to which false-negative results that arise from the Pap test are continuously being reported even now. Laboratory misinterpretations, preparation errors, and improper sampling are the main causes of erroneous negative results (4). Although the basic treatment for cervical cancer is surgery or chemoradiation therapy, patients with advanced-stage tumor have poor disease prognosis with severe side effects. Hence, substitute screening approaches are required in underdeveloped and developing countries (5).

It has been reported that *KRAS* and phosphoinositide 3-kinases, upon activation *via* different receptors, e.g., G protein-coupled receptors (GPCRs) and receptor tyrosine kinase (RTK), cause the activation of the major downstream signaling pathways. Various studies have confirmed the interactions of Kruppel-like factor 9 (*KLF9*), protein kinase C epsilon (*PKCϵ*), tumor protein D52 (*TPD52*), and microRNA 223 (miR-223) with the downstream components of these signaling pathways, which eventually lead to carcinogenesis (6–9).

TPD52 (CR542034.1) is situated at the 8q21 chromosome, on an area that is commonly amplified in numerous cancers particularly in humans (10). The primary evidence of the importance of an altered expression of *TPD52* in various cancers was obtained from the position of this gene on chromosome 8q, and during the mid-1990s, it became widely understood that the expression of *TPD52* increases in certain tumor types, as well as in *MYC* oncogene. Nevertheless, the role of *TPD52* in the onset of cancer is still debatable (11). The expression of *TPD52* is upregulated in certain types of cancers, such as breast, prostate, ovarian, and pancreatic cancer, Burkitt's lymphoma, multiple myeloma, and melanoma (12). On the other hand, the expression of *TPD52* is also downregulated in other cancer types such as leiomyosarcoma, papillary renal cell cancer, clear cell renal cell cancer, lung cancer, and liposarcoma. Due to its altered expression in various cancers, it is referred to as a controversial gene (13). Several studies have reported evidence of the role of *TPD52* in various signaling pathways of cancers, i.e., in the PI3K/Akt signaling pathway (14), protein kinase B/Akt signaling pathway (15), and nuclear factor-κB transactivation (16).

KLF9 (NM_001206.4) is a regulator of transcription in cellular adhesion, differentiation, and proliferation in the endometrium (17). Irregular expression of *KLF9* may contribute toward the onset of several carcinomas and their proliferation (18). *KLF9* is known to interact substantially with the Akt pathway. One of the studies validated the involvement of *KLF9* in the Akt pathway and indicated that *KLF9* substantially inhibits AKT activation and abrogates tumor growth in prostate cancer (19, 20).

PKCϵ (NM_005400.3) is one of the members of the protein kinase C family. Out of 10 isoforms of serine/threonine kinases, PKCϵ is the most widely studied for its contribution to malignant transformation (21). A recent study has revealed the interaction of PKCϵ with Akt, suggesting that the downregulation of *PKCϵ* causes the inhibition of Akt in breast cancer cells, thus increasing drug efficacy in breast cancer patients (22). The overexpression of *PKCϵ* has been reported in a wide range of carcinomas, including breast cancer, lung cancer, prostate cancer (23, 24), and brain tumors (25).

Similarly, recent studies have suggested the reduced expression of miR-223 (NC_000023.11) in metastatic and end-stage osteosarcoma patients, indicating the inhibitory role of miR-223 in osteosarcoma. An increased expression of miR-223 revokes atherosclerosis advancement by activating the PI3K/AKT pathway through blockade of *TLR4* signaling. Its dysregulation is also associated with aberrant Akt/mTor pathway in various diseases such as myocardial infarction (26), colorectal cancer (27), and pancreatic cancer (28).

Kruppel-like factor (KLF) proteins have been found in diverse species and are known to have evolved by gene duplication (29, 30). However, the structures of all KLFs, except that of *KLF4* (PDB ID: 2BWU), remain unpredicted. The prediction of the first ever structure of *KLF4* provided new insights toward a better understanding of the molecular basis and functional anatomy of *KLF4* and the other members of the KLF family (31) The three-dimensional (3D) structure of proteins helps in understanding their functions and their interactions with their binding partners (32). Our study describes the approaches to identify and determine the conserved domains and 3D structure of *KLF9* and the development of a genetic pathway, thus establishing a crosstalk between *KLF9* and its upstream and downstream targets. Additionally, although the individual expression status of *TPD52*, *KLF9*, miR-223, and *PKCϵ* has been previously studied in various tumors, no study has investigated the co-expressions of *TPD52*, *KLF9*, miR-223, and *PKCϵ* in any cancer type. Hence, our study also aimed to identify the combined expression patterns of *TPD52*, *KLF9*, miR-223, and *PKCϵ*, and their relationship with clinicopathological features, and to investigate the diagnostic and prognostic value of these genes in cervical cancer patients.

METHODS

Blood Sample Collection

Blood samples were collected only from those patients who gave approval to collect their blood voluntarily in Combined Military Hospital (CMH), Rawalpindi, after approval by the Ethical Committee of Combined Military Hospital and ASAB, National University of Science and Technology, Islamabad, Pakistan. All participants were informed about the study objectives and signed the informed consent. The study

protocol was carried out in accordance with the principles of the Declaration of Helsinki (33).

Blood samples were collected from female patients with histologically confirmed diagnosis of localized and/or metastasized carcinoma of the cervix (n = 100) and currently were on chemotherapy, radiotherapy, or chemoradiotherapy. Patients with co-infection of HIV were excluded from our study. The median age of cervical cancer patients was 47.5 years (range, 35–60 years). Furthermore, a control group was also included in the present study, which comprised blood samples from healthy individuals (n = 100), for accurate interpretion of the results.

RNA Extraction and cDNA Synthesis

RNA was extracted from whole blood drawn from peripheral veins of cancer patients using the TriZol reagent (Thermo Fischer Scientific, Waltham, MA, USA). The reaction was conducted on ice to avoid RNA degradation. For cDNA synthesis, 20 µl of the reaction mixture was prepared by adding 1 µl of Oligo dT20 [Random Hexamer, 1 µl dNTP mix (2.5 mM)], <5 µg of RNA, and RNAse-free water up to 10 µl. The reaction mixture was incubated at 65°C in a thermocycler for 5 min. In the next step, 10× reaction buffer (2 µl), 100 mM DTT (1 µl), RNase inhibitor (0.5 µl), and RTase (1 µl) were added into the PCR tube (same) and placed in a thermocycler for 50 min at 42°C and for 10 min at 70°C. The synthesized cDNA was stored at −20°C.

Real-Time PCR

For analysis of the expression of the candidate gene and microRNA (miRNA), real-time PCR was used. Real-time reaction mixture was made by adding 10 µl of Wiz pure qPCR master mix (SYBR), 6 mM of forward and reverse primers, and 10 µg of cDNA with RNAse-free water up to a volume of 20 µl. The conditions for quantitative PCR (qPCR) amplification were 40 cycles with an initial temperature of 95°C for 10 min, which basically activated Hot Start DNA polymerase, followed by 95°C for 15 s and then amplification for 1 min for 61°C, followed by real-time analysis for 45 s at 75°C. The primer sequences and the GC (guanine–cytosine) content are presented in **Table 1**. The specificity of primers was confirmed by observing the melt curve analysis of qPCR. The reagent and software used for real-time PCR were SYBR Green dye and 7300 SDS software, respectively.

For quantifying the gene expression, the $2^{-\Delta\Delta CT}$ method was performed. Moreover, the Livak method was used for conversion of the cycle threshold (C_t) values, obtained for real-time PCR, into fold change. β-actin was used as a control, and the experiment was performed in triplicate. The C_t values obtained in triplicate for each sample was found to be almost the same, hence confirming the validity of the results.

Statistical Analysis

Statistical analysis was performed with one-way and two-way ANOVA in order to show the relationship of the expressions of *TPD52*, *KLF9*, miR-223, and *PKCε* with the different clinicopathological features of cervical cancer. Spearman's rho correlation was used to test the association of age and the stage of the disease. All these statistical tests were performed using GraphPad Prism 6.0 software. Similarly, GraphPad prism was employed for generating the receiver operating characteristic (ROC) curve.

Kruppel-Like Factor 9: Three-Dimensional Structure Prediction

The 3D structure of KLF9 protein (NP_001197.1) was determined through homology modeling *via* SWISS-MODEL, a bioinformatics web server. For prediction of the 3D structure of KLF9, the first amino acid sequence of the *KLF9* gene was retrieved from the National Center for Biotechnology Information (NCBI) in FASTA format. In order to find the conserved domains and the evolutionary relationships between all the 17 members of the KLF family, multiple sequence alignment was done using Clustal Omega. For a better understanding of the evolutionary histories and conservation of the different members of the KLF family, phylogenetic analysis was performed using MEGA 7. The secondary structure of KLF9 was predicted *via* different servers, i.e., UCL Bioinformatics Group (34), SPIDER2 (35), and Predict Protein (36). For 3D structure predictions, *KLF4* was chosen as a template due to the fact that its structure has already been crystallographically predicted in RCSB-PDB (Research Collaboratory for Structural Bioinformatics Protein Data Bank). Hence, the structure of *KLF4* (PDB ID: 2BWU) was taken from RCSB-PDB. After acquistion of the template (*KLF4*) structure, the sequence of *KLF9* in FASTA format was aligned to the crystallographically determined structure of *KLF4 via* the SWISS-MODEL and a 3D model of *KLF9* was generated.

TABLE 1 | Sequences and parameters of primer used for qPCR.

Name	Sequence	GC content (%)	Annealing temperature (°C)
KLF9 forward	5′-TGGCTGTGGGAAAGTCTATGG-3′	52.4	60
KLF9 reverse	5′-CTCGTCTGAGCGGGAGAACT-3′	60	60
TPD52 forward	5′-GCTGCTTTTTCGTCTGTTGGCT-3′	50	60
TPD52 reverse	3′-TCAAATGATTTAAAAGTTGGGGAGTT	30	60
miR223 forward	5′-AGCCGTGTCAGTTTGTCAAAT-3′	42.9	60
miR-223 reverse	5′-GTGCAGGGTCCGAGG TC-3′	70.6	60
PKCε forward	5′-AGCCTCGTTCACGGTTCT-3′	55.6	60
PKCε reverse	5′-TGTCCAGCCATCATCTCG-3′	55.6	60

Pathway Construction

In order to construct a genetic pathway depicting the crosstalk between understudied genes, the Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used and STRING analysis was performed to study the gene linkage, while the genetic pathway was obtained *via* DAVID software.

RESULTS

Kruppel-Like Factor 9: Three-Dimensional Structure Prediction

Multiple Sequence Alignment

The results of the multiple sequence alignment of *KLF9* with the rest of the members of the KLF family *via* Clustal Omega (37) depicted the conserved domains across all KLF family members. **Figure 1** depicts the results of multiple sequence alignment using Clustal Omega. Three tandem C_2H_2 zinc finger domains, 1, 2, and 3, were found to be conserved throughout the members of the KLF family.

Phylogenetic Tree Construction

Phylogenetic analysis of the KLFs performed by MEGA 7 (38) using the UPGMA (unweighted pair group method with arithmetic mean) phylogenetic tree placed *KLF9* in group 3 based on its transcription repression activity (**Figure 2**). Like in earlier studies, the KLF family members were divided into three groups based on their evolutionary histories, structural characteristics, and binding domains, which help define their functions. Group 1 includes *KLF3*, *KLF8*, and *KLF12*. These members serve as repressors of transcription by mediating interactions with the co-repressors Sin3A and CtBP. Group 2 includes *KLF1*, *KLF2*, and *KLF4–KLF7*. These members act as activators of transcription. Group 3 includes *KLF9–KLF11*, *KLF13*, *KLF14*, and *KLF16*. These members serve as repressors of transcription by mediating interactions with the co-repressors Sin3A and CtBP (41).

Functional Binding Domains

Each member of KLF family, despite having highly conserved consensus sequences at the C-terminal region, has unique functions involved in cellular processes. This is due to great variations in sequences at the N-terminus region of KLFs that mediate interactions with diverse activators and repressors of transcription. The KLF sequences contain conserved motifs, at the N-terminus, comprising CtBP and Sin3A binding sites (41). Co-repressor C-terminal binding protein (CtBP) is a co-repressor of transcription. The main mechanism by which CtBP proteins

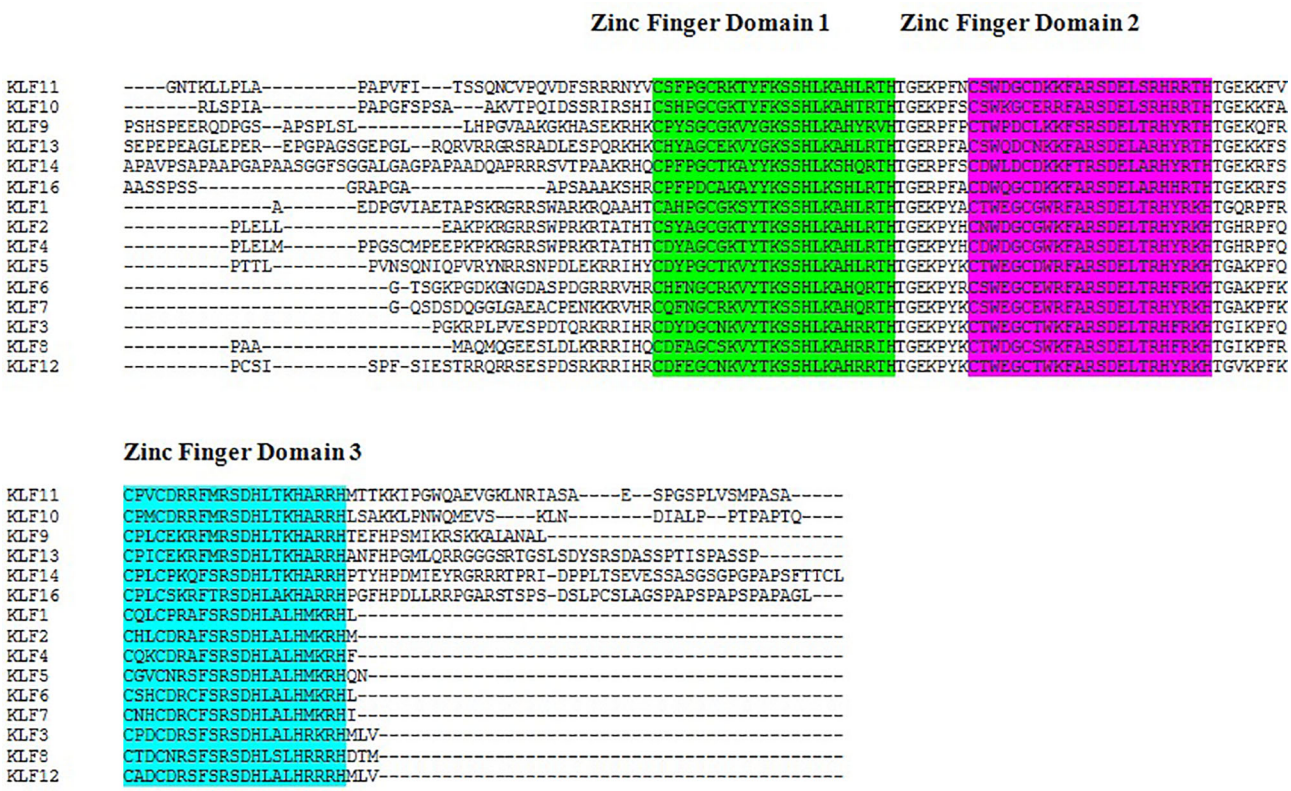

FIGURE 1 | Sequence alignment of Kruppel-like factors depicting the conserved domains obtained from Clustal Omega. Certain sequence alignments have been deleted for formatting. Zinc figure domain 1 (labeled *green*), zinc figure domain 2 (labeled *purple*), and zinc figure domain 3 (labeled *blue*) are highlighted.

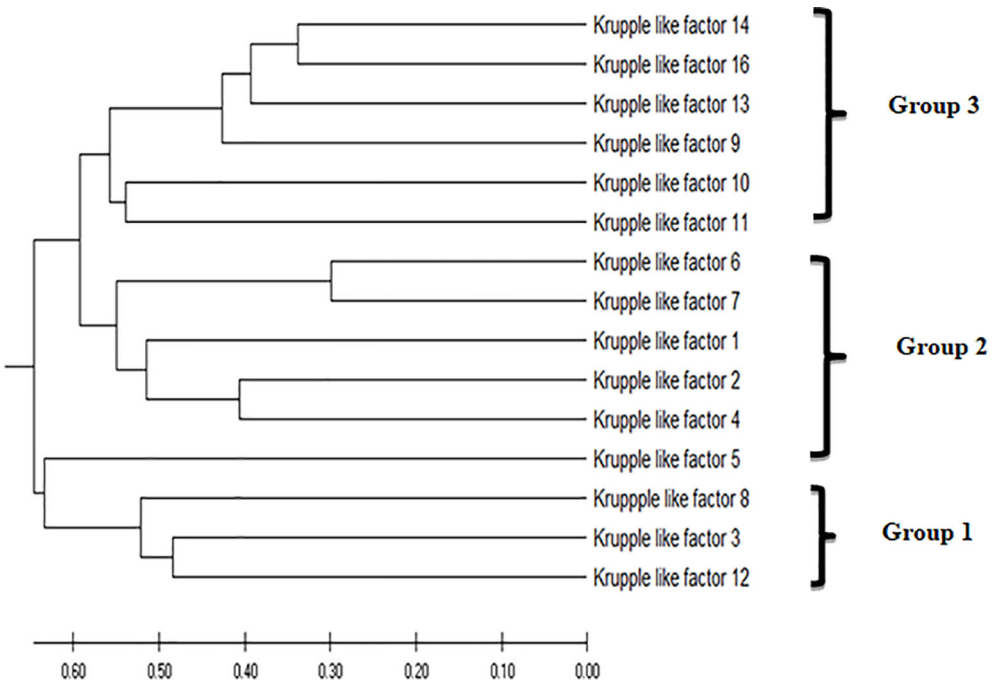

FIGURE 2 | The evolutionary history was inferred using the unweighted pair group method with arithmetic mean (UPGMA). The optimal tree with the sum of branch length = 7.54632578 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method (39) and are in the units of the number of amino acid substitutions per site. This analysis involved 15 amino acid sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 602 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (40).

suppress transcription is by recruiting histone methyl transferases and histone deacetylases (HDACs) to transcriptional complexes, which causes chromatin compaction and transcriptional silencing by the methylation and deacetylation of proteins, respectively (42, 43). *KLF3*, *KLF5*, *KLF8*, and *KLF12* contain the conserved motif CtBP binding site. *KLF3*, *KLF8*, and *KLF12* contain the conserved sequence PXDLS that mediates the interaction between KLFs and CtBP. This interaction facilitates the functions of *KLF3* and *KLF8* in co-repression and the activity of *KLF12* in repressing *AP-2α* gene expression (44). Sin3A is a protein that functions as a repressor of transcription. It is involved in the recruitment and binding of HDACs (45). *KLF9*, *KLF10*, *KLF11*, *KLF13*, *KLF14*, and *KLF16* possess binding sites for Sin3A. These KLFs possess the R1 domain that enclose a Sin3-interacting domain (SID), an α-helical hydrophobic structure that meditates binding with the PAH domain of Sin3 proteins (46). It was found that *KLF9*, *KLF10*, *KLF11*, *KLF13*, *KLF14*, and *KLF16* possess a conserved α-helical motif in their structure, i.e., AA/VXXL, a binding site for Sin3A that facilitates interaction with Sin3A and causes transcriptional repression (47). Unexpectedly, *KLF1* possesses no SID, but still interacts with Sin3A and acts as a co-repressor (48).

Sin3A Binding Site in *KLF9*

KLF9 contains the conserved hydrophobic motif AAQCL in its amino acid sequence, as shown in **Figure 3**. It serves as a SID and is able to recruit and bind Sin3A. Sin3A proteins bind HDAC1, HDAC2, and other proteins, probably assembling multi-unit complexes (HDAC1 and HDAC2), altering chromatin compaction and so repressing transcription. A number of studies have justified the presence of such conserved motifs in *KLF9* (49).

Subcellular Localization

Subcellular localization of *KLF9* was found to be inside the nucleus (**Figure 4**). By modeling the functional domain features and the hidden associations of gene ontology, different servers gave different nuclear signals. Hum-mPLoc 3.0 showed a nuclear signal of 1.88, while DeepLoc-1.0 showed a nuclear signal of 0.99.

3D Structure Visualization and Assessment of KLF9 Protein

The similarity index between the structures of the template (KLF4) and target (KLF9) was found to be 62%. The 3D structure of KLF9 is shown in **Figure 5A**. Using Chimera, the structure of KLF9 obtained *via* the SWISS-MODEL was superimposed on KLF4 (template) for the anlysis of structural conservation between the target (KLF9) and template (KLF4). The template is labeled red, while target is labeled blue. **Figure 5B** illustrates the superimposed structures of the template (KLF4) and target (KLF9) proteins. Ramachandran plots were used to analyze the quality of the model obtained

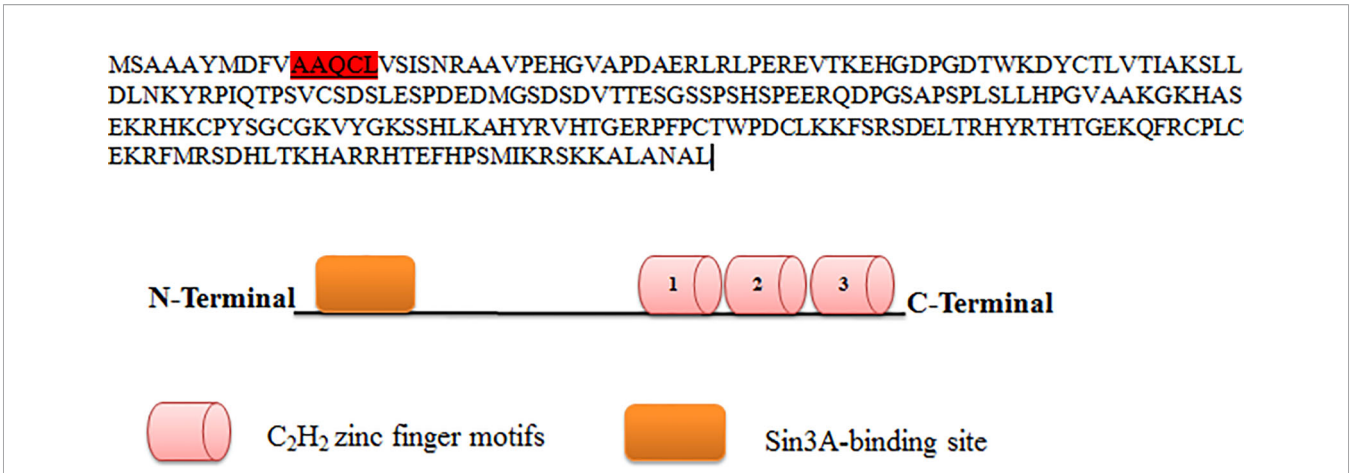

FIGURE 3 | Protein sequence of *KLF9*. *KLF9* is highly homologous to other members of the Kruppel-like factor (KLF) family at carboxy-terminal DNA-binding regions, which contain three C_2H_2 zinc finger motifs. At the N-terminal region is the Sin3A binding region.

via the SWISS-MODEL. These plots were used for visualization of the dihedral angles, i.e., phi (φ) and psi (ψ) angles of the amino acids. It was found that most of the amino acids were found to be lying in favorable regions, i.e., 95.06%, and Ramachadran outliers were 1.23% (A146 PRO). Bad bonds in the structure were 0/721, while bad angles were 16/965. **Figure 5C** illutrates the Ramachadran plot.

Expressions of *TPD52, KLF9*, miR-223, and *PKCϵ* in Blood of Cervical Cancer Patients

In this study, we observed a significantly increased expression of *TPD52* (23.8 ± 0.42) in understudied samples of cervical cancer compared to the controls. The expression of *KLF9* was found to be downregulated in the blood of cervical cancer patients (0.14 ± 1.6) relative to healthy controls. There was an elevation of miR-223 expression in cervical cancer patients (2.0 ± 1.8) relative to controls. In the case of *PKCϵ*, its expression was found to be significantly reduced in cervical cancer patients (0.05 ± 5.7). Overall, we found that the expressions of *TPD52* and miR-223 were increased 23- and 2-fold in peripheral blood of cervical cancer patients, respectively, whereas expressions of *KLF9* and *PKCϵ* were 0.14- and 0.05-fold reduced in cervical cancer patients relative to healthy individuals (**Figure 6**).

Relative Expressions of TPD52, KLF9, miR-223, and PKCϵ With Clinical Features in Cervical Cancer

The clinicopathological features of cervical cancer patients are shown in **Table 2**. The relative expressions of *TPD52*, *KLF9*, miR-223, and *PKCϵ* in cervical cancer patients were measured with respect to their clinical features. The fold change and expression status of *TPD52*, *KLF9*, miR-223, and *PKCϵ* for each clinicopathological feature, i.e., low tumor stage groups I–II and advanced tumor stage groups III–IV, distant metastatic *vs.* non-metastatic group, and treatment status of patients (e.g., chemotherapy, radiotherapy, or chemoradiotherapy), are shown in **Table 3**. Significant results ($p < 0.001$) were found between all groups of patients. The expression of *TPD52* was found to be significantly higher in the lower tumor stage and non-metastatic groups of patients in comparison to its high expression in the advanced tumor stage and distant metastatic groups of patients (**Figures 7A, B**). A similar trend was found for miR-223 (**Figures 7E, F**). In the case of *KLF9*, its expression was much more significantly reduced in the advanced tumor stage and distant metastatic groups relative to its less reduced expression in the lower tumor stage and non-metastatic groups (**Figures 7C, D**). On the other hand, for *PKCϵ*, its expression was much more significantly reduced in the lower tumor stage and non-metastatic groups relative to its less reduced expression in the advanced tumor stage and distant metastatic groups (**Figures 7G, H**).

We also found that the expression of *TPD52* was lowest in patients undergoing chemoradiotherapy relative to its higher expression in patients receiving a combination of chemotherapy and radiotherapy (**Figure 8A**). A similar trend was followed in the expression profile of miR-223 (**Figure 8C**), whereas for *KLF9* and *PKCϵ*, patients undergoing chemoradiotherapy showed higher expressions relative to patients on chemotherapy and radiotherapy, where their expressions were significantly reduced (**Figures 8B, D**). However, it is to be noted that the expressions of *TPD52* and miR-223 were higher relative to healthy controls and that the expressions of *KLF9* and *PKCϵ* were lower in comparison to healthy controls in each group of patients.

Specificity of *TPD52*, *KLF9*, miR-223, and *PKCϵ* for Cervical Cancer Diagnosis

For verification of the relationship between these blood-based biomarkers (*TPD52*, *KLF9*, miR-223, and *PKCϵ*) and cervical cancer, ROC curves were generated (**Figure 9**). The area under the ROC curve (AUC) was calculated and 95% confidence intervals were determined.

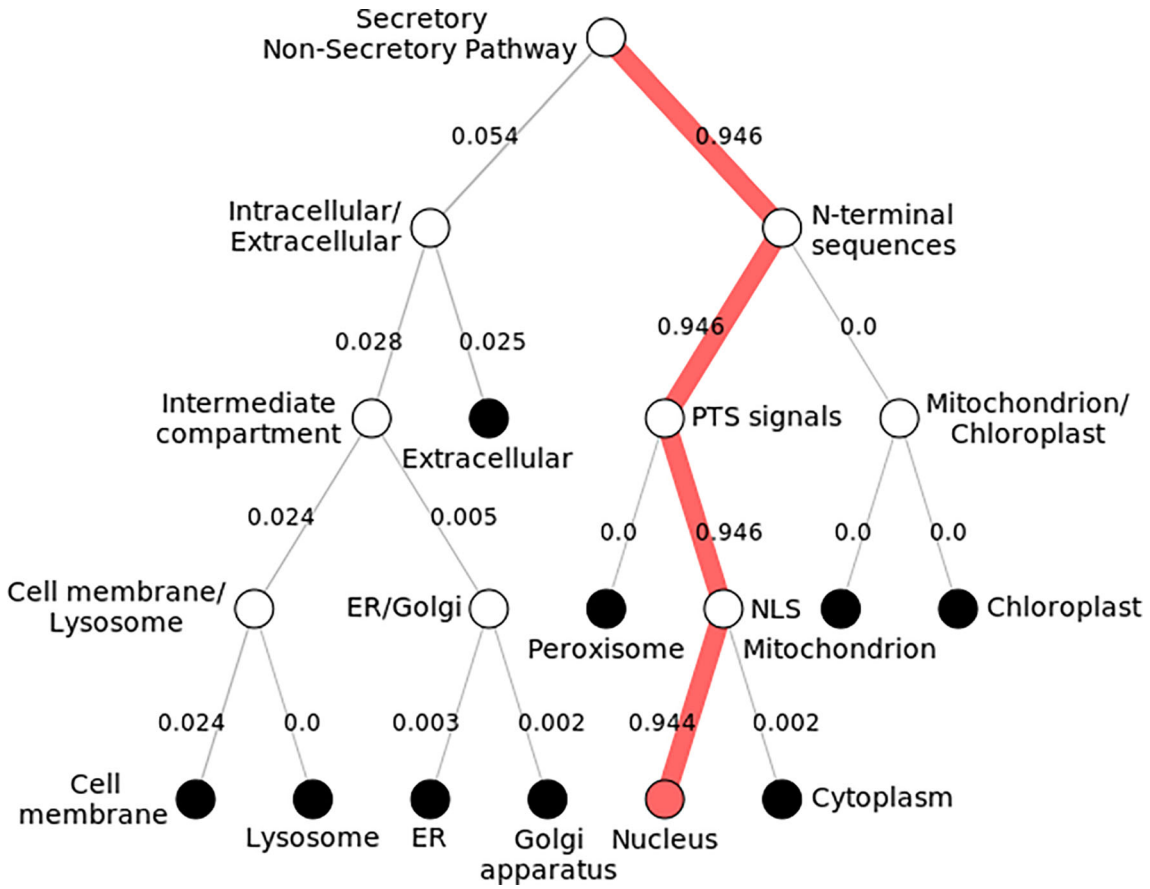

FIGURE 4 | Subcellular localization of KLF9. Pathway following subcellular localization of KLF9 generated by DeepLoc-1.0. Numerous locations are shown, and each follows a distinct pathway and score. The KLF9 protein is localized inside the nucleus (depicted by 0.9 score). It directs toward the nucleus by executing peroxisomal targeting signals (PTS) and nuclear localization signals (NLS).

Association Between Patient Age and Cancer Stage

The association between patient's age and cancer stage is shown in **Figure 10**. Participants diagnosed with stage IV were significant older than those in the early stages. Furthermore, age showed a significant positive correlation with stage (r = 0.503, $p < 0.001$).

DISCUSSION

Tumors arising in the genital tract of females were found to be the fourth most frequent set of malignancies among females. The absence of screening methods, diagnostic techniques, and treatment and the lack of proper knowledge are the leading causes of cervical cancer incidences (50). The late diagnosis of the illness results in increased mortality rates (51). Although various screening techniques are being used for the diagnosis of cervical cancer, the death rates in developing states continue to be high, i.e., 87%. Pap smear is currently used for screening cervical neoplasia at an early stage. However, the false-negative results that are often produced by the Pap test is one of its major drawbacks (50). Hence, discovering the biological and molecular mechanisms of tumor progression and identifying diagnostic biomarkers have become essential in cancer research studies.

With improvements in technology, there has been a significant increase in the structure determination of numerous proteins. Still, the prediction of protein structures remains a challenging task. However, certain theoretical models can be used to assess the topological characteristics of proteins. The 3D structure of protein helps in understanding their functions and their interactions with their binding partners. Homology modeling can help in predicting low-resolution structures. Hence, in this study, the 3D structure of *KLF9* was predicted *via* the SWISS-MODEL Workspace. The template used for 3D structure predictions was *KLF4*. The server used for the visualization of the 3D structures was Chimera. The similarity index between the structure of a template (*KLF4*) and a target (*KLF9*) was known to be 62%, and no bad bonds were found in the predicted structure. This study also predicted the possible crosstalk of *KLF9* with *TPD52*, miR-223, and *PKCε*. KEGG and STRING were used to determine gene linkage with neighboring

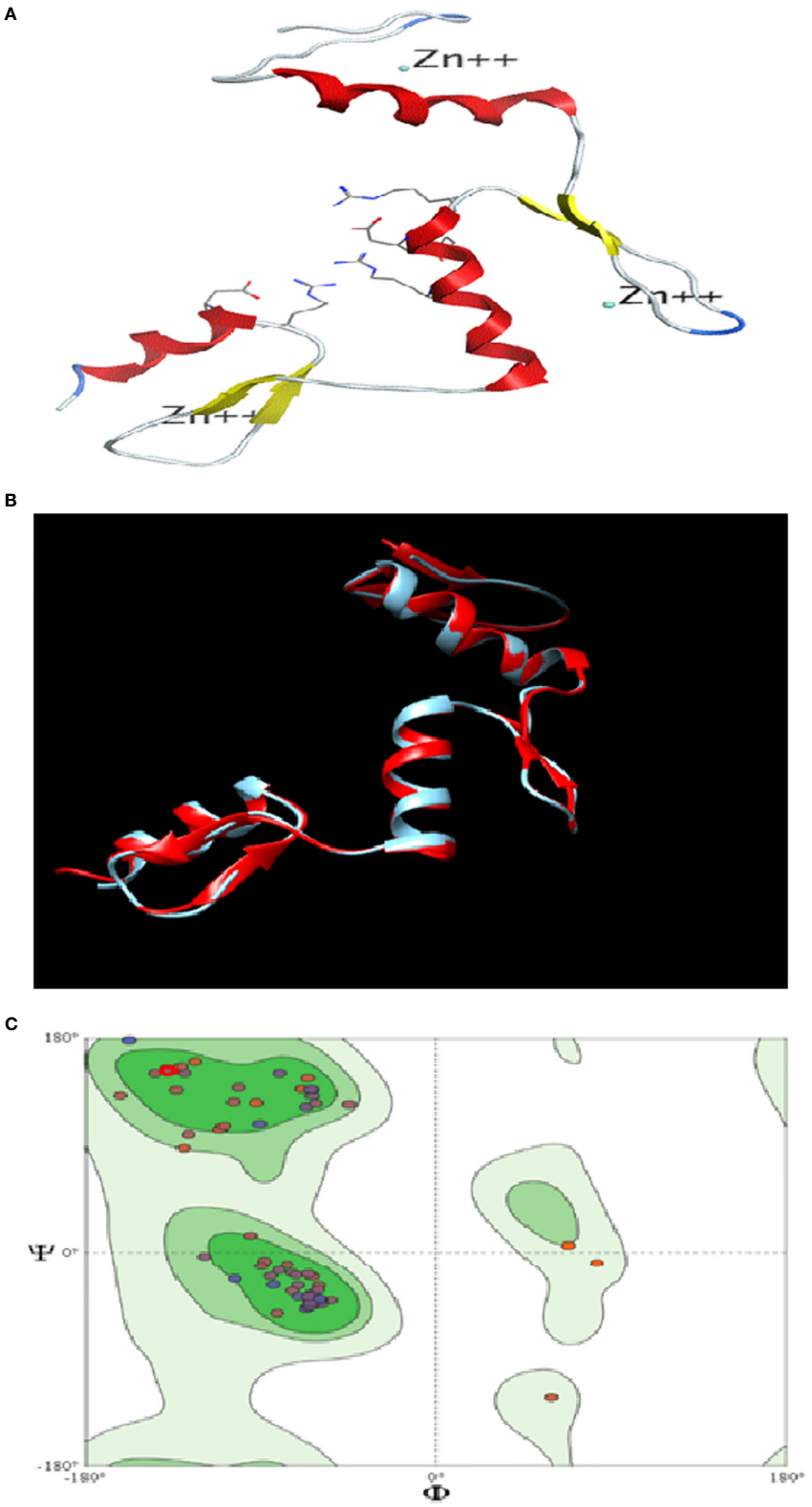

FIGURE 5 | *In silico* analysis of *KLF9*. **(A)** Three-dimensional structure of *KLF9*. **(B)** Comparison of the crystallographically determined structure 2bwu (labeled *red*) and the predicted structure *KLF9* (labeled *blue*) for the analysis of structure conservation. **(C)** Ramachandran plot analysis determining the quality of the model. Most amino acids (95%) were found in favored regions, showing that the model is of good steriochemical quality.

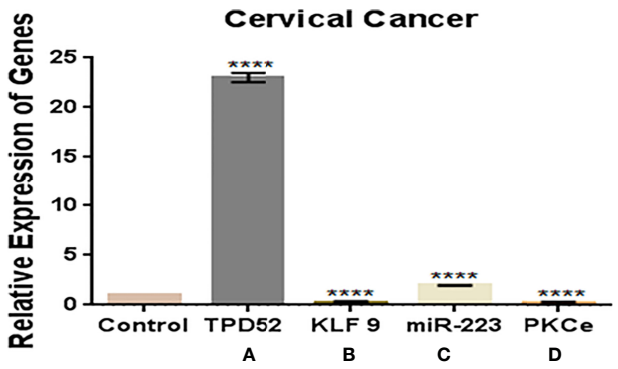

FIGURE 6 | Expressions of *TPD52*, *KLF9*, miR-223, and *PKCϵ* in blood of cervical cancer patients. **(A)** *TPD52* expression was increased 23-fold. **(B)** *KLF9* expression was decreased 0.4-fold. **(C)** miR-223 expression was increased 2-fold. **(D)** *PKCϵ* decreased 0.05-fold. Fold change is plotted on the *y*-axis and study groups on the *x*-axis. Illustrative data are presented as the mean ± SEM of triplicate experimentations. Statistical significance was measured by ordinary two-way ANOVA (****$p < 0.0001$).

genes, while DAVID 6.8 was used to dig out the biological meaning from a large set of genes.

Gene linkage analysis *via* KEGG and STRING is shown in **Figure 11**. Our genetic pathway depicted that all the understudied genes are linked to each other and are involved in the Akt pathway. The pathway obtained *via* DAVID software depicted that *PKCϵ* is found is upstream to the Ras/Raf pathway and bridges the activation of this pathway by GPCRs. Some studies have also described the involvement of *PKCϵ* in the Ras/Raf pathway and have revealed that *PKCϵ* activates GPCR coupled Ras/Raf pathway and helps in the remodeling of cardiomyocytes (24). We also found that the regulation of *PKCϵ* by the *STAT3* gene (signal transducer and activator of transcription 3) stimulates the activity of cyclin D in the nucleus *via* activation of o-myc (family of transcription regulatory genes), which leads to enhanced cell cycle progression. A regulatory link of *PKCϵ* with *STAT3* has also been established in prostate adenocarcinoma (52). A few studies also depicted the activation of *STAT3 via* Rho kinases, which validates our results (53). Moreover, *TPD52* also activates *STAT3*. A recent study has ascertained the activation of *STAT3 via TPD52* (16). Hence, the transcriptional activity of *STAT3* is regulated by *PKCϵ*, *TPD52*, and Rho-kinases. *PKCϵ* involvement was also found in the Rho signaling pathway, which eventually leads to metastasis. According to a recent study, *PKCϵ* also facilitates metastasis in breast cancer by activating Rho-GTPases (54). Our genetic pathway showed that Rho-GTPases are found downstream of *PKCϵ*, and ERK phosphorylation in the Ras/Raf pathway occurs due to the activation of a downstream target of PKCϵ (Rho GTPases). Our finding is in agreement with the previously published report by Pan et al. (55), who also found the same phosphorylation mechanism of ERK in the Ras/Raf pathway. Our genetic pathway also depicted the involvement of *PKCϵ* in the Akt pathway. We found that *PKCϵ* is located upstream of *TPD52*, and both of these genes activate the Akt pathway, which promotes tumor proliferation and invasion. The role of *PKCϵ* in Akt activation, by phosphorylating Akt at serine 473, has already been established (56). Akt is known to regulate proliferation and the cell cycle by targeting cyclin D1, p21, p53, and p27 (57). Forkhead box O (FOXO) is a transcription factor that serves as a downstream target of Akt (protein kinase B). Akt inhibits FOXO by phosphorylating it, and hence promoting cell survival, growth, and proliferation. *TPD52* and *PKCϵ* block the transcriptional activity of FOXO, activate cyclin D, and inactivate p27 (regulator of the cell cycle), leading to enhanced cellular proliferation. According to Zhang et al. (58), the PI3K/Akt signaling pathway inactivates FOXO and, hence, cause the downregulation of cell cycle controls, i.e., CDKI and p27. Our results manifested that the decreased expression of *KLF9* inhibits the progesterone growth hormones (progesterone receptor gene, PGR), which in return directly blocks FOXO and ultimately promotes tumorigenesis. Pabona et al. (59) validates our finding by demonstrating *KLF9* as a regulator of PGR. Loss of *KLF9* leads to the inhibition of PGR and FOXO signaling, hence leading to oncogenesis and tumor invasion in endometrial cells. The genetic pathway constructed in the current study also proposes that the increased expression of miR-223 causes the activation of STMN1 and inhibition of FOXO. In gastric cancer, overexpression of miR-223 also leads to a reduced expression of FOXO and the inhibition of cyclin D, p21, and p27 (60). Moreover, miR-223 is also involved in the activation of phosphatidylinositol 3-kinase (PI3K), which in return produces phosphatidylinositol triphosphate (PIP3) in the cell membrane. PIP3 activates Akt signaling. Zhu et al. (8) also reported on the role of overexpressed miR-223 in the activation of Akt and onset of tumorigenesis in cervical cancer.

TABLE 2 | Clinicopathological features of the cancer patients enrolled in the study.

Clinicopathological characteristics		Cervical cancer *N* (%)
Age (years)	≤50	52 (52)
	>50	48 (48)
Stage	I–II	48 (48)
	III–IV	52 (52)
Metastasis	Metastatic	38 (38)
	Non-metastatic	62 (62)
Treatment	Chemotherapy	16 (16)
	Radiotherapy	32 (32)
	Chemotherapy + radiotherapy	52 (52)

TABLE 3 | Relationship between *TPD52*, *KLF9*, *PKCϵ*, and miR-223 expression and clinicopathological features of cervical cancer.

Clinical–pathological characteristics of cervical cancer patients			***TPD52* expression**			***KLF9* expression**			**miR-223 expression**			***PKCϵ* expression**		
Features	**Groups**	***N* (%)**	**Expression status**	**Fold change**	***p*-value**	**Expression status**	**Fold change**	***p*-value**	**Expression status**	**Fold change**	***p*-value**	**Expression status**	**Fold change**	***p*-value**
Stage	I–II	48 (48)	High	27.0614	0.0001	High	0.68388	0.0001	High	1.2246	0.0001	High	0.05228	0.0001
	III–IV	52 (52)	Low	1.62668	0.0001	Low	0.01752	0.0001	Low	2	0.0001	Low	0.10324	0.0001
Metastasis	Metastatic	40 (40)	High	5.25275	0.0001	High	0.00733	0.0001	High	5	0.0001	High	0.08387	0.0001
	Non-metastatic	60 (60)	Low	14.2051	0.0001	Low	0.13664	0.0001	Low	2.7869	0.0001	Low	0.07114	0.0001

This study also aimed to identify new biomarkers and critical genes linked to the prognosis and diagnosis of cervical cancer. In our study, we have measured the co-expressions of *TPD52*, *KLF9*, miR-223, and *PKCϵ* in cervical cancer. Expression dysregulation of the biomarkers *PKCϵ*, *TPD52*, miR-223, and *KLF9* was determined by comparing the expression fold change with the expression profile of the healthy group. Previously, numerous studies that determine the expressions of biomarkers in patient blood using real-time-PCR were conducted. For instance, the prognostic significance of *KLF7* was studied in tongue cancer (61). The plasma levels of several miRNAs, such as miR-218, miR-223, miR-7, miR30, and miR-21, were studied in hepatocellular carcinoma and gastric and ovarian cancer (62–64). Recently, the relative expressions of matrix metalloproteinases (MMPs) in blood of breast cancer patients were investigated to determine their role in cancer progression (65), hence indicating their possible application in disease prognosis. The current study also evaluated the mRNA expression of these molecules in blood of cervical cancer patients and provided a foundation for conducting an in-depth, proteome-level analysis *in vitro* and *in vivo*. The outcome of the current study indicated the prognostic significance of these molecules for cervical cancer. The diagnostic specificity of these biomarkers was also determined through ROC curve analysis. However, further evaluation on a larger cohort size and at the protein level is required to determine its clinical significance.

Earlier, the role of understudied genes had been independently studied in various tumors, which confirmed the involvement of these genes in cancer, metastasis, and expansion and in resistance

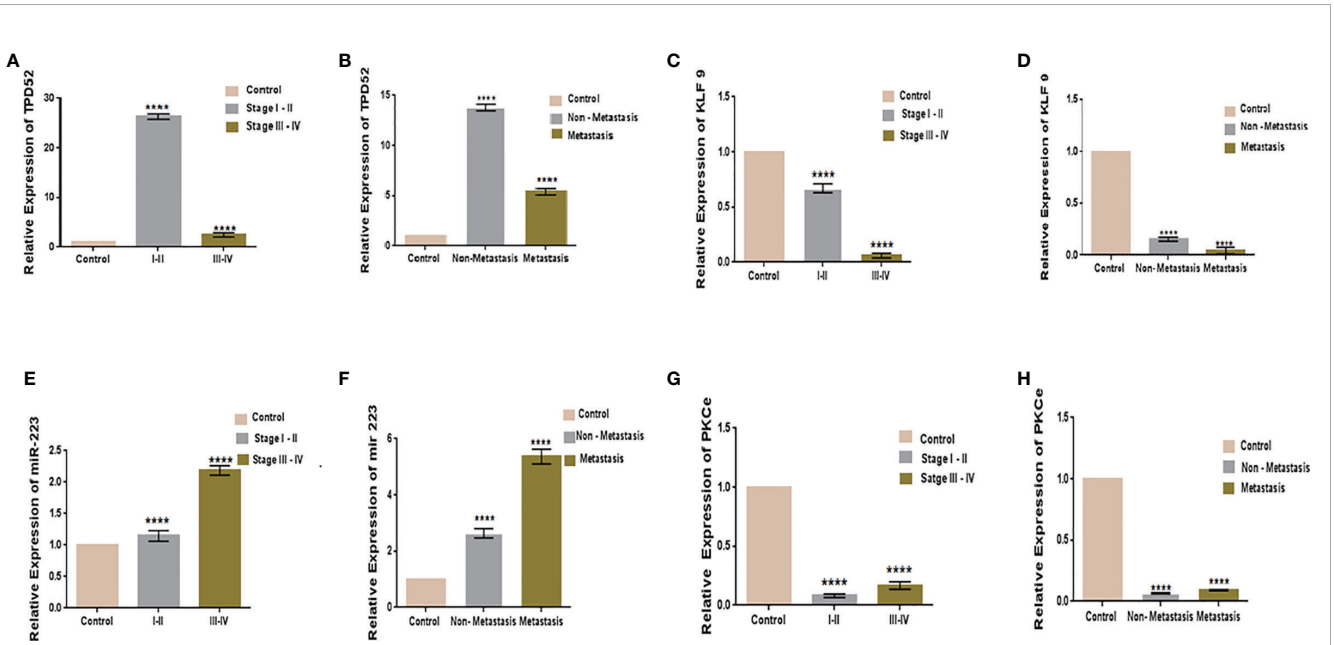

FIGURE 7 | Relative gene expression with clinical features of cervical cancer. Relative *TPD52* expression with tumor stage **(A)** and metastasis **(B)**. Relative *KLF9* expression with tumor stage **(C)** and metastasis **(D)**. Relative miR-223 expression with tumor stage **(E)** and metastasis **(F)**. Relative *PKCϵ* expression with tumor stage **(G)** and metastasis **(H)**. Fold change is plotted on the *y*-axis and study groups on the *x*-axis. Illustrative data are presented as the mean ± SEM of triplicate experimentations. Statistical significance was measured by ordinary one-way ANOVA (****$p < 0.0001$).

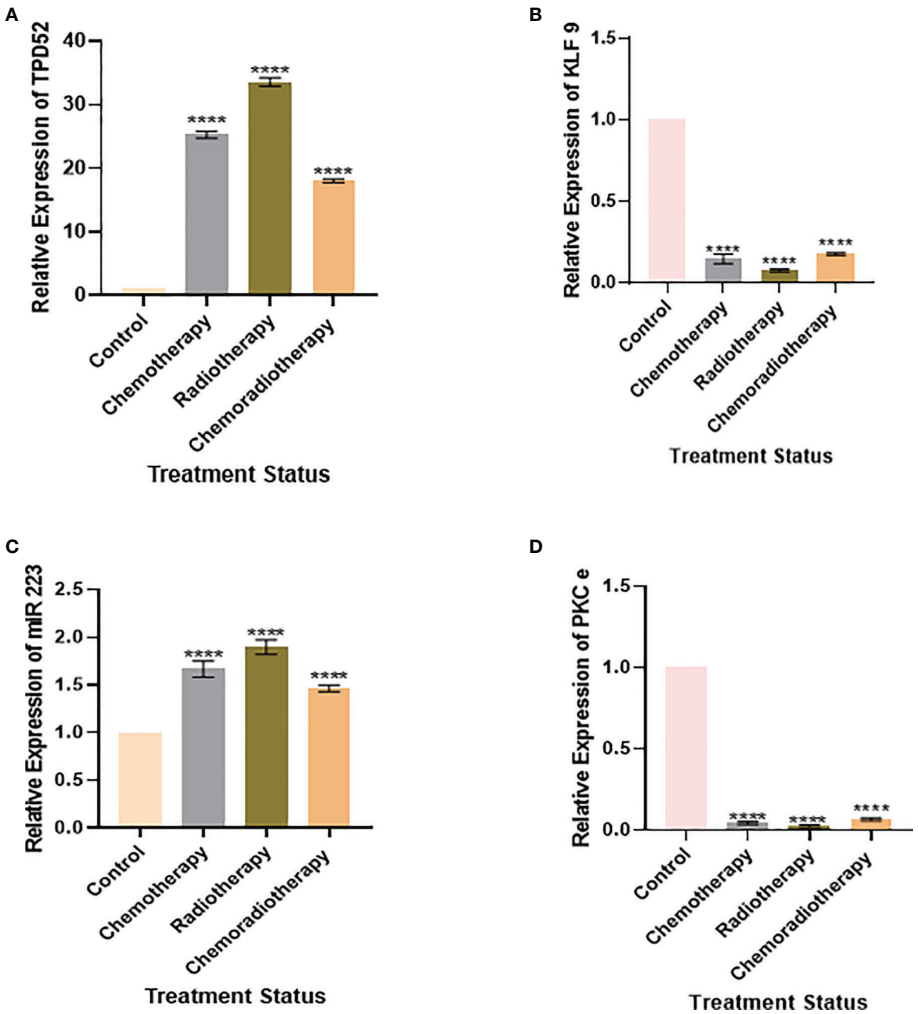

FIGURE 8 | Relative gene expression with treatment status. **(A)** Relative *TPD52* expression. **(B)** Relative *KLF9* expression. **(C)** Relative miR-223 expression. **(D)** Relative *PKCε* expression. Illustrative data are presented as the mean ± SEM of triplicate experimentations. Fold change is plotted on the *y*-axis and study groups on the *x*-axis. Statistical significance was measured by ordinary one-way ANOVA (****$p < 0.0001$).

to therapy. To the best of our knowledge, the co-expression of these genes in cervical cancer has not been studied yet. We observed an increased expression of *TPD52* in cervical cancer patients relative to healthy controls who have very low levels of the *TPD52* gene in their blood. Various studies reported the upregulation of *TPD52* expression in quite a few cancers, such as breast, prostate, and pancreatic cancer, Burkitt's lymphoma, multiple myeloma, and melanoma (12). On the other hand, the expression of *TPD52* is downregulated in some cancers, such as papillary renal cell cancer, lung cancer, and liposarcoma (13). In the case of *KLF9*, we observed its significantly reduced expression in cervical cancer patients relative to healthy controls. Similar downregulation of *KLF9* has been reported in endometrium cancer, where its downregulation is linked to estrogen-mediated growth control (66). The reduced expression of *KLF9* has also been reported in breast cancer, human colorectal tumors, and hepatocellular carcinoma (67). Various studies have discovered that expression profiling of various circulating miRNAs in the blood may probably be used in therapeutic interventions and in identifying different tumor types (68). We have found an upregulation of miR-223 in cervical cancer patients relative to the healthy individuals. According to a recent study, the expression of miR-223 is significantly elevated in gastric adenocarcinoma cells. The upregulation of miR-223 encouraged cell proliferation and reduced apoptosis in gastric adenocarcinoma cells, while the downregulation of miR-223 expression has been linked to various cancer subtypes, including leukemia and gastric, esophageal, and colorectal cancer (69). In the case of *PKCε*, we observed its reduced expression in cervical cancer patients relative to healthy controls who had significantly high levels of this gene in their blood. On the contrary, an upregulation of *PKCε* has been reported in a large number of carcinomas, including breast, lung, and prostate cancer (70). Various reports have confirmed the role of this gene as an oncogene and its involvement in tumor

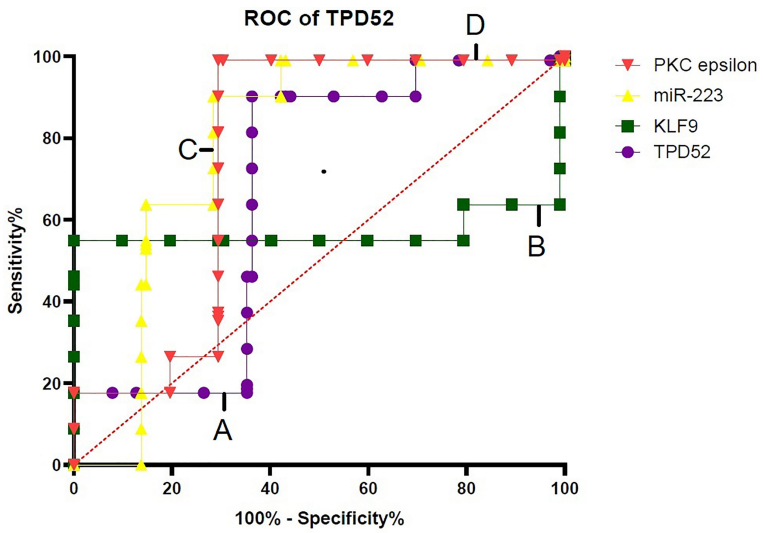

FIGURE 9 | Specificity of *TPD52*, *KLF9*, miR-223, and *PKCε* in the diagnosis of cervical cancer. Receiver operating characteristic (ROC) curve for *TPD52*, *KLF9*, miR-223, and *PKCe* predicted high risk of cervical cancer. **(A)** *TPD52*: area under the ROC curve (AUC) = 0.6685 and 95% confidence interval (CI) = 0.5880–0.7490. **(B)** *KLF9*: AUC = 0.5706, 95%CI = 0.4775–0.6638. **(C)** miR-223: AUC = 0.7884, 95%CI = 0.7184–0.8583. **(D)** *PKCε*: AUC = 0.7595, 95%CI = 0.6852–0.8338.

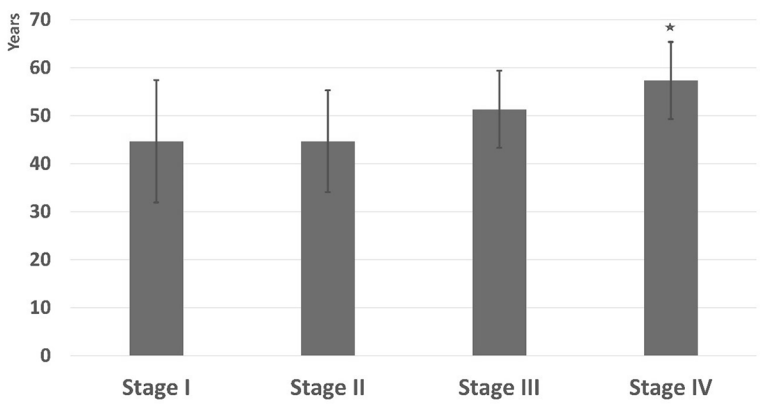

FIGURE 10 | Age of participants in different clinical stages. *Significant *vs*. stage I.

metastasis (55). Our study found that the expression of *TPD52* was upregulated in the advanced-stage tumor group (1.62 ± 0.4) and in the distant metastatic group of patients (5.25 ± 0.42) relative to lower stage tumor and non-metastatic groups, where its expression levels were increased 27.0 ± 1.68- and 14.2 ± 1.68-fold, respectively. Hence, *TPD52* may serve as a potent early diagnostic biomarker in cervical cancer. A recent study has reported the decreased expression of *TPD52* in tumorous tissues of hepatic cellular carcinoma (HCC) in comparison to healthy tissues. Further correlation analysis exposed that the reduced expression of *TPD52* in HCC was suggestively linked to advanced stage tumor, signifying that a reduced *TPD52* expression may promote tumor metastasis (71). These results are inconsistent with our study. Furthermore, in the case of *KLF9*, we observed its reduced expression in advanced tumor stage (0.01 ± 1.6) and in distant metastasis (0.007 ± 1.39). A downregulated expression of *KLF9* was suggestively found in the lower stage tumor group (0.68 ± 1.6) and the non-metastatic group (0.13 ± 1.82). Our result is encouragingly inconsistent with recent findings that point to the fact that a reduced expression of *KLF9* is linked to poor survival and prognosis in pancreatic ductal adenocarcinoma and leads to tumor metastasis (9). Our study found that the expression of miR-223 was increased in the advanced tumor stage (2.07 ± 3.9) and distant metastasis (5.8 ± 4.25) groups, while its expression was decreased in the lower tumor stage group (1.2 ± 43.9) and the non-metastatic group (2.7 ± 4.5). Further studies have revealed that miR-223 plays a significant part in the metastasis of cervical cancer. The

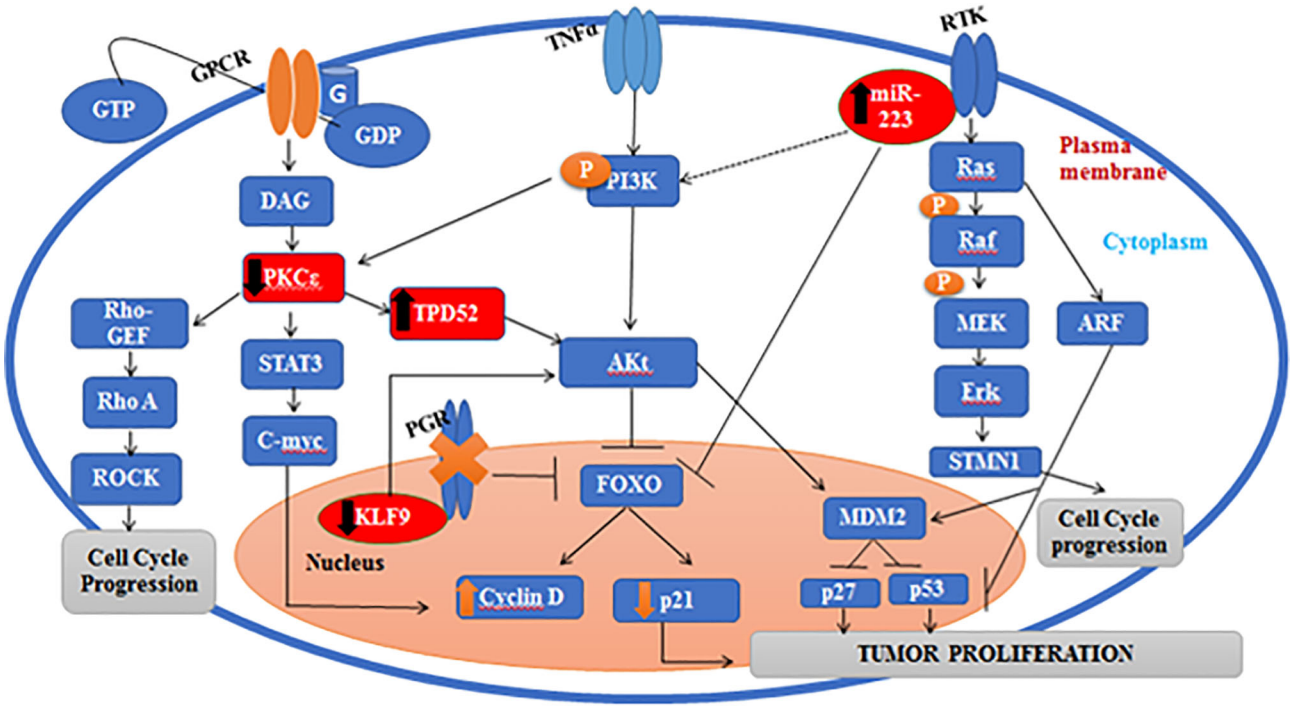

FIGURE 11 | Pathway showing the correlations between *TPD52*, *KLF9*, miR-223, and *PKCε*.

upregulation of miR-223 promotes metastasis in cervical cancer cells (72). These results validate our results showing that the increased expression of miR-223 in cervical cancer patients causes metastasis and poor prognosis. The expression of *PKCε* was much more downregulated in the advanced tumor stage (0.10 ± 5.8) and distant metastasis (0.08 ± 6.36) groups relative to the lower tumor stage group and the non-metastatic group, where its expression was reduced 0.05 ± 6.0- and 0.07 ± 5.87-fold, respectively. According to recent studies, *PKCε* causes tumor metastasis to the bone by promoting translation increase and causes osteosarcoma metastasis (73). These findings contradict our study as *PKCε* inhibited metastasis in cervical cancer. The contradictory results may be due to the different cancer types.

Our study also discovered the effect of treatment on the expression profiles of understudied genes. It was found that patients undergoing chemoradiotherapy showed better prognosis. In the case of *TPD52*, patients undergoing chemoradiotherapy showed the lowest expression (18.52 ± 1.84) relative to patients on chemotherapy (26.2 ± 1.5) and radiotherapy (34.7 ± 1.83). Likewise, patients on chemoradiotherapy showed the lowest miR-223 expression (1.51 ± 3.8) relative to patients undergoing chemotherapy (1.76 ± 3.7) and radiotherapy (2.03 ± 4.2). These results show patients' response to treatment and indicate that chemoradiotherapy has better prognosis, while radiotherapy is linked to poor prognosis in cervical cancer. During treatment expression profiling, *KLF9* and *PKCε* were found to be slightly less reduced in patients treated with chemoradiotherapy, who showed better prognosis, relative to chemotherapy and radiotherapy. The expression patterns of *KLF9* in patients undergoing chemotherapy and radiotherapy were found to be 0.10 ± 0.60 and 0.08 ± 1.85, respectively. In the case of *PKCε*, these were found to be 0.06 ± 0.1 and 0.04 ± 6.48, respectively. Hence, it was deduced that chemoradiotherapy is linked to better survival of cervical cancer patients.

To further validate our findings, Spearman's rho correlation was used to test the association of age and the stage of the disease. The association of age and stage of the disease was found in line with the frequency found in the literature in adults (74) and children (75). However, some studies showing evidence of a relationship between age and cancer in adults (76) have reported that cancer does not have to be a consequence of old age.

All the involved genes and miRNAs in our study are known to be implicated in various cancer signaling pathways, such as the PI3K/Akt, nuclear factor-κB, Wnt/β-catenin, and Ras signaling pathways. Hence, these genes and miRNAs may serve as potential diagnostic and prognostic biomarkers. Moreover, these genes can further be investigated as targets for anticancer therapy.

CONCLUSION

In the present study, we identified the conserved domains and the 3D structure of *KLF9* and developed a genetic pathway establishing the crosstalk between *KLF9* and its upstream and downstream targets. Moreover, upregulation of the expressions of *TPD52* and miR-223 and downregulation of the expressions

of *KLF9* and *PKCε* were found in peripheral blood of cervical cancer patients. Altered expressions of these genes have been found to be related to tumor progression. Alterations in the expression levels of the understudied genes in cervical cancer may serve as a potential circulating biomarker for cancer diagnosis and prognosis. Hence, understanding the functions, signaling pathways, and genetic networks of *TPD52, KLF9*, miR-223, and *PKCε* may synergistically reveal the mechanisms of disease progression and serve as a target for inhibitors, therefore assisting in the development of effective anticancer therapy.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved. This experimental protocol for the use of human was approved (ref. no. IRB-110) by the Ethical Committee of Combined Military Hospital and ASAB, NUST. The patients/participants provided 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

MS, SS, KZ, YB, KK, NA and SR designed and conceived the study and analyzed the results. ED, SR, TA, MS, KK and AA conceived an initial part of the study, performed the experiment and histology, and helped in compiling the results. MS, KZ, and SS performed experiments. MS, SR, ED, AA, NA and TA helped in writing the results. SR, TA, DD, and AA wrote the paper with input from all other authors. MS, KZ, SR, YB, DD, SS, TA, KK, NA and AA made a substantial contribution in the interpretation of data and revised the manuscript for intellectual content. All authors read and approved the final manuscript.

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Assessment of ESGO Quality Indicators in Cervical Cancer Surgery: A Real-World Study in a High-Volume Chinese Hospital

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The ESGO developed a list of fifteen quality indicators for cervical cancer surgery in order to audit and improve clinical practice in 2020. However, data from the developing countries with high incidence rates of cervical cancer is still lacking. Therefore, we conducted a retrospective study of 7081 cases diagnosed as cervical cancer between 2014 and 2019 in a Chinese single center according to the quality indicators proposed by ESGO. A total of 5952 patients underwent radical procedures, with an average of 992.0 per year. All surgeries were performed or supervised by a certified gynecologic oncologist as surgical qualification grading system has been established. Compared with the low-volume group, patients in the high-volume group (≥15 cases/year) had a shorter hospital stay (P<0.001), more free surgical margins (P=0.031), and less complications (P<0.001), but the 5-year recurrence-free survival and overall survival rates were similar (P>0.05). Treatment was not planned at a multidisciplinary team meeting but with the consultation system. The required preoperative workup was incomplete in 19.7% of patients with pelvic MRI and 45.7% of patients with PET-CT. A total of 1459 (20.6%) patients experienced at least one complication after surgery. The CDC grade IIIb or higher complications occurred in 80 patients, accounting for 5.5% complications. The urological fistula rate within 30 postoperative days were 0.3%. After primary surgical treatment, 97.4% patients had clear vaginal and parametrial margins. After restaging FIGO 2009 to FIGO 2018 system, 14.7% patients with a stage T1b disease were T-upstaged. After a median follow-up of 42 months, recurrence occurred in 448 patients, and 82.1% patients recurred within 2 years. The 2-year RFS rate of patients with pT1b1N0 was 97.3% in 2009 FIGO staging system. Lymph node staging was performed in 99.0% patients with a stage T1 disease. After a primary surgical treatment for a stage pT1b1N0 disease, 28.3% patients received adjuvant chemoradiotherapy. Above all, most of quality indicators reached the targets, except four quality indicators. The quality indicators of ESGO should be popularized and applied in China to guarantee quality of surgery.

Keywords: cervical cancer, oncological outcome, quality assurance, quality of treatment, gynecologic oncologists

INTRODUCTION

Cervical cancer is the fourth most common cancer in women worldwide, with an estimated 604,000 new cases and 342,000 deaths in 2020 (1). In China, the age-standardized incidence and mortality rates of cervical cancer have been constantly increasing over last 20 years, with 109,741 new cases and 59,060 deaths of cervical cancer in 2020, approximately accounting for 18% and 17% that of the world respectively (2). Surgery is the preferred treatment for patients with early-stage cervical cancer. Clear evidence was found that implementation of a quality improvement program helped to reduce both morbidity and costs, and improve the quality of life of cancer patients. Moreover, the quality of surgical care has been shown to improve outcomes in patients with other malignances such as breast cancer, lung cancer, gastric cancer, colorectal cancer, soft tissue sarcoma, ovarian cancer, and so on (3–7). Thus, it is likely that implementation of a quality management program could improve survival of patients with cervical cancer. In 2020, the European Society for Gynecologic Oncology (ESGO) then developed a list of fifteen quality indicators (QIs) in an easy and practicable way in order to audit and improve the surgical treatment of cervical cancer (8).

To our knowledge, few studies assessed the quality of cervical cancer surgery based on the ESGO list of quality indicators. A retrospective study including 1156 cases from 126 institutions belonging to 29 European countries evaluated the ESGO quality indicators for surgical treatment of cervical cancer (9). And another multicenter retrospective study in Europe assessed the oncological outcomes of 239 patients diagnosed with cervical cancer according to the quality indicators (10). However, data from the developing countries with high incidence rates of cervical cancer is still lacking. Therefore, we conducted a retrospective real-world study involving patients diagnosed as cervical cancer between 2014 and 2019 in the Obstetrics and Gynecology Hospital of Fudan University, Shanghai, China, so as to audit the surgery quality of cervical cancer in this high-volume single center according to the quality indicators proposed by ESGO.

MATERIAL AND METHODS

Study Population

It was a retrospective study under real-world conditions. The study was approved by the Institutional Review Board (IRB) of the Obstetrics and Gynecology Hospital of Fudan University (No.2021-15). All patients who diagnosed with cervical cancer and underwent surgical treatment from January 1, 2014 to December 31, 2019 in the Obstetrics and Gynecology Hospital of Fudan University were included. Exclusion criteria were: ① no surgical management during the period of inclusion, ② just biopsy or conization for diagnose but not for surgical treatment, and ③ undergoing other surgical treatment but not related to the cervical cancer therapy.

Data Collection

Using the International Classification of Diseases Tenth Revision (ICD-10) code C53.9 or the diagnosis of "cervical cancer" as the keyword for the search, data were extracted from the hospital information system and the outpatient information system. The tumors were classified according to the Federation International of Gynecology and Obstetrics (FIGO) staging system. Between 2014 and 2018, patients were diagnosed with the 2009 FIGO staging system, while the 2018 FIGO staging system began to be used in 2019 (11, 12). In principle, patients underwent operations based on different stages according to the National Comprehensive Cancer Network (NCCN) guidelines at that time. All the procedures were accomplished with the use of a uterine manipulator and without vaginal closure and tumor exclusion before the colpotomy before 2018. But after the report of the Laparoscopic Approach to Cervical Cancer (LACC) trial, the uterine manipulator was banned, and the tumor was enclosed before the colpotomy in the hospital. Some of the patients with bulky (≥4 cm) stage IB or IIA cervical carcinoma were treated by neoadjuvant chemotherapy at the discretion of the treating gynecologist. The patients received paclitaxel and platinum for 1-2 courses, and then underwent surgical treatment. We extracted the information of complications through the identical information of patients and reanalyzed them according to the Clavien-Dindo classification (CDC) system (13) and the comprehensive complication index (CCI) (14). The CCI values were computed from the CCI calculator at website (http://www.assessurgery.com).

A patient was considered to be treated by a certified gynecologist if her gynecologist had a corresponding surgery qualification. The surgical qualification grading system has been established in the hospital since 2013 according to the provisions of the National Health Administration, which is similar to the Endoscopic Surgical Skill Qualification System (ESSQS) in Japan (15). According to the system, surgical qualifications are classed into four grades and authorized by the Surgical Qualification Examination Committee. Surgical Grade IV are subdivided into pelvic lymphadenectomy (IVa), radical hysterectomy (IVb), and paraaortic lymphadenectomy (IVc). Since the minimum required number of radical procedures per year was 15, we classified those who qualified for surgical Grade IVb and performed more than 15 cases of radical procedures per year as the high-volume surgeons, while those who qualified for surgical Grade IVb but performed <15 cases/year radical procedures, or those who did not qualify for surgical Grade IVb and performed radical procedures under supervision as the low-volume surgeons.

After surgery, patients underwent adjuvant therapy if they presented any high-risk factors (positive margin, parametrial involvement, or lymph node metastasis) or intermediate-risk factors met the Sedlis criteria (16) or the "four-factor model" (17). According to the NCCN guidelines, patients were followed up every 3 months for 2 years, every 6 months for the next 3 years, and once per year thereafter. The follow-up information was recorded in the follow-up information system and can be obtained after searching for the identical information of

the patient. The last follow-up date was December 2020. Recurrence-free survival (RFS) was defined as the length of time (in months) from the primary surgery to initial diagnosis of recurrence or date of last follow-up. Overall survival (OS) was calculated (in months) as the difference between the primary surgery date and the date of death from cervical cancer or last contact, whichever came first.

Statistical Analyses

Statistical analyses were performed with SPSS v23.0 (IBM Corp., Armonk, NY). Student's t-test or analysis of variance (ANOVA) was used to compare continuous variables, whereas chi-square test was used to compare categorical variable. Oncological outcomes, RFS and OS were calculated with the Kaplan-Meier method, with differences in the probability of survival analyzed with the log-rank test. Differences were considered to be statistically significant at P <0.05.

RESULTS

A total of 7081 patients with diagnosis of cervical cancer between January 2014 and December 2019 were finally enrolled as the study population. The clinical characteristics of all patients were shown in **Table 1**. The mean age of all patients was 48.1 years old. Majority of patients (99.0%) were FIGO stage <IIB, and more than half patients (51.0%) were stage IB1. A total of 6891 (97.3%) surgeries were performed by minimally invasive surgery. Of these, 6489 (94.2%) patients had a laparoscopic approach, and 402 (5.8%) patients had robotic surgery. Only 135 (1.9%) patients underwent by laparotomy. Another 55 (0.8%) patients underwent transvaginal repeat cone biopsy because of fertility sparing. The surgical procedure was described as radical surgery in 5952 (84.0%) cases. A total of 5985 (84.5%) patients underwent lymphadenectomy, mostly (89.8%) with pelvic lymphadenectomy. While only 24 (0.4%) cases underwent sentinel lymph node biopsy.

TABLE 1 | Clinical characteristic of patients with cervical cancer in different years.

Variables	N = 7081
Age (years), median (range)	48.1 ± 10.0 (8-84)
FIGO 2009 stage, n (%)	
IA1	1203 (17.0)
IA2	182 (2.6)
IB1	3614 (51.0)
IB2	640 (9.0)
IIA1	884 (12.5)
IIA2	485 (6.9)
≥IIB	73 (1.0)
Surgical approach, n (%)	
Laparoscopy	6489 (91.6)
Robotic surgery	402 (5.7)
Laparotomy	135 (1.9)
Transvaginal surgery	55 (0.8)
Type of surgical resection, n (%)	
Radical surgery	5952 (84.0)
Radical hysterectomy	5653 (95.0)
Modified radical hysterectomy	188 (3.2)
Trachelectomy	73 (1.2)
Parametrectomy	38 (0.6)
Cone biopsy	55 (0.8)
Hysterectomy	1068 (15.1)
Local recurrence resection	6 (0.1)
Type of lymph node dissection, n (%)	5985 (84.5)
Sentinel lymph node biopsy	24 (0.4)
pelvic lymphadenectomy	5373 (89.8)
pelvic and para-aortic lymphadenectomy	588 (9.8)

All results of the ESGO quality indicators in the hospital year by year were shown in **Table 2**.

Quality Indicators Related to Caseload in the Center, and Training and Experience of the Surgeon

QI 1 is a structural indicator, which means the number of radical procedures in cervical cancer performed per center per year. The optimal target is ≥30 cases and the minimum required target is ≥15 cases. As shown in **Table 2**, a total of 5952 patients underwent radical procedures, with an average of 992.0 ± 207.3, which significantly exceeded the optimal target. The number of radical procedures increased significantly year by year (P<0.001).

QI 2 is a process indicator, which means surgery performed or supervised by a certified gynecologic oncologist or a trained surgeon dedicated to gynecological cancer (accounting for 80% of his or her practice) or having completed an ESGO-accredited fellowship. The target is 100%. This indicator was performed 100% in our center.

A total of 40 surgeons underwent radical procedures, while 36 of these qualified for surgical Grade IVb. Among them, 18 surgeons were divided into the high-volume group as they underwent radical procedures ≥15 cases/year, with a total of 5016 (84.3%) patients. Ten surgeons who qualified for the robotic radical hysterectomy were all in the high-volume group, and underwent 390 cases since 2015. As seen in **Table 3**, patients in the high-volume group were younger (48.3 *vs* 49.7, P<0.001), and more likely to be stage IB1 or ≥IIB (P<0.001). They had a higher incidence of superficial stromal infiltration (41.9% *vs* 38.6%, P=0.042), no lymphovascular space incision (LVSI) (55.5% *vs* 51.5%, P=0.023), and free surgical margins (93.1% *vs* 91.2%, P=0.031). Furthermore, the patients in the high-volume group had a shorter hospital stay (11.0 *vs* 12.5 days, P<0.001), and less intraoperative complications as well as postoperative severe complications (P<0.001), especially in the incidence of urological injury and fistula. But there was no significant difference between the two groups in the cumulative 5-year RFS rates (91.4% *vs* 92.4%, P=0.456) and OS rates (93.3% *vs* 91.4%, P=0.654) (**Figure 1**).

Quality Indicators Related to the Overall Management

QI 3 is a structural indicator, which means the center participating in ongoing clinical trials in gynecological cancer. The target is ≥1. Twenty clinical trials had been conducted from 2014 to 2019, with an average of 3 clinical trials ongoing every year. The target was performed 100%.

TABLE 2 | Evaluation of the ESGO quality indicators in the hospital.

	Quality indicators	Target	Total result	2014	2015	2016	2017	2018	2019	*P-value*
1	Radical procedures performed per year	≥30	992.0 ± 207.3	705	841	915	1101	1126	1264	<0.001
2	Certified surgical specialist	100%	100%	100%	100%	100%	100%	100%	100%	
3	Ongoing clinical trials	≥1	3.3 ± 2.7	1	1	2	3	5	8	0.002
4	Multi-disciplinary team meeting	100%	0%	0%	0%	0%	0%	0%	0%	
5	Required pre-operative investigation	100%	54.3%	40.1%	43.2%	50.8%	52.2%	61.0%	78.5%	<0.001
6	Required elements in surgical reports	100%	100%	100%	100%	100%	100%	100%	100%	
7	Required elements in pathology reports	≥90%	100%	100%	100%	100%	100%	100%	100%	
8	Structured prospective reporting of the follow-up and 30-day postoperative morbidity	≥90%	90%	90%	90%	90%	90%	90%	90%	
9	Urological fistula rate within 30 days after a radical parametrectomy	≤3%	0.3% (19/5952)	0.3%	0.4%	0.4%	0.2%	0.3%	0.4%	0.919
10	Negative vaginal and parametrial margins	≥97%	97.4% (6897/7081)	97.8%	97.8%	97.5%	97.4%	97.6%	96.6%	0.314
11	T-upstaged after surgery in T1b disease	<10%	14.7% (626/4254)	12.4%	14.8%	12.6%	17.9%	16.5%	13.0%	0.010
12	Recurrence rate at 2 years in patients with pT1b1N0	<10%	2.7%	2.7%	4.2%	3.6%	3.7%	1.7%	0.6%	0.002
13	Lymph node staging in T1 disease	≥98%	99.0% (3467/3501)	99.8%	98.9%	99.3%	99.5%	99.4%	97.7%	0.001
14	Counseling about fertility-sparing treatment	100%	100%	100%	100%	100%	100%	100%	100%	
15	Adjuvant chemoradiotherapy in pT1b1N0 disease	<15%	28.3% (876/3098)	24.1%	24.5%	23.4%	30.8%	32.2%	31.0%	0.001

QI 4 is a process indicator, which means treatment discussed at a multi-disciplinary team (MDT) meeting. The target is 100%. But there was no MDT meeting in our hospital before 2020. Instead, the consultation system was performed. The target was totally not performed.

QI 5 is a process indicator, which means required preoperative investigation. The target is 100%. As seen in **Table 4**, all patients underwent pelvic examination, and the average of clinical tumor size was 20.0 mm. All patients underwent pelvic ultrasound, with an average size of 20.6 mm. But pelvic MRI with contrast was performed in 80.3% of patients with stage ≥ IB1, and the mean tumor diameter measured by MRI was 24.2mm. Whole-body PET-CT or chest/abdomen/pelvic CT was performed in 54.3% of patients in locally advanced cervical cancer and higher. Actually, the main problem of the preoperative workup was the whole-body imaging. Fortunately, the completion rate of imaging was increasing year by year (P<0.001). All patients in locally advanced cervical cancer and higher performed urinary examination. Nearly all patients underwent a cervical biopsy except 7 (0.1%) patients were found incidentally after hysterectomy. As indicated, 2466 (99.7%) patients underwent cone biopsy except nine patients who were so elder with cervical atrophy that difficult to operate.

Quality Indicators Related to Recording Pertinent Information

QI 6 is a process indicator, which means minimum required elements in surgical reports. The target is 100%. All required elements as defined in the ESGO-ESTRO-ESP guidelines were present in the patient surgical report. The target was performed 100%.

QI 7 is a process indicator, which means minimum required elements in pathology reports. The target is ≥90%. Three tumor dimensions were all measured, with the average maximum tumor size of 23.8 ± 19.3 mm. All the other required elements as defined in the ESGO-ESTRO-ESP guidelines were present in the patient pathology report, as seen in the **Table 3**. The target was performed 100%.

QI 8 is an outcome indicator, which means structured prospective reporting of the follow-up and 30-day post-operative morbidity using a validated surgical complication scoring system. The optimal target is ≥90% and the minimum required target is that selected cases are discussed at morbidity and mortality conferences. The target was performed 90% in our hospital. A total of 1459 (20.6%) patients experienced at least one complication after surgery. The type, occurrence time, reason, and management of complications as well as recovery of the patient were all reported. Every complication which leaded to organ injury or function permanent damage and even death of a patient would be discussed and defined as grade of medical events in the meeting. However, the CDC system or the CCI had never been used in the hospital. Therefore, the data in the complication reporting system were reviewed and reanalyzed in **Table 5**. Bladder injury (0.2%) was the most common intraoperative complications. Leg lymphedema (17.5%), bladder dysfunction (9.8%), and fever (7.2%) were the most common postoperative complications. The CDC grade IIIb or higher complications occurred in 80 (1.1%) patients, accounting for 5.5% complications. The mean CCI was 18.2 ± 8.0.

Quality Indicators Related to the Quality of Surgical Procedures

QI 9 is an outcome indicator, which means urological fistula rate within 30-post-opetative days after a radical parametrectomy in the preceding 3 years. The target is ≤3%. As seen in **Table 3**, a total of 40 (0.7%) patients had urologic complications in 6 years. Furthermore, urinary injury and bladder injury occurred in 0.4% (22/5952) and 0.3% (18/5952) of patients, respectively. Of these, 19 patients (0.3%) had urological fistula after radical procedures. The incidence of urological fistula was similar every year.

QI 10 is an outcome indicator, which means proportion of patients after primary surgical treatment who have clear vaginal and parametrial margins in the preceding 3 years. The target is ≥97%. In the center, 6897 (97.4%) cases had clear surgical

TABLE 3 | Comparison of clinical, pathologic and operative characteristics between the high-volume and the low-volume groups.

	Total (n = 5952)	High-volume (n = 5016)	Low-volume (n = 936)	*P-value*
Age (years)	48.5 ± 10.0	48.3 ± 9.9	49.7 ± 10.2	<0.001
FIGO 2009 stage (n,%)				<0.001
IA1	127 (2.1)	111(2.2)	16 (1.7)	
IA2	174 (2.9)	141 (2.8)	33 (3.5)	
IB1	3588 (60.3)	3076 (61.3)	512 (54.7)	
IB2	636 (10.7)	537 (10.7)	99 (10.6)	
IIA1	884 (14.9)	731 (14.6)	153 (16.3)	
IIA2	479 (8.0)	360 (7.2)	119 (12.7)	
≥IIB	64 (1.1)	60 (1.2)	4 (0.5)	
Type of surgery (n,%)				<0.001
Laparoscopy	5440 (91.4)	4541 (90.5)	899 (96.0)	
Robotic surgery	390 (6.6)	390 (7.8)	0 (0.0)	
Laparotomy	122 (2.0)	85 (1.7)	37 (4.0)	
Histological type (n,%)				0.660
SCC	4753 (79.9)	3996 (79.7)	757 (80.9)	
AC	715 (12.0)	614 (12.2)	101 (10.8)	
ASC	376 (6.3)	315 (6.3)	61 (6.5)	
Other type	108 (1.8)	91 (1.8)	17 (1.8)	
Tumor size, mm (n,%)				0.059
≤20	2418 (40.6)	2069 (41.2)	349 (37.3)	
(20-40]	2237 (37.6)	1873 (37.3)	364 (38.9)	
>40	1297 (21.8)	1074 (21.4)	223 (23.8)	
Stromal infiltration (n,%)				0.042
<1/3	2464 (41.4)	2103 (41.9)	361 (38.6)	
[1/3 -2/3)	287 (4.8)	230 (4.6)	57 (6.1)	
≥2/3	3201 (53.8)	2683 (53.5)	518 (55.3)	
LVSI (n,%)				0.023
No	3267 (54.9)	2785 (55.5)	482 (51.5)	
Yes	2685 (45.1)	2231 (44.5)	454 (48.5)	
Parametrial involvement (n,%)				0.850
No	5522 (92.8)	4655 (92.8)	867 (92.6)	
Yes	430 (7.2)	361 (7.2)	69 (7.4)	
Uterine involvement (n,%)				0.956
No	4951 (83.2)	4173 (83.2)	778 (83.1)	
Yes	1001 (16.8)	843 (16.8)	158 (16.9)	
Vaginal involvement (n,%)				0.163
No	4071 (68.4)	3449 (68.8)	622 (66.5)	
Yes	1881 (31.6)	1567 (31.2)	314 (33.5)	
Ovarian involvement (n,%)				0.353
No	5952 (99.5)	4995(99.6)	930 (99.4)	
Yes	27 (0.5)	21 (0.4)	6 (0.6)	
Lymph node metastasis (n,%)				0.519
No	4664 (78.4)	3938 (78.5)	726 (77.6)	
Yes	1288 (21.6)	1078 (21.5)	210 (22.4)	
Number of lymph node (n)	22.1 ± 7.7	22.0 ± 7.7	22.3 ± 7.9	0.201
Surgical margin status (n,%)				0.031
Free margins	5524 (92.8)	4670 (93.1)	854 (91.2)	
Free but close margins (<5mm)	50 (0.8)	40 (0.8)	10 (1.1)	
Positive margins (pre-invasive disease)	203 (3.4)	172 (3.4)	31 (3.3)	
Positive margins (invasive disease)	175 (2.9)	134 (2.7)	41 (4.4)	
NACT				0.910
No	5809 (97.6)	4895 (97.6)	914 (97.6)	
Yes	143 (2.4)	121 (2.4)	22 (2.4)	
Adjuvant treatment (n,%)				0.185
No	2655 (44.6)	2256 (45.0)	399 (42.6)	
Yes	3297 (55.4)	2760 (55.0)	537 (57.4)	
Operative time (min)	172.6 ± 65.6	171.2 ± 63.6	176.4 ± 70.7	0.214
Estimated blood loss (ml)	233.3 ± 192.1	232.7 ± 187.8	236.4 ± 213.7	0.594
Hospital stays (day)	12.3 ± 5.8	11.0 ± 5.6	12.5 ± 5.8	<0.001
Intraoperative complications (n,%)	31 (0.5)	11 (0.2)	20 (2.1)	<0.001
Ureteral injury	8 (0.1)	0 (0.0)	8 (0.9)	
Bladder injury	13 (0.2)	7 (0.1)	6 (0.6)	
Bowel injury	6 (0.1)	2 (0.0)	4 (0.4)	

(Continued)

TABLE 3 | Continued

	Total (n = 5952)	High-volume (n = 5016)	Low-volume (n = 936)	*P-value*
Vascular injury	3 (0.1)	2 (0.0)	1 (0.1)	
Obsturator nerve injury	1 (0.0)	0 (0.0)	1 (0.1)	
Postoperative complications (n,%)	29 (0.5)	12 (0.2)	17 (1.8)	<0.001
Bowel obstruction	2 (0.0)	0 (0.0)	2 (0.2)	
Hemorrhage	3 (0.1)	2 (0.0)	1 (0.1)	
Vesicovaginal fistula	5 (0.1)	2 (0.0)	3 (0.3)	
Ureteral fistula	14 (0.2)	6 (0.1)	8 (0.9)	
rectovaginal fistula	1 (0.0)	0 (0.0)	1 (0.1)	
Deep venous thrombosis	4 (0.1)	2 (0.0)	2 (0.2)	

LVSI, lymphovascular space incision; SCC, squamous cell carcinoma; AC, adenocarcinoma; ASC, adenosquamous carcinoma; NACT, neoadjuvant chemotherapy.

margins after primary surgical treatment in 6 years. There was no significant difference every year.

QI 11 is an outcome indicator, which means proportion of patients with a stage T1b disease T-upstaged after surgery. The target is <10%. All patients were reclassified following the 2018 FIGO staging system based on pathology report. As seen in **Table 6**, a total of 2527 (35.7%) patients were restaged. Of these, 2107 patients were upstaged: 1300 (61.7%) due to lymph node metastasis, 453 (21.5%) due to vaginal involvement, 232 (11.0%) due to tumor size, 117 (5.6%) due to parametrial involvement and 5 (0.2%) due to ovarian involvement or distant metastasis. Of these, 14.7% (626/4254) patients with a stage T1b disease were T-upstaged after surgery, which did not reach the target.

QI 12 is an outcome indicator, which means recurrence rate at 2 years in patients with a stage pT1b1 with negative lymph nodes after primary surgical treatment. The target is <10%. After a median follow-up of 42 months (range 0-85), 5844 (82.5%) patients remained free of disease, 448 (6.3%) patients occurred recurrence, and 316 (4.5%) patients had died. The 5-year RFS and OS rate were respectively 91.9% and 94.3%. The 2-year RFS and OS rate were respectively 93.4% and 95.0%. Most of patients (82.1%) recurred within 2 years after surgery. The RFS rate was analyzed in the 2009 and 2018 FIGO staging systems by Kaplan-Meier analysis (**Figure 2**). The 2-year RFS of patients with T1b1N0 was 97.3%, and the 5-year RFS rate was 96.2% in the 2009 FIGO staging system. While in the 2018 FIGO staging system, the 2-year RFS of patients with stage IB1and IB2 was 97.6%, and the 5-year RFS rate was 96.7%. The recurrence rate was significantly reduced after 2018 (P=0.002). Compared minimally invasive radical hysterectomy to open surgery for early-stage cervical cancer, there was no significant difference in patients with T1b1N0 in the 2-year RFS rate (97.3% *vs* 96.7%, P=0.721), or the 5-year RFS rate (96.2% *vs* 96.7%, P=0.721). Similarly, there was no significant difference in patients with T1 disease in the 2-year RFS rate (95.4% *vs* 95.1%, P=0.613), or the 5-year RFS rate (94.0% *vs* 91.0%, P=0.613).

Quality Indicators Related to the Compliance of Management With the Standards of Care

QI 13 is an outcome indicator, which means proportion of patients with a stage T1 disease treated by primary surgery who have undergone lymph node staging according to the ESGO-ESTRO-ESP guidelines. The target is ≥98%. Before surgery, all patients with stage T1 were scheduled to undergo lymph node staging according to guidelines. During surgery, five

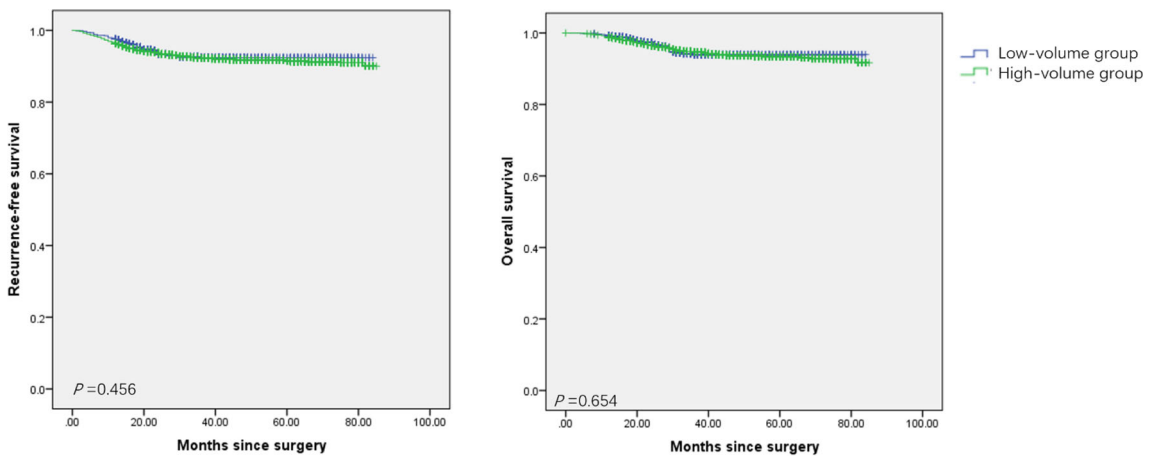

FIGURE 1 | Survival of patients with cervical cancer treated with radical procedures. **(A)** the Kaplan-Meier estimates of recurrence-free survival between the high-volume group and the low-volume group. **(B)** the Kaplan-Meier estimates of overall survival between the high-volume group and the low-volume group.

TABLE 4 | Characteristics of the preoperative assessment of patients.

Items	Number (%)
Pelvic examination	7081
Yes	7081 (100.0)
No	0 (0.0)
Tumor clinical size, mm	20.0 ± 17.7
≤20	3521 (49.7)
>20	3560 (50.2)
Preoperative pathology	7081
Cervical biopsy	7075 (99.9)
No cervical biopsy	6 (0.1)
Cervical conization as indicated	2473
Yes	2466 (99.7)
No	7 (0.3)
Pelvic ultrasound	7081
Yes	7081 (100.0)
No	0 (0.0)
Max diameter of US, mm	20.6 ± 19.7
Pelvic MRI with *contrast* in FIGO stage ≥ IB1	5696
Yes	4575 (80.3)
No	1121 (19.7)
Max diameter of MRI in FIGO stage ≥ IB1, mm	24.2 ± 19.1
Whole-body PET-CT or chest/abdomen/pelvic CT in locally advanced cervical cancer and higher	2082
Yes	1130 (54.3)
No	952 (45.7)
Urinary ultrasound or CTU in locally advanced cervical cancer and higher	2082
Yes	2082 (100.0)
No	0 (0.0)

MRI, magnetic resonance imaging; CT, computed tomography; PET-CT, positron emission tomography/computed tomography; CTU, computed tomography urography.

TABLE 5 | Complications analysis according to the CDC and the CCI.

CDC grade	Number of CDC	CCI scores	Number of CCI
Grade I	2281	8.7	231
		12.2	375
		15.0	154
		17.3	122
Grade II	580	20.9	69
		22.6	115
		24.2	98
		29.6	136
		30.8	45
		32.0	34
Grade IIIa	25	26.2	9
		27.6	11
		33.5	5
Grade IIIb	53	33.7	48
		39.7	5
Grade IVa	2	51.7	1
		58.1	1
Grade IVb	0		
Grade V	0		

CDC, the Clavien–Dindo classification; CCI, the comprehensive complication index.

patients were found to upgrade from stage IA1 to stage ≥IA2 according to the results of frozen sections, yet the agents of the patients refused to expand the operative extent but to choose radiation. After surgery, the final pathologic diagnosis showed that 25 patients were upstaged from high grade squamous intraepithelial lesions, carcinoma in situ, or stage IA1 without LVSI. Furthermore, four patients were found cervical cancer unexpectedly according to the postoperative pathology. Therefore, a total of 34 patients did not undergo lymph node staging. In other words, there were 99.0% (3467/3501) patients with T1 disease underwent lymph node staging. The patients in 2019 had the lowest rate of lymph node staging (P=0.001).

QI 14 is a structural indicator, which means counseling about a possibility of fertility-sparing treatment (FST). The target is 100%. All eligible patients with stage T1 were counseled about the possibility of FST. A total of 128 patients underwent FST.

QI 15 is a structural indicator, which means proportion of patients receiving adjuvant chemoradiotherapy after a primary surgical treatment for a stage pT1b1pN0 disease. The target is <15%. There were 3098 patients with pT1b1N0 according to the 2009 FIGO staging system. Of these, 1100 (35.5%) patients with high risk or intermediate risk required adjuvant therapy. In fact, 876 out of 1100 (28.3%) patients received adjuvant therapy at last. There was no significant difference between the completed group and the uncompleted group in 5-year RFS rates (96.3% *vs* 93.3%, P=0.097) as well as in OS rates (94.9% *vs* 91.5%, P=0.077). Whereas 897 out of 2954 (30.4%) patients with stage IB1 and IB2 according to FIGO 2018 staging system required adjuvant chemoradiotherapy, while 685 (23.2%) patients received adjuvant therapy actually. The rate of patients with pT1b1N0 receiving adjuvant therapy varied significantly from year to year (P=0.001), but neither reached the target.

DISCUSSION

Implementation of a quality management program in surgery has a major impact on survival of cancer patients. The ESGO developed a list of quality indicators for cervical cancer surgery with the aim of auditing clinical practice in 2020. Therefore, we retrospectively analyzed the quality of cervical cancer surgery for 7081 cases from 2014 to 2019 in our hospital according to the ESGO quality indicators for self-assessment and improvement. It showed that most of quality indicators achieved the target, except four quality indicators which were MDT, preoperative investigation, T-upstaged and adjuvant therapy. To the best of our knowledge, this is the first study comprehensively evaluating the quality of surgical treatment of cervical cancer in a single institution, especially in such a high-risk area of cervical cancer as China. Moreover, the large sample size and relatively long duration of follow-up are also the strength of research.

The Quality of Hospital Management

As the incidence rate of cervical cancer has been increasing in China, nearly 1000 patients of cervical cancer every year were treated in the hospital, which contributed to almost the largest number in Shanghai. The effect of hospital volume on outcomes of surgery is related to a surgeon's skill and experience as well as the supporting team (8). Radical surgery performed by a gynecologic oncologist is recommended to be the preferred treatment modality in early-stage disease by ESGO. Different

TABLE 6 | Shift in stage for cervical cancer patients from FIGO 2009 to FIGO 2018.

2009 FIGO	2018 FIGO												
	IA1	IA2	IB1	IB2	IB3	IIA1	IIA2	IIB	IIIC1	IIIC2	IVA	IVB	Total
IA1	1154	17	23	1		3			5				1203
IA2		169	10	1		2							182
IB1			1749	855	120	280	62	31	493	23	1		3614
IB2			20	84	174	42	64	26	215	15			640
IIA1			80	100	21	321	60	30	263	9			884
IIA2			7	22	40	36	107	30	215	26		2	485
IIB				2	1	5	6	11	29	7	1	1	63
IVA											3		3
IVB												7	7
Total	1154	186	1889	1065	356	689	299	128	1220	80	5	10	7081

from the sub-specialty training program in gynecologic oncology in Europe, the surgical qualification grading system has been established for years in China. Increasing studies showed that high surgical volume of cervical cancer was a favorable prognostic factor for operative outcomes and peri-operative complication rates (18–21). Latest studies stressed that a steady trend of reduction in disease recurrence risk is associated with increased surgeon experience (22, 23). The 3-year RFS was significantly lower at the beginning of a surgeon's learning path compared to the time he had been adequate experience. Hence, we classified the surgeons who were at the beginning of learning path or did not perform the radical treatment frequently into the low-volume group. We found that there was no significant difference in treatment outcomes no matter what surgeons were in the learning path. This may because surgeons in either group could meet adequate surgical standards after training of the surgical qualifications grading system. However, surgeons who had adequate experience conferred significant benefit in terms of a shorter hospital stay, more free surgical margins, and lower risks of complications.

The Quality of Management Before Surgery

An accurate diagnosis guides patient management and informs prognosis. In our study, not all the patients reached the goals, especially whole-body assessment in patients of locally advanced cervical cancer. There may be some reasons. First, surgeons may not be fully aware of the importance of imaging. Second, imaging diagnoses were not accurate interpretation so that surgeons could not get effective information. Third, the examination of MRI, CT or PET-CT was expensive for some of patients in China. Adequate clinical staging with imaging and vaginal assessment is crucial for decisions on choice of treatment and tailoring of surgery. On contrary, inaccurate preoperative assessment led to increasing rates of postoperative upgrading and adjuvant chemoradiotherapy. Fortunately, the completion rate of imaging was increasing year by year. Overall preoperative evaluation should be more emphasized and improved in our hospital.

Multi-disciplinary care is internationally recognized as best practice in treatment planning and care. However, the consultation system but not MDT was performed in our

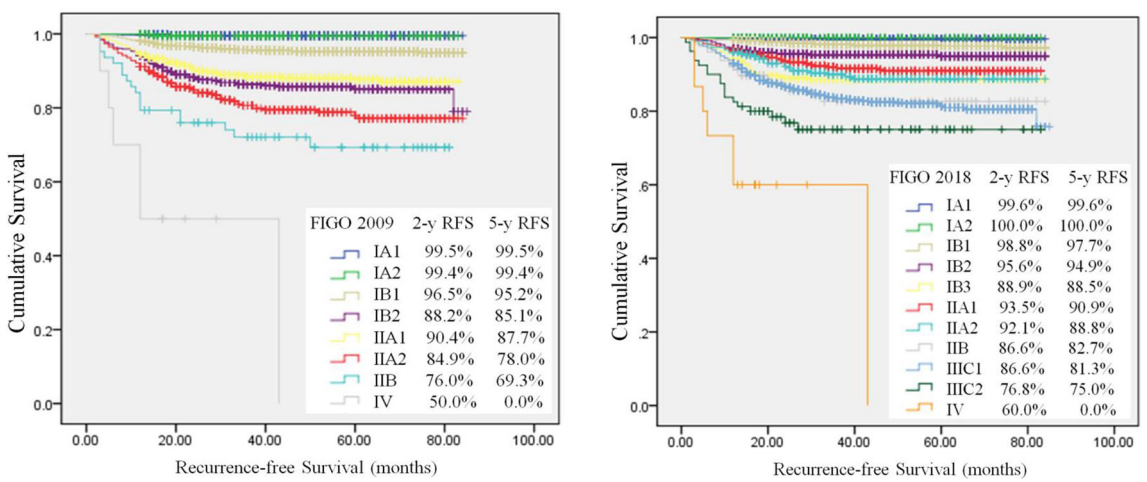

FIGURE 2 | Kaplan-Meier analysis of recurrence–free survival. Differences between use of 2009 FIGO (log-rank test, $P<0.001$) and 2018 FIGO (log-rank test, $P < 0.001$) staging systems.

hospital if the patient was in sophisticated or dangerous situation and need to discuss with different departments. For example, all patients who met the standard of FST were informed and discussed with anesthesiologists, obstetricians, or endocrinologists to provide a whole-process treatment plan. The MDT system should be established in our hospital.

The Quality of Management During Surgery

In the study, only 1.0% patients did not perform lymph node staging in the primary surgery due to upstaging or incidental finding of cervical cancer. Accurate preoperative evaluation could avoid missing lymph node staging. Identification of sentinel lymph nodes and its ultra-staging is highly recommended because it increases staging accuracy (24–26). But sentinel lymph nodes biopsy was attempted just in 24 patients. The ABRAX trial recently showed that if lymph node involvement is detected intra-operatively, further pelvic lymph node dissection and radical hysterectomy should be avoided (27). But all the patients who were found positive lymph nodes underwent radical hysterectomy further in the hospital, and even the patients with ≥IIB underwent radical hysterectomy. There may be some reasons. First, most Chinese patients had a deeply rooted prejudice that surgery was the best treatment for cancer. Second, compared with radiologist, gynecologists were more likely to recommend surgery. Third, the problem of side effects of radiotherapy, especially the long-term side effects, has not been solved, which directly affects the subsequent quality of life, especially for young patients. Fourth, it had been a great challenge for doctors to treat the recurrence after radiotherapy. Fifth, lack of radiotherapy equipment leads to the choice of surgery for patients but not wait for radiotherapy.

The Quality of Management After Surgery

The surgical complications had been still reported in the ranking system in the hospital, while the ESGO recommend the CDC system and CCI, which are widely applied in many fields of surgery, including cervical cancer (13, 14, 28, 29). Thereafter, the CDC and the CCI should be introduced in the hospital so as to improve patient management. Urologic complication is an important quality indicator because it may lead to increased rates of reoperation and readmission, an increased length of stay, and increased litigations. The incidence of urologic complications varies from 0% to 6.0% (ureteral), 0.1% to 3.0% (bladder) and 0.4% to 4.5% (fistula) (30). In our hospital, urologic complications were seen in 0.7% of the cohort, and the postoperative genitourinary fistulas was 0.3%. The significant lower incidence rate may be attributed to the patient characteristics, and the surgeon's operative experience. Previous studies showed that the proportion of urinary fistulas was twice that of the intraoperative urinary injuries (30, 31). However, we found that the proportion of intraoperative and postoperative of urinary injuries was similar. This may be due to the prophylactic placement of ureteral stent during operation and the control of postoperative infection, which reducing the ischemic damage of ureter and bladder. Furthermore, the incidence of ureteral injury and bladder injury was also similar in the study, which was consistent with previous studies (32, 33).

The study showed that most of patients recurred within 2 years after surgery, and the 2-year recurrence rate of patients with pT1b1N0 was 2.7% in our study, which was similar to previous studies that the recurrence rate of patients with pT1b1N0 was less than 10% within 2 years of primary surgery, irrespective of the neo-adjuvant or adjuvant treatment strategy (8, 34, 35). Furthermore, the LACC trial in 2018 (34) showed that minimally invasive radical hysterectomy was associated with lower RFS rates than open radical hysterectomy (91.2% *vs* 97.1%, HR 3.74). A resent respective study (35) also found that the recurrence rate in the open surgery was significantly lower than that in minimally invasive radical hysterectomy (7.5% *vs* 9.1%, P=0.43). However, in this study there was no significant difference in the RFS rate for patients with T1b1N0 or T1 between open surgery and minimally invasive radical hysterectomy. This may be related to the small number of patients who underwent open surgery. In fact, patients had been carefully and fully counseled about the surgical outcomes and oncologic risks of the different surgical approaches after the LACC trial according to the NCCN guidelines. But open surgery was still limit (1.9%). Here are some reasons. Minimally invasive surgery was associated with reduced blood loss, shorter hospital stays, and fewer postoperative complications compared to open surgery. Some measures such as no use of uterine manipulator and tumor enclosing before colpotomy had been taken to improve tumor-free technology in the minimally invasive radical hysterectomy. A multicenter, retrospective, observational cohort study showed that avoiding the uterine manipulator and using maneuvers to avoid tumor spread at the time of colpotomy in minimally invasive surgery was associated with similar outcomes to open surgery (36). Several prospective clinical trials on the outcome of different surgical approaches have been also launched in the hospital. It turned out that the recurrence rate was significantly lower after 2018. A pilot study of forty-eight patients with early-stage who underwent vaginal-assisted gasless laparoendoscopic single-site radical hysterectomy also showed no relapsed in the hospital (37). Prospective studies and longer follow-up periods should be performed to further evaluate the oncological outcomes.

In the study, more than 30% patients with T1b1N0 were required adjuvant therapy. On the contrast, 20% patients chose to observe rather than receiving adjuvant therapy. In fact, observation is an alternative option in experienced teams when adequate type of radical hysterectomy has been performed according to the ESGO-ESTRO-ESP guidelines (38). Actually, there was no significant difference between the completed group and the uncompleted group in 5-year RFS rates in the study. So accurate preoperative assessment, appropriate treatment options, adequate radical surgery, and close follow-up will reduce the incidence of adjuvant therapy.

Limits of the Study

There are several limitations of this study. First, it is a retrospective study and there may be unrecognized bias. Second, the objectivity of the current study is dependent on accurate charting and documentation, which could be incomplete or inaccurate sometimes. Third, some patients received adjuvant chemotherapy or radiotherapy after surgery.

But not all patients received adjuvant therapy in the same institution, so the effect of variation in irradiation technique and chemotherapeutic regimens cannot be eliminated. Fourth, our data only reflect a single center experience. Further investigation at multiple centers is needed.

CONCLUSION AND FUTURE RESEARCH

In this Chinese cohort, we found that most of quality indicators achieved the goals even before the publication of the ESGO quality indicators, except four quality indicators which concentrated on MDT, preoperative investigation, T-upstaged and adjuvant therapy after operation. In future, the MDT, the CDC system, and the CCI should be established. Overall preoperative evaluation should be emphasized and improved in the hospital. Multicenter prospective studies and longer follow-up periods should be performed to further evaluate the oncological outcomes. Furthermore, such a study could conveniently be conducted at a hospital level in order to draw up an inventory of strategies and recommend lines of improvement. The ESGO quality indicators should be popularized and applied in China to guarantee quality of surgery and homogeneous treatment throughout the country to patients with cervical cancer.

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 human participants were reviewed and approved by the institutional review board of the Obstetrics and Gynecology Hospital, Fudan University. Written informed consent was exempted because of a retrospective study.

AUTHOR CONTRIBUTIONS

KH and JZ conceived and designed the study. YD designed the study, performed the statistical analysis, and drafted the manuscript. JZ, XZ, and JQ performed the data collection and analysis. All authors read and critically revised the manuscript for intellectual content and approved the final manuscript.

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Trends in Surgical Morbidity and Survival Outcomes for Radical Hysterectomy in West China: An 11-Year Retrospective Cohort Study

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Objectives: To vertically analyze the trend of surgical approaches, demographics, surgical morbidity, and long-term survival outcomes of early-stage cervical cancer over the past 11 years and to determine whether there have been any significant changes.

Methods: A total of 851 patients with consecutive International Federation of Gynecology and Obstetrics (FIGO) 2009 stage IA–IIA cervical cancer diagnosed between January 2008 and June 2018 at a single center in China were included in this retrospective study. Trends in the rate of minimally invasive surgery (MIS), demographics, surgical morbidities, and long-term survival outcomes were determined. We categorized patients into two groups according to their year of operation. The demographics, pathological factors, surgical morbidity, and long-term survival outcomes were compared between these two groups.

Results: Regarding the surgical approach, there was a significant increase in the rate of laparoscopic radical hysterectomy (LRH) performed over the study period, from 7.8% in 2008 to 72.5% in 2018 ($p < 0.0001$). The mean age of patients who underwent abdominal radical hysterectomy (ARH) has increased slightly from 2008 to 2018, and those who underwent ARH in the second half of the study period (2014–2018) were significantly older (45.01 vs. 47.50 years; $p = 0.001$). The most impressive changes over the past 11 years have occurred in the surgical morbidity in both the ARH and LRH groups. The overall surgical morbidity decreased from 29.2% in 2008 to 11.9% in 2018, with an annual rate of 1.57%. The median estimated blood loss volume of the ARH group was 500 ml (range 50–2,000) in the first few years compared to 400 ml (30–2500) in the last few years of the study period ($p < 0.0001$), which in the LRH group was 350 ml (range 150–800) and 150 ml (range 5–1,000), respectively ($p < 0.0001$). Similarly, allogeneic blood transfusions and hospital stay have all decreased dramatically over time in both approaches. On the other hand, our study did not reveal any significant statistical changes in long-term survival outcomes over the follow-up period in either group.

Conclusions: The findings of our study demonstrate that great progress in surgically managed cervical cancer has been made over the last decade in West China. Our retrospective study demonstrated that the year of operation does not appear to influence the long-term survival, but the surgical morbidity impressively decreased over the study period in both the ARH and LRH groups, which reflects that the higher hospital surgical volume for radical hysterectomy (RH) was not associated with lower survival outcomes but related to the reduction of surgical morbidity.

Keywords: surgical morbidity, radical hysterectomy, cervical cancer, oncology, survival

INTRODUCTION

Globally, cervical cancer (CC) continues to be the fourth most common cancer among females, and 85% of new cases and 90% of deaths occur among people from socioeconomically weaker sections of society (1). China reported 98,900 new cases of CC and 30,500 deaths in 2015 (2). Previous guidelines (3) indicate that either open or minimally invasive surgery (MIS) is an acceptable surgical treatment to radical hysterectomy (RH) in patients with early-stage (IA2 to IIA) CC. These recommendations have led to the widespread use of the MIS approach in recent years after the implementation of laparoscopy during the 1990s. However, Ramirez et al. (4) reported a multicenter randomized controlled trial (RCT), namely, the LACC trial, which showed that MIS was associated with lower 4.5-year disease-free survival (DFS), progression-free survival (PFS), overall survival (OS), and disease-specific survival rates and a higher local recurrence rate than the laparotomic approach. Several multicenter retrospective studies from different countries have validated this finding (5–8). Reasons beyond these results are unclear. Some studies focused on the learning curves of the surgeons and discussed that the learning curves of MIS probably caused the decline in survival outcomes (9, 10).

However, the management of surgical patients involved the whole medical team, not only surgeons. We wondered whether team proficiency affects the survival outcomes. Previous studies involving women with early or locally advanced CC have demonstrated improvements in guideline compliance and outcomes at high-volume centers (11–16). However, there is currently no study involving the change of survival by years in a single center.

Therefore, this study aims to vertically analyze the trend of demographics, surgical approaches, and long-term survival outcomes of early-stage CC over the past 11 years, determine whether there have been any significant changes, and investigate the prognostic impact of different surgical year groups in patients with early CC undergoing RH in open and laparoscopic approaches.

METHOD

Study Design and Patient Enrollment

A total of 1,765 patients with consecutive International Federation of Gynecology and Obstetrics (FIGO) (2009) stage IA–IIA CC diagnosed between January 2008 and June 2018 at a single center in China were screened for eligibility in this retrospective study.

Inclusion criteria were the following: 1) patients underwent standard surgical treatment according to the National Comprehensive Cancer Network (NCCN) guidelines, a modified RH with pelvic lymphadenectomy (PLND) in stage IA1 with LVSI and stage IA2, and an RH with PLND with/without para-aortic lymphadenectomy in stage IB to IIA. 2) Patients have a histological subtype of squamous cell carcinoma, adenocarcinoma, or adenosquamous carcinoma regardless of histological grading.

Exclusion criteria were the following: 1) patients with incomplete follow-up data and 2) patients with severe fundamental diseases or pregnant.

Complete information, including demographics, clinical, and pathological information, was extracted from the Hospital Information System by two investigators. The demographics included age, menstruation, and body mass index (BMI); the clinical information included diagnosis, FIGO (2009) stage, surgical approach, date of surgery, hospital stay, duration of surgery, estimated blood loss, number of lymph node resected, and adjuvant treatment; the pathological information included histologic subtype, grading, lymphovascular space invasion (LVSI), stromal invasion depth, parametrial involvement, vaginal margin involvement, and lymph node metastasis. Recurrence was defined by clinical findings and imaging examinations, and all recurrences were confirmed by pathological analysis. This study was approved by the Institutional Ethics Committee of West China Second University Hospital (2019078), and all participants orally consented to the use of their medical records during telephone follow-up.

Study Outcomes

The primary outcome of interest was PFS and OS in the whole study period and different phases. Secondary outcomes included the rate of the MIS approach versus the open approach for CC over the study period and trends in demographics and perioperative outcomes. Perioperative outcomes included blood transfusion, estimated blood loss, hospital stay, operation time, and postoperative complications, which are defined as those occurring during hospitalization, including urinary tract complications, paralytic ileus, incisional hernia, and deep venous thrombosis.

Statistical Analysis

SPSS 23.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. $p < 0.05$ was set to indicate statistical significance.

Comparisons of continuous variables were conducted with parametric methods if assumptions of normal distribution were confirmed. Non-normally distributed variables and categorical data were compared between laparoscopic RH (LRH) and abdominal RH (ARH) groups with the use of non-parametric tests. Survival curves were generated by the Kaplan–Meier (K-M) method analyzed with log-rank test and multivariable Cox proportional hazards regression models. The enumeration data were analyzed *via* the chi-square test. The measurement data were analyzed *via* t-test and the Mann–Whitney U test between two groups while *via* ANOVA and the Kruskal–Wallis H test between multiple groups.

RESULT

After exclusions, a total of 851 women who were diagnosed with CC and had an RH (Querleu and Morrow type C2) in West China Second Hospital between January 1, 2008, and June 31, 2018, were included in this study. Among them, 581 (68.3%) had an abdominal approach, and 270 (31.7%) had a minimally invasive approach. All included operations were completed by five surgeons in our department. Regarding the surgical approach, there was a significant increase in the rate of LRH performed over the study period, from 7.8% in 2008 to 72.5% in 2018 ($p < 0.0001$). Our hospital began to carry out a large number of LRH operations in 2014. In the following years, it has increased at a stable rate, with an average annual increase of 13.2% of patients doing LRH (**Figure 1**).

As shown in **Table 1** and **Figure 2**, the mean age of patients who underwent ARH has increased slightly from 2008 to 2018, and those who underwent ARH in the second half of the study period (2014–2018) were significantly older (45.01 vs. 47.50 years; $p = 0.001$). The mean BMI of patients who underwent ARH had no upward or downward trend over time, which fluctuated in the range of 21.5–23 ($p = 0.064$). On the other hand, the age and the BMI of patients who underwent LRH had no statistically significant change over the past 11 years.

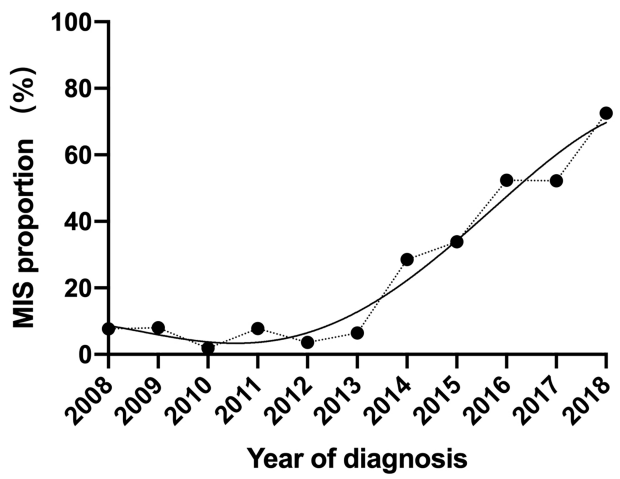

FIGURE 1 | Crude rate of radical MIS for cervical cancer over study period.

There was no significant shift in the proportion of patients with squamous cell carcinoma versus adenocarcinoma or the proportion of each FIGO stage in the ARH group over the past 11 years. Patients subjected to ARH have been diagnosed as more proportion of G1/G2 (11.9 vs. 19.2; $p = 0.024$) and more parametrial invasion (9.4 vs. 18.7; $p = 0.002$) in the second half of the study period, with no statistically significant change in the stromal invasion, incidence of pelvic lymph node metastases, or positive LVSI. Similarly, the pathological variables were analyzed in the LRH group, and the FIGO stage was the only variable that significantly changed over time ($p = 0.032$), with more stage IB1 in the last few years of the study period.

The most impressive changes over the past 11 years, however, have occurred in the operative and postoperative short-term outcomes in both the ARH and LRH groups (**Table 2**). The median estimated blood loss volume of the ARH group was 500 ml (range 50–2,000) in the first few years compared to 400 ml (30–2,500) in the last few years of the study period ($p < 0.0001$), which in the LRH group was 350 ml (range 150–800) and 150 ml (range 5–1,000), respectively ($p < 0.0001$). Estimated blood loss volume, allogeneic blood transfusions, and hospital stay have all decreased dramatically in both approaches **Figure 3**. The median length of hospital stay of patients undergoing the open approach was 9 days (range 6–46) compared to 10 days (range 5–27) for the MIS approach in the first few years. By the last few years of the study period, the median length of hospital stay had significantly decreased to 7 days (range 3–24) following the open approach compared to 6 days (range 3–19) for MIS ($p < 0.0001$ in both approaches). The proportion of allogeneic blood transfusions of patients undergoing the open approach was 25.1% in the first few years compared to 6.2% in the last few years of the study period ($p < 0.0001$), which in the LRH group was 16.7% and 1.4%, respectively ($p = 0.025$). Although there were fluctuations, the median operation time of ARH remained stable over the past 11 years, floating around 220 min (3 h 40 min), whereas, in the LRH group projected, there was a downward trend, 275 min in 2008 compared to 240 min in 2018, but not statistically significant.

The median follow-up duration was 77.2 and 62.5 months in the ARH and LRH groups, respectively. The overall 3- and 5-year OS of the ARH group is 94.1% and 92.3%, respectively. The overall 3- and 5-year OS of the LRH group is 95.6% and 94.8%, respectively. When stratified by years of diagnosis, the chi-square test did not reveal any significant statistical changes of long-term survival outcomes over the follow-up period in either group (**Table 3** and **Figure 4**). Similarly, K-M survival analysis revealed no statistically significant differences between the 2008–2013 group and the 2014–2018 group in OS and PFS regardless of the surgical approaches (all p-value >0.05) (**Figure 5**).

DISCUSSION

In this research, we provided no differences in survival across the years despite the spread of the MIS approach to perform the RH; however, the surgical outcomes significantly improved over the years regardless of approach. Our study was based on a

TABLE 1 | Baseline characteristics in ARH and LRH groups.

Characteristics	ARH (N = 581)			LRH (N = 270)		
	2008–2013 (N = 295)	2014–2018 (N = 286)	p-Value	2008–2013 (N = 18)	2014–2018 (N = 252)	p-Value
Age (years), mean ± SD	45.01 ± 8.85	47.50 ± 8.76	**0.001**	46.61 ± 10.97	46.66 ± 9.11	0.982
BMI (kg/m^2), mean ± SD	22.11 ± 3.74	22.64 ± 2.98	0.064	22.44 ± 3.52	23.13 ± 3.81	0.460
FIGO stage, N (%)			0.522			**0.032**
IA	21 (7.1)	14 (4.9)		5 (27.8)	24 (9.5)	
IB1	121 (41)	118 (41.3)		7 (38.9)	160 (63.5)	
IB2-IIA	153 (51.9)	154 (53.8)		6 (33.3)	68 (27.0)	
Grade, N (%)			**0.024**			0.778
G1/G2	35 (11.9)	55 (19.2)		6 (33.3)	64 (25.4)	
G3	240 (81.4)	206 (72)		10 (55.6)	151 (59.9)	
Gx	20 (6.8)	25 (8.7)		2 (11.1)	37 (14.7)	
Histology, N (%)			0.357			0.696
Squamous carcinoma	251 (85.1)	230 (80.4)		15 (83.3)	215 (85.3)	
Adenocarcinoma	30 (10.2)	34 (11.9)		3 (16.7)	29 (11.5)	
Adenosquamous carcinoma	13 (4.4)	21 (7.3)		0	6 (2.4)	
Other	1 (0.3)	1 (0.3)		0	2 (0.8)	
Stromal invasion			0.235			0.511
>1/2	178 (66.9)	151 (61.9)		10 (58.8)	99 (50.5)	
<1/2	88 (33.1)	93 (38.1)		7 (41.2)	97 (49.5)	
Positive lymph node metastasis	54 (22.0)	49 (19.5)	0.504	2 (18.2)	46 (23.2)	0.462
Parametrial invasion	25 (9.4)	46 (18.7)	**0.002**	4 (26.7)	20 (10.0)	0.069
Lymphovascular space invasion	133 (45.1)	105 (36.7)	0.536	5 (27.8)	96 (38.1)	0.382

ARH, abdominal radical hysterectomy; LRH, laparoscopic radical hysterectomy; BMI, body mass index; FIGO, International Federation of Gynecology and Obstetrics.
The bold values mean statistically significant (p< 0.05).

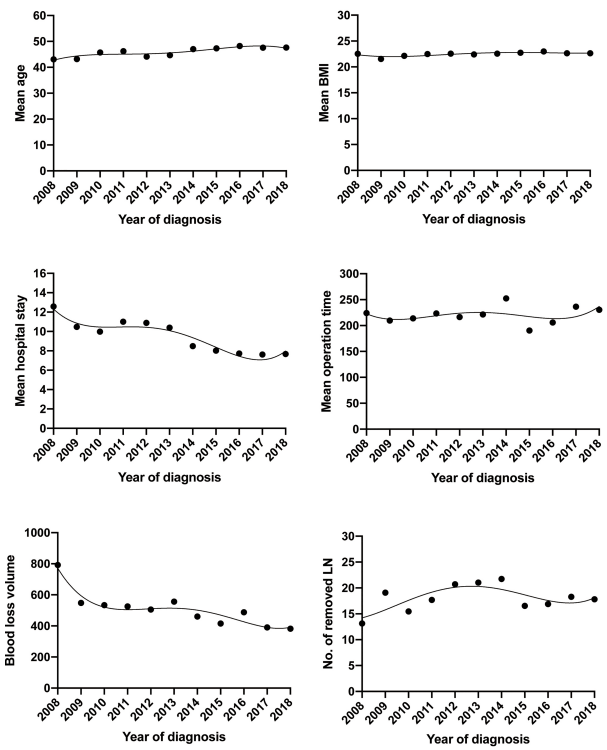

FIGURE 2 | Trends of demographics and surgical morbidities with open approach of early-stage cervical cancer over the past 11 years.

TABLE 2 | Perioperative outcomes in ARH and LRH groups.

Characteristics	ARH (N = 581)			LRH (N = 270)		
	2008–2013 (N = 295)	2014–2018 (N = 286)	p-Value	2008–2013 (N = 18)	2014–2018 (N = 252)	p-Value
Blood transfusion	62 (25.1)	16 (6.2)	**<0.001**	2 (16.7)	3 (1.4)	**0.025**
Hospital stay, median (range, days)	9 (6–46)	7 (3–24)	**<0.001**	10 (5–27)	6 (3–19)	**<0.001**
Operation time, median (range, min)	210 (90–510)	200 (55–2500)	0.105	255 (150–360)	235 (65–450)	0.122
Estimated blood loss, median (range, ml)	500 (50–2000)	400 (30–2500)	**<0.001**	325 (100–800)	150 (5–1000)	**<0.001**
Postoperative complication						
No	209 (70.8)	252 (88.1)	**<0.001**	12 (66.7)	226 (89.7)	**<0.001**
Urinary tract complications	56 (19.0)	22 (7.7)		3 (16.7)	18 (7.2)	
Paralytic ileus	15 (5.1)	7 (2.5)		2 (11.1)	4 (1.5)	
incisional hernia	8 (2.7)	2 (0.7)		0 (0.0)	2 (0.8)	
Deep venous thrombosis	7 (2.4)	3 (1.0)		1 (5.6)	2 (0.8)	

ARH, abdominal radical hysterectomy; LRH, laparoscopic radical hysterectomy.
The bold values mean statistically significant (p< 0.05).

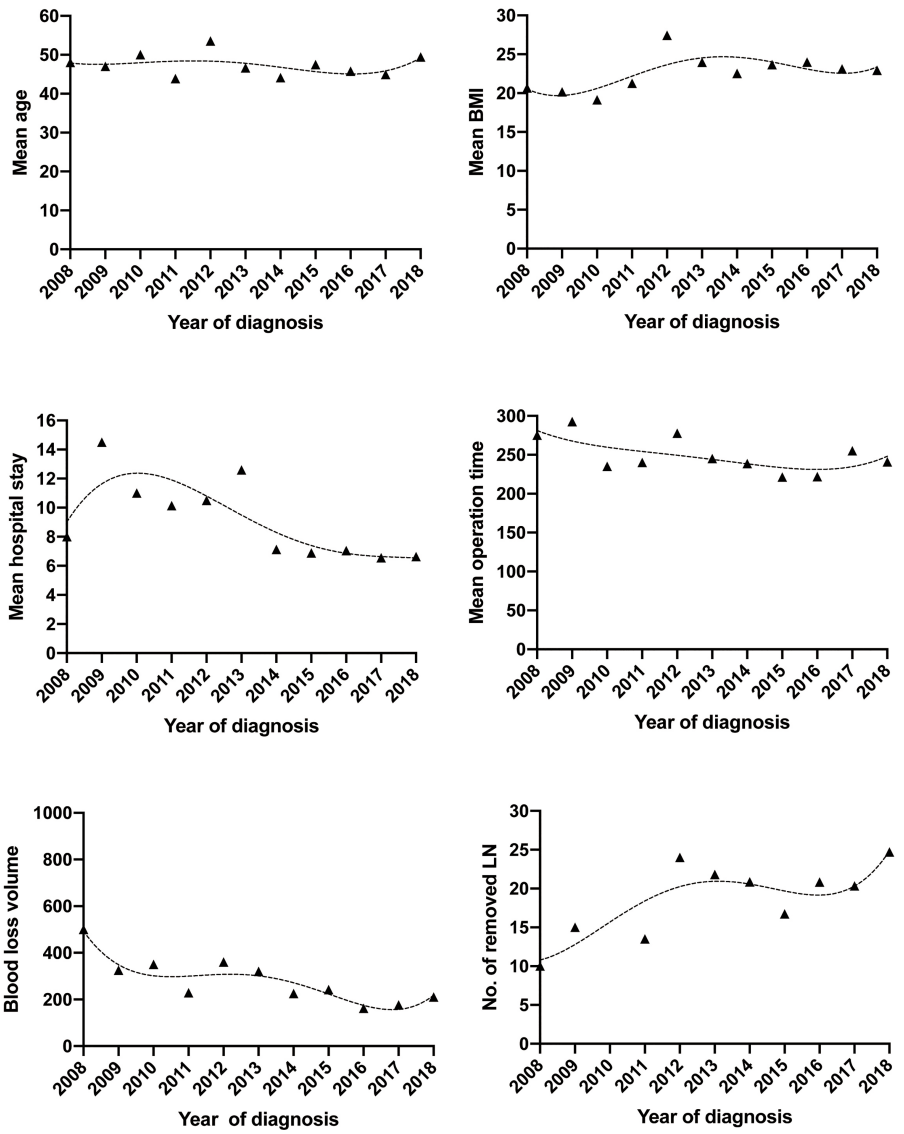

FIGURE 3 | Trends of demographics and surgical morbidities with MIS approach of early-stage cervical cancer over the past 11 years. MIS, minimally invasive surgery.

TABLE 3 | Survival outcomes of different years in ARH and LRH groups.

		2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total	p-Value
	Laparotomy cases	12	23	52	83	83	72	75	82	61	43	25	581	
ARH group	Three-year PFS (%)	100	95.7	94.2	90.4	94.3	94.4	92.0	91.5	91.8	100	92	93.3	0.763
	Three-year OS (%)	100	95.7	96.2	91.6	94.3	94.4	93.3	92.7	93.4	100	92.0	94.1	0.845
	Five-year PFS (%)	100	91.3	94.2	89.2	94.3	94.4	89.3	88.7	90.2	100	92.0	92.2	0.483
	Five-year OS (%)	100	91.3	94.2	89.2	94.3	94.4	89.3	89.0	91.8	100	92.0	92.3	0.489
	Laparoscopy cases	1	2	2	5	3	5	30	42	67	47	66	270	
LRH group	Three-year PFS (%)	–	–	–	–	–	–	90.0	95.2	98.5	95.7	92.4	94.8	0.429
	Three-year OS (%)	–	–	–	–	–	–	93.3	95.2	100	95.7	92.4	95.6	0.195
	Five-year PFS (%)	–	–	–	–	–	–	90.0	92.9	98.5	95.7	92.4	94.4	0.405
	Five-year OS (%)	–	–	–	–	–	–	93.3	92.9	98.5	95.7	92.4	94.8	0.540

ARH, abdominal radical hysterectomy; LRH, laparoscopic radical hysterectomy; OS, overall survival; PFS, progression-free survival.

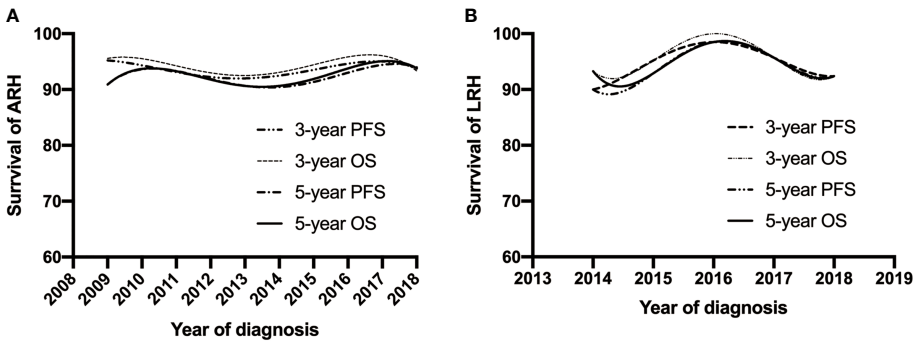

FIGURE 4 | Trends of the OS and PFS adjusted for clinicopathological factors for patients with open approach **(A)** and MIS approach **(B)** of early-stage cervical cancer over the past 11 years. MIS, minimally invasive surgery; OS, overall survival; PFS, progression-free survival.

hypothesis of the surgical volume–outcome relationship that was originally reported in 1979 (17). The central concept of the volume–outcome relationship is that a larger surgical volume is associated with decreased surgical morbidity and mortality. Thus, we want to know whether the surgical morbidity and survival outcome of patients will be improved with the accumulation of the surgical volume and surgeons' proficiency in our center.

The selection criteria for RH remained relatively stable over the research period, which allowed us to describe the change of patient demographics, pathology characteristics, surgical morbidity, and long-term survival outcomes with a small selection bias. These findings suggest that the year of operation does not appear to influence long-term survival. However, surgical morbidity has impressively decreased over the past 11 years in both the ARH and LRH groups.

The finding that surgical morbidity has decreased over the research period is not surprising. Many studies have indicated the same finding, which is almost indisputable (18, 19). The LRH for early CC has been utilized in developed countries since the early 1990s. However, in the underdeveloped areas of Western China, the introduction of this technology is about in the early 2010s. According to this study, our center, the most influential and technologically advanced tertiary hospital in this region of China, began to develop LRH rapidly in 2013–2014, and there were a small number of cases per year before 2014. Unlike LRH, ARH has been the standard approach for surgical treatment of early-stage CC for several decades. The hospital stay length and blood loss volume had still decreased slightly with the operation time remaining stable over the past 11 years. These changes have occurred with no improvement in the surgical procedure.

In our study, 5-year PFS and 5-year OS had no clear upward or downward trend over the study period, and the K-M survival curve showed no difference in the two groups divided by year. Whether the surgical volume affects survival remains controversial. Matsuo et al. (19) indicated that the hospital volume for RH may be a prognostic factor for early-stage CC and that high-volume centers are associated with decreased local recurrence risk and improved survival. A systematic review and meta-analysis suggested an association between high surgical volume and improved oncologic outcomes in MIS-RH for CC (20). However, Aviki et al. (21) indicated that there was no association between hospital volume and survival. A recent study also suggested that high-volume surgeon is not associated with better 5-year DFS and OS in cervical patients undergoing LRH (22).

The findings of our study demonstrate that great progress in surgically managed CC has been made over the last decade. The surgeon's learning curve may be the explanation for the reduction in blood loss and blood transfusion. Previous studies

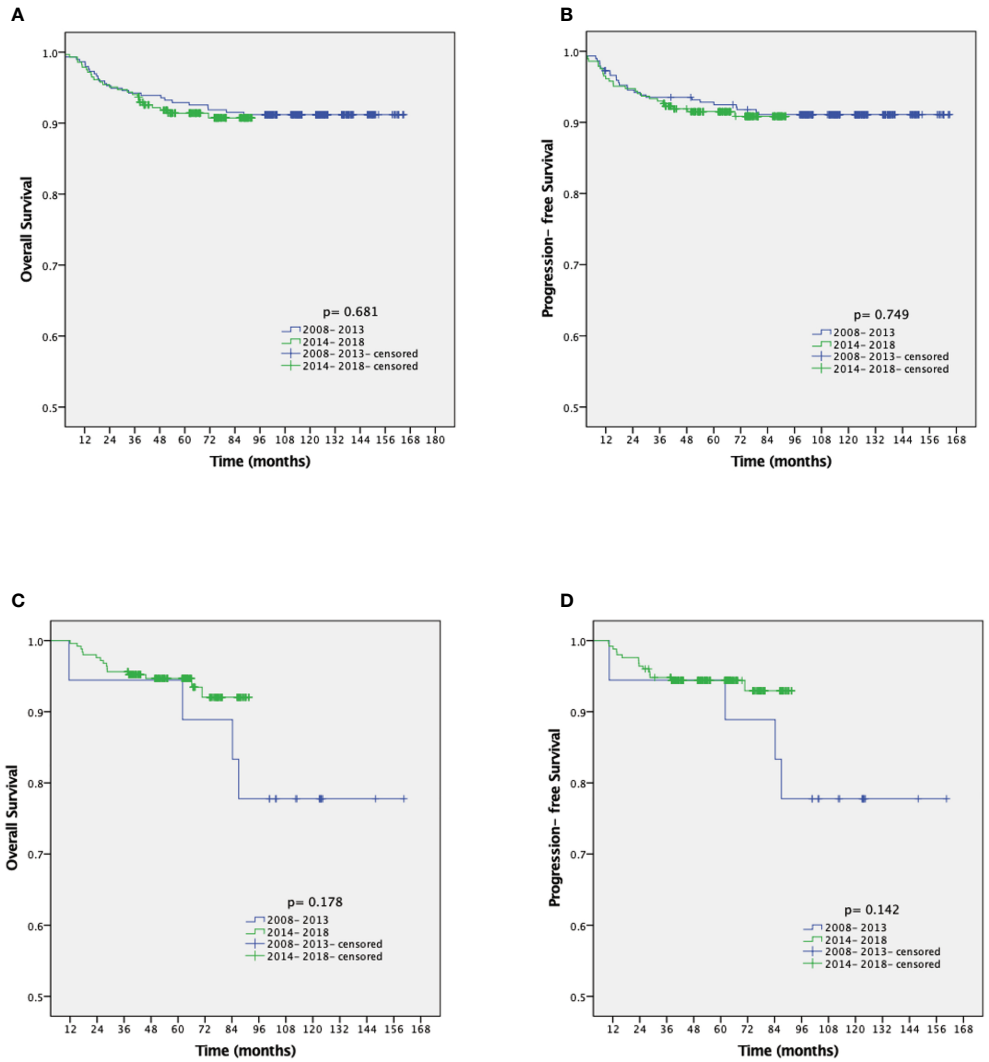

FIGURE 5 | Kaplan-Meier analysis of the OS and PFS for patients with open approach stratified by year of diagnosis **(A, B)**. Kaplan-Meier analysis of the OS and PFS for patients with MIS approach stratified by year of diagnosis **(C, D)**. OS, overall survival; PFS, progression-free survival; MIS, minimally invasive surgery.

have examined the learning curve in terms of the number of cases needed to obtain a relatively low hemorrhage volume, which is usually less than 50 cases (9, 23, 24). On the other hand, according to our data, blood loss has actually been decreasing slightly over the past 11 years. This may be explained by the assumption that surgeons are continuing to improve their surgical technique with time and experience after the early stage of the learning curve.

The main strength of this study is the large sample size. In addition, this is the first study to vertically analyze the trend of demographics, surgical approaches, surgical morbidity, and long-term survival outcomes of early-stage CC in West China. However, our study has several limitations. First, this is a retrospective study, and there may be unmeasured factors that confound the findings. Due to the nature of the retrospective study, it is difficult to achieve a balanced baseline between the two groups. In the ARH group, there is a higher rate of stage IB2-IIA (52.8% vs. 27.4%) and Grade 3 (76.8% vs. 59.6%). This is a subjective tendency based on experience that surgeons tend to choose laparotomy for patients with more severe conditions and laparoscopy for patients with lighter conditions. Second, tumor size data are not available in most cases, which may significantly impact the surgical outcome. Last, this is a single-center study, and the significant differences of institutional variables are unknown.

In conclusion, our retrospective study demonstrated that the year of operation does not appear to influence the long-term survival, but the surgical morbidity impressively decreased over the study period in both the ARH and LRH groups, which reflects that the higher hospital surgical volume for RH was not associated with lower survival outcomes but related to the reduction of surgical morbidity.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Medical Ethics Committee of West China Second Hospital, Sichuan University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HJ and ZL contributed to the data collection and study design. YY, PZ, and YL contributed to the data analysis. All authors contributed to the data interpretation, manuscript preparation, editing, and review.

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Implications of Persistent HPV52 and HPV58 Positivity for the Management of Cervical Lesions

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Objective: This study aimed to compare the variability of HPV16/18/52/58 subtype infections in patients with different cervical lesions, to explore the guiding significance of persistent positive HPV subtypes 52 and 58 in the stratified management of cervical lesions, and to determine the appropriate management model.

Method: This study was conducted through a retrospective analysis of 244,218 patients who underwent HPV testing at the Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University from September 2014 to December 2020 to examine the distribution of different types of HPV infection. From March 2015 to September 2017, 3,014 patients with known HPV underwent colposcopy to analyze high-risk HPV infection for different cervical lesions. Meanwhile, from September 2014 to December 2020, 1,616 patients positive for HPV16/18/52/58 alone with normal TCT who underwent colposcopy in our hospital were retrospectively analyzed for the occurrence of cervical and vulvovaginal lesions, with colposcopic biopsy pathology results serving as the gold standard for statistical analysis.

Result: Analysis of 244,218 patients who had HPV tested revealed that the top 3 high-risk HPV types were HPV52, HPV58, and HPV16. Further analysis of 3,014 patients showed that 78.04% of patients referred for colposcopy had HPV16/18/52/58 alone. Among high-grade squamous intraepithelial lesions (HSIL) and cervical cancer, the most common is HPV16, followed by HPV58 and then HPV52 ($p < 0.05$). A total of 1,616 patients with normal TCT who were referred for colposcopy due to HPV16/18/52/58 infection were further analyzed. Based on pathological findings in lesions of HSIL and CC, HPV16 is the most common, followed by HPV58 and then HPV18 ($p < 0.05$). In the 1,616 patients analyzed, high-grade vulvovaginal lesions were detected, with HPV58 being the most common, followed by HPV16 and then HPV52 ($p < 0.05$).

Conclusion: 1. In patients with positive HPV58 alone and normal TCT, the indications for colposcopy may be relaxed, with particular attention paid to the possibility of vulvar and vaginal lesions.2. Patients with a positive HPV type 52 alone and normal TCT may be considered for a follow-up review and, if necessary, a colposcopy.3. The development of a more suitable HPV vaccine for the Asian population, such as HPV16/18/52/58, may better protect women's health.

Keywords: cervical lesions, human papillomavirus (HPV), colposcopic biopsy, human papillomavirus 52 (HPV52), human papillomavirus 58, cervical cancer

INTRODUCTION

Cervical cancer is the 4th most common cancer among women (1) and is more prevalent in developing countries than in developed countries (2).

Persistent high-risk human papillomavirus (HR-HPV) infection with HPV is a major cause of cervical precancer and cervical cancer. By the time the HPV vaccine for cervical cancer became available in 2006, human research on the correlation between HPV and cervical disease had come a long way in just a few decades, with tremendous achievements in the understanding and study of HPV, with studies reported from all over the world. However, studies on cervical disease due to different subtypes of HPV infection have been reported inconsistently, and the interactions between the various subtypes of infection are inconsistent. More than 150 types of HPV have been identified, with more than 40 of which can cause cervical lesions. HPV testing is widely used to screen for cervical cancer and has significantly reduced the incidence and mortality rate of the disease.

This paper focuses on the analysis of four high-risk subtypes of HPV16/18/52/58 in different cervical lesions to understand the HPV infection in different cervical lesions. According to studies, the ranking of HR-HPV subtypes varies depending on the level of cervical lesions, with HPV52, HPV58, and HPV16 having the greatest impact on the health of Chinese women (3). HPV18, HPV16, HPV52, and HPV58 are more prevalent in patients with high-grade squamous intraepithelial lesions (HSIL) and invasive cervical cancer (4). HPV52 and HPV58 are more prevalent in squamous intraepithelial lesions and cervical cancer from East Asia than in other parts of the world (5). It has an important role in the development of cervical cancer in Chinese women (6). The implementation of stratified management of high-risk groups is important in reducing the incidence of cervical cancer and precancerous lesions, and both the ASCCP/ACS/ASCP and the CSCCP advocate immediate referral for colposcopy for those positive for HPV16/18 infection. However, there is uncertainty about the significance of HPV52 positivity and HPV58 positivity in the stratified management of cervical lesions. To investigate the guiding role of HPV52 and HPV58 in the stratified management of cervical lesions, this study was conducted. This study was conducted through a retrospective analysis of the patient population attending the gynecology department of our hospital and is reported below.

MATERIALS AND METHODS

General Information

From September 2014 to December 2020, 244,218 patients aged 17–91 years, with a mean age of 39.9 ± 10.47 years, were tested for HPV at the Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University. Of the 244,218 patients, 1,616 patients aged 18–83 years, with a mean age of 41 ± 10.59 years, underwent colposcopy for HPV16/18/52/58 alone positive with normal TCT. Meanwhile, from March 2015 to September 2017, 3,014 patients with known HPV aged 17–82 years, with a mean age of 41.38 ± 10.67 years, underwent colposcopy.

Method

HPV Testing

Cervical scrape specimens were gently collected from the squamocolumnar junction of the cervix using a sampling brush and were stored at 4°C prior to HPV genotyping. The genotype of HPV was determined using a 27-HPV genotyping kit from TellgenplexTMxMAP™ (TELLGEN Life Sciences Ltd., Shanghai, China), which detects 17 high-risk types (HPV16, HPV18, HPV26, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV53, HPV56, HPV58, HPV59, HPV66, HPV68, HPV82) according to the manufacturer's instructions. Patients were grouped according to HPV16, HPV18, HPV52, and HPV58 infections. The HPV16/18/52/58 group indicates positivity for HPV species alone, whereas other groups include other types of infections or mixed infections.

Cervical Liquid-Based Cytology Tests

Cells were collected from the ectocervix and the cervical canal using a cervical canal brush, and the cells attached to the small brush were eluted in vials containing cell preservation solution and sent to the pathology department (Haoluojie, Zhejiang, China). The cytological diagnosis was made using The Bethesda System (TBS) classification criteria (TBS 2001 Revised). The cytological diagnosis was made using The Bethesda System (TBS) classification criteria (TBS 2001 Revised). The TBS classification included the following: 1. Negative, no intraepithelial lesion cells and malignant cells (NILM); 2. Abnormal epithelial cells: (1) Atypical squamous cells (ASC), including squamous epithelial cells of undetermined significance (ASC-US) and atypical squamous epithelial cells in

which HSIL cannot be excluded (ASC-H),squamous low-grade intraepithelial lesions (LSIL), HSIL, and squamous cell carcinoma (SCC); (2) Abnormal glandular epithelial cells: atypical glandular cells (AGC), adenocarcinoma *in situ* (AIS), and adenocarcinoma; and (3) other malignant neoplasms.

Colposcopy Indications

Those with normal cytology but positive for HPV16 and HPV18 or those with HPV52 and HPV58 infection for more than 6 months with 2 consecutive positive tests will most likely need to have a colposcopy.

Colposcopy Methods

The standard procedure for colposcopy is used. A full colposcopic assessment of the cervical transformation zone is performed, and a multipoint biopsy plus endocervical curettage (ECC), if necessary, is performed on suspicious areas. For those without significant colposcopic abnormalities but at high risk of precancerous lesions or invasive cancer, a routine 4-point random biopsy of the cervix at 3, 6, 9, and 12 points plus endocervical curettage, if necessary, is performed. All tissues taken were sent separately for histological examination, and the pathological diagnosis was the gold standard for evaluation. The classification criteria for evaluation include the following: (1) normal or inflammatory; (2) LSIL; (3) HSIL; and (4) cervical cancer, including cervical invasive squamous carcinoma and adenocarcinoma. The colposcopic biopsy pathology results were the gold standard for statistical analysis.

Statistical Processing

SPSS22.0 software was used for the statistical data analysis. Statistics data are presented as frequencies and proportions. The Chi-square or Fisher's exact test was used for the comparison of proportions between groups. A Spearman correlation was used for statistical and correlation analyses, and differences were considered statistically significant at $p < 0.05$.

RESULTS

General Information on the Various HPV Subtype Groups

Of the 3,014 patients, the highest proportion of patients referred for colposcopy had HPV16 infection, followed by HPV52 and then HPV58 and HPV18. There were no statistical differences between the age groups.

Distribution of HPV Subtypes in Different Cervical Lesions

As shown in **Table 1**, of the 3,014 patients, 78.04% were suggestive of HPV16/18/52/58 infection. As shown in **Table 2**, in HSIL and cervical cancer, the most common was HPV16, followed by HPV58 and then HPV52 ($p < 0.05$). Among normal and LSIL, HPV16 was relatively common with HPV52, followed by HPV18 and HPV58, but no statistically significant difference ($p > 0.05$) was observed.

TABLE 1 | Age distribution of HPV subtype groups.

Group	Age	Number	Percentage (%)
HPV16	40.56 ± 10.60	883	29.30
HPV18	41.32 ± 9.70	419	13.90
HPV52	42.01 ± 10.42	588	19.51
HPV58	42.56 ± 10.65	462	15.33
Others	41.46 ± 10.44	662	21.96
Total	41.38 ± 10.67	3,014	100.00

HPV Infection in the Population

As shown in **Figure 1**, the most common HR-HPV genotype detected among the 244,218 patients was HPV52 (3.58%), followed by HPV58 (2.23%), HPV16 (2.01%), and HPV18 (1.02%) further down the list at 7th.

Patient's Colposcopic Biopsy Results in the 1,616 Patients

The previous data showed that HPV52 and HPV58 had a high prevalence of infection in the population (**Figure 1**) and were second only to HPV16 in the proportion of lesions of HSIL and cervical cancer (**Table 1**). To further explore the role of HPV52 and HPV58 in cervical disease, we analyzed 1,616 patients and identified various pathological findings, with most patients having normal or LSIL. The percentage of pathological findings were high-grade and cervical cancer: HPV16 positive (21.81%) > HPV58 positive (10.71%) (Chi-square: 31.957, $p < 0.05$), HPV58 positive (10.71%) > HPV18 positive (5.96%) (Chi-square: 4.456, $p < 0.05$), and HPV18 positive (5.96%) > HPV52 positive (5.17%) (Chi-square: 0.241, $p = 0.624$) (**Table 3**).

Detection of High-Grade Lesions and Cancers of the Vulva and Vagina in the 1,616 Cases

As shown in **Table 4**, among the 167 patients with high-grade lesions and cancer, no high-grade vaginal or vulvar lesions developed in the HPV18-positive patients (0%), 10 cases in the 82 HPV16-positive patients (2.66%), 8 cases in the 36 HPV58-positive patients (2.38%), and 4 cases in the 32 HPV52-positive patients (0.65%). The most common was HPV16 positivity, followed by HPV58 positivity (Chi-square: 38.27, $p = 0.003$).

DISCUSSION

The occurrence of cervical cancer or precancerous lesions is closely linked to persistent infection with high-risk HPV types,

TABLE 2 | Distribution of HPV subtypes in different cervical lesions.

Pathology	Normal and LSIL	HSIL and Ca
Type (%)		
HPV16	420 (19.36)	325 (38.46)
HPV18	284 (13.09)	51 (6.04)
HPV52	396 (18.26)	93 (11.00)
HPV58	261 (12.04)	128 (15.15)
Other	808 (37.25)	248 (29.35)
Total	2,169 (100)	845 (100)

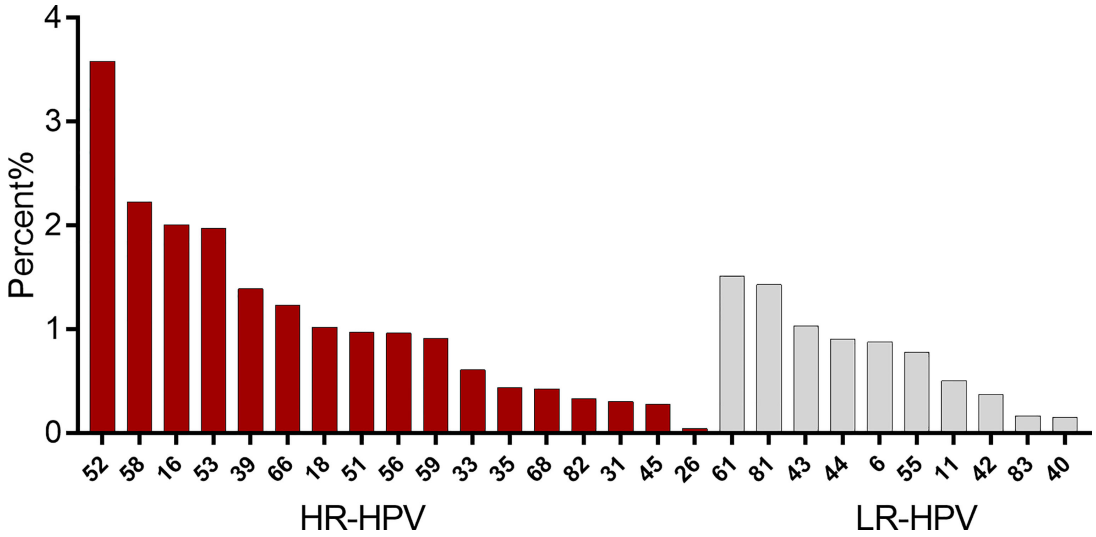

FIGURE 1 | Distribution of the total infection rate (%) for the 27 HPV subtypes (left, high-risk subtypes; right, low-risk subtypes).

TABLE 3 | Colposcopic biopsy results by HPV typing (*n* (%)).

	HPV16	HPV18	HPV52	HPV58	Total	χ (2)
Normal and LSIL	294 (78.19)	268 (94.04)	587 (94.83)	300 (89.29)	1,449	
HSIL and cancer	82 (21.81)	17 (5.96)	32 (5.17)	36 (10.71)	167	77.2*
Total	376 (100)	285 (100)	619 (100)	336 (100)	1616	

*Comparison of HSIL and cervical cancer in each group. *p <0.001.*

TABLE 4 | Distribution of high-grade lesions and cancer (*n* (%)).

Types	HPV16	HPV18	HPV52	HPV58	Total	χ (2)
Group						
Vulvar vagina	10 (2.66)	0 (0)	4 (0.65)	8 (2.38)	22 (1.36)	38.27*
Cervical	72 (19.15)	17 (5.96)	28 (4.52)	28 (8.33)	145 (8.97)	
Total	82 (21.81)	17 (5.96)	32 (5.17)	36 (10.71)	167 (10.33)	

*Comparison of high-grade lesions and cancers of the vulva and vagina in each group. *p < 0.05.*

and according to the World Health Organization, there are nearly 500,000 new cases of cervical cancer each year, of which more than 100,000 occur in China, home to about one-third of the world's population (5).

In our data analysis, the top 3 HPV infections in the population were HPV52, HPV58, and HPV16, indicating that HPV52 and 58 infections are more common in the population. The relatively high detection rate of HPV52 and HPV58 among pathological findings suggesting high-grade cervical lesions and cervical cancer again indicates the presence of a higher rate of HPV52 and HPV58 infection in high-grade cervical lesions in addition to HPV16 and HPV18 types (7). In addition, studies have shown a relatively high prevalence of HPV58 and HPV52 in invasive cervical cancer (ICC) in Asia (8). The HPV nine-valent vaccine (HPV16/18/31/33/45/52/58/6/11), therefore, offers more protection against HPV than the quadrivalent HPV vaccine (HPV16/18/6/11) while also being safer and more cost-effective (9); this may be important to the inclusion of HPV52/58 in the HPV nine-valent vaccine. Although expanded HPV vaccination may reduce the incidence of cervical cancer, HPV vaccination rates remain low in developing countries (10, 11) due to factors such as age, education, and cost; thus, the development of a new HPV16/18/52/58 quadrivalent vaccine may help to increase vaccination rates and reduce the incidence of cervical cancer.

A meta-analysis showed that the prevalence of HR-HPV infection in women with normal cervical fluid-based cytology

was 11.7% (12). In this study, we analyzed 1,616 patients who were positive for HPV16/18/52/58 alone with TCT normal, with pathological findings suggestive of normal and inflammatory conditions accounting for 64.89%–78.51%, in line with a TCT specificity of 58%–76% (13). HPV screening can compensate for the lack of liquid-based cytology and can be combined with liquid-based cytology to improve sensitivity (14).The pathological findings suggest that HPV58-positive infection is more prevalent in patients with high-grade and above lesions than HPV52 positive and also higher than HPV18 positive. This is consistent with the Kaidar–Person study, which found that HPV58 has a 1.8-fold higher association with invasive cervical cancer than HPV52 (15). We can consider HPV type 58 an important pathogen, especially in Asia (16). Therefore, in patients who are positive for HPV type 58, especially those with persistent infection despite normal TCT results, attention needs to be paid and the indications for colposcopy may be relaxed as appropriate.

There is no screening strategy for vulvovaginal high-grade lesions and cancer, given that many vaginal (17) and vulvar (18) cancers are the result of HPV infection. HPV testing can be used as a primary screening tool for the disease, and vaccination may be the only effective means of prevention (18). In this study, the detection rate of high-grade and above vulvovaginal lesions was as high as 2.66%, suggesting the need for concomitant biopsies to avoid missing lesions when we suspect vaginal or vulvar lesions. In particular, persistent infection with high-risk subtypes other than HPV types 16 and 18, such as HPV types 52 and 58, cannot exclude the possibility of vulvovaginal disease despite a normal TCT test result.

There is a limitation in this study. According to the guideline, patients with HPV16/18 infection are immediately referred for colposcopy, but patients with HPV52/58 infection are not all referred for colposcopy and tissue biopsy if TCT test results are normal. The sample included in this study is only a portion of the overall total number of samples. Thus, there is bias here. We will further expand the sample size.

CONCLUSION

1. In patients with positive HPV58 alone and normal TCT, the indications for colposcopy may be relaxed, with particular attention paid to the possibility of vulvar and vaginal lesions.
2. Patients with a positive HPV type 52 alone and normal TCT may be considered for a follow-up review and, if necessary, a colposcopy.
3. The development of a more suitable HPV vaccine for the Asian population, such as HPV16/18/52/58, may better protect women's health.

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

BY proposed the research object, collected the data, analyzed the data, and wrote the article. QX has contributed equally to this Work, is considered as co-first author. ZZ collected and analyzed the data. JZ, YX, and LH collected the data. YH proposed the research object and analyzed the data. QT proposed the research, collected and analyzed the data, is considered as co-corresponding author. JC collected, analyzed, wrote portion the manuscript and found the right journal, is the corresponding author of this paper. All authors listed have made a substantial, direct, and intellectual contribution to the work.

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SYSTEMATIC REVIEW
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Emerging Role of MicroRNAs in the Therapeutic Response in Cervical Cancer: A Systematic Review

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Cervical cancer is a common female cancer, with nearly 600,000 cases and more than 300,000 deaths worldwide every year. From a clinical point of view, surgery plays a key role in early cancer management, whereas advanced stages are treated with chemotherapy and/or radiation as adjuvant therapies. Nevertheless, predicting the degree of cancer response to chemotherapy or radiation therapy at diagnosis in order to personalize the clinical approach represents the biggest challenge in locally advanced cancers. The feasibility of such predictive models has been repeatedly assessed using histopathological factors, imaging and nuclear methods, tissue and fluid scans, however with poor results. In this context, the identification of novel potential biomarkers remains an unmet clinical need, and microRNAs (miRNAs) represent an interesting opportunity. With this in mind, the aim of this systematic review was to map the current literature on tumor and circulating miRNAs identified as significantly associated with the therapeutic response in cervical cancer; finally, a perspective point of view sheds light on the challenges ahead in this tumor.

Systematic Review Registration: PROSPERO (CRD42021277980).

Keywords: cervical cancer, miRNAs, radiotherapy, chemotherapy, HPV, therapeutic response

INTRODUCTION

Cervical Cancer

Cervical cancer (CC) is the cancer with the greatest incidence in developing countries, with over 300,000 deaths worldwide each year (1). It recognizes an etiology in most cases associated with infection and cell integration of Human Papilloma Virus (HPV) (2). Given that, it is quite clear that the disease is largely preventable, and about 90% of the CC cases occur in low-income and middle-income countries that do not provide widely planned screening or HPV vaccination programs (2, 3). The most common histological type of CC is squamous cell carcinoma (SCC) with a percentage

ranging between 75% and 90%, while the remaining portion is represented by adenocarcinomas (4, 5) and few rare types.

From a clinical point of view, surgery plays an important role in early cancer management, whereas advanced stages are treated with chemotherapy and radiation as adjuvant therapies to eliminate the disease (1, 6). In some cases, neoadjuvant chemotherapy (NAC) can also be employed to reduce tumor mass before surgical approaches (7). However, when cancer does not respond to concomitant therapies, salvage surgery is carried out with demolition procedures that in most cases require emptying the pelvis and definitive urostomies and colostomies (8, 9). Predicting the degree of cancer response to chemotherapy/radiation therapy from the diagnosis to personalize the clinical approach represents the biggest challenge in locally advanced cancers. The feasibility of such predictive models has been repeatedly assessed using histopathological factors, imaging and nuclear methods (10, 11), tissue and fluid scans, however with poor results. In this context, the identification of novel potential biomarkers remains an unmet clinical need.

MicroRNAs

MicroRNAs (miRNAs) are short non-coding RNAs that are able to regulate the expression of several target genes by complementary binding to specific seed sequences (12–14). Considering that the seed sequence can be formed by 2–8 nucleotides and that the complementarity may also be imperfect, a single miRNA may potentially modulate hundreds of mRNAs (15). Physiologically, mRNAs and miRNAs work in concert and basically control every biological process. However, in pathological conditions, miRNA levels can be deregulated both as a cause and as a consequence of the disease itself, promoting altered conditions including cancer. Over time, the role of miRNAs has been progressively clarified and, even if some aspects have not been completely understood, miRNAs may represent biomarkers or surrogate markers of diagnosis and prognosis (16). Moreover, it has been widely reported that miRNAs can affect the response to a variety of therapeutic treatments, and their expression can be associated with chemosensitivity and radiosensitivity (17, 18).

In recent years, compelling evidence showed that, besides tumor tissue, miRNAs are detectable in every type of body fluid, including but not limited to blood, saliva, tears, and urine. It has been supposed that cancer cells, as well as normal cells, release circulating miRNAs as messengers to send specific messages and communicate with distant cells.

Since their discovery, an increasing number of research groups have demonstrated the involvement of miRNAs in cancer (12, 15), and CC has not been excluded (19, 20). Indeed, many studies have identified different miRNAs as potential diagnostic and prognostic biomarkers in CC. However, in most papers, deregulation was detected when comparing tumor with a normal counterpart or healthy tissue. On the other hand, reports evaluating miRNA expression in relation to pharmacological response are limited and with small consensus. Given these premises, the aim of this review is to provide an overview of the current literature on tumor tissue and circulating miRNAs that are identified to be significantly associated with the therapeutic response in CC.

METHODS

Systematic Review of Studies Investigating Tumor Tissue and Circulating miRNAs in Therapeutic Response in Cervical Cancer Patients

For this purpose, we systematically searched for papers analyzing the expression of tissue and circulating miRNAs in CC in relation to the therapeutic response.

The systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement principles (21, 22). The research question was "Can miRNAs be used as biomarkers to monitor therapeutic response in cervical cancer?," and it was determined using the PICOS process (Population, Intervention, Comparison, Outcomes, Study design) (23). The protocol was registered in the PROSPERO international register on October 10, 2021 (CRD42021277980). PubMed, Cochrane library, and Scopus databases were systematically searched for original articles analyzing the miRNAs associated with drug response in cervical cancer (last updated search December 1, 2021). The papers included in this revision are summarized in **Table 1**. Relevant studies were selected using the Boolean combination of the following key terms: "treatment AND response" OR "therapy AND response" AND "cervical AND cancer" AND "circulating microRNA" OR "microRNA OR miRNA." Additionally, the reference lists of reviews, meta-analyses, and all original studies were hand-searched to acquire further relevant studies missed from the initial electronic search (**Figure 1**).

Eligible studies were required to meet the following inclusion criteria: studies evaluating tissue and circulating miRNAs in relation to the therapeutic response in CC. Exclusion criteria were: 1) meta-analyses, reviews, and editorials; 2) non-human studies; 3) *in vitro* studies; 4) non-English articles.

After removing duplicate studies, two investigators (GR and FG) independently checked titles and abstracts of the retrieved articles and judged their eligibility. Then, the entire text of potentially eligible studies was evaluated to assess the appropriateness of inclusion in this systematic review. The same two authors independently extracted the following data from the selected papers: 1) first author, publication year, and aim; 2) sample size; 3) CC stage; 4) evaluation of HPV genotype; 5) type of therapy; 6) type of biological material used for the analysis (tissue/blood/plasma/serum); 7) techniques used and (8) validations; 9) main findings of the report. Disagreements were resolved by discussion with a third reviewer (AMP). Results of the review were discussed with all authors for multidisciplinary topics.

The methodological quality of the cohort studies was evaluated by two investigators (GR and FG) based on an adapted "Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies" proposed by the NIH (37).

TABLE 1 | Studies included in the systematic review.

Author, year, [ref]	Aim of the study	Number of patients	Stage	HPV genotype	Therapy	Biological matrix	Technique/s used	Validation of the results	Most important findings	Response definition
miRNA expression in cervical cancer and chemotherapy + radiotherapy										
Fekete et al., 2020 (24)	To identify predictive miRNAs in platinum-treated SCC	n = 94 SCCs (from GDC data portal): n = 16 non-responders vs. n = 78 responders	Unknown	NE	Platinum-based chemo	T	MiRNA-seq	\	↑ let-7g, miR-150, miR-155, miR-342, miR-378a, miR-378c, miR-378d-2, miR-502, miR-5586, miR-7702 in responders vs. in non-responders	Response defined based on disease progression at 18 months.
Liu et al., 2018 (25)	To define the role of miR-492 in SCC	-discovery cohort (n = 6: n = 3 sensitive vs. n = 3 resistant pts) - validation cohort (n = 104 CCs: n = 78 sensitive vs. n = 26 resistant pts)	n = 57: stage IIb, n = 47: stage IIIb	NE	Platinum-based chemo + radio	T	RT-PCR based approach (TaqMan Array and assay)	Y - In pts - In cell models - In animal models	↑ miR-492 in sensitive vs. resistant pts ↑ associated with LNM	Resistance defined after 12 months after completion of first-line therapy
Pedroza-Torres et al., 2016 (26)	To identify a set of miRNAs to predict the response in locally advanced CC pts receiving radiation and chemotherapy treatment.	n = 41 CCs: -discovery cohort (n = 10: n = 5 NR vs. n = 5 CR) - validation cohort (n = 31: n = 15 NR vs. n = 16 CR)	IIb/IIIb	E	Platinum-based chemo + radio	T	RT-PCR based approach (miScript miRNA PCR Array and Taqman assay)	Y In pts	↓ miR-100-5p, miR-125a-5p, miR-125b-5p, miR-200a-5p, miR-342 in NR vs. CR. ↑ miR-31-3p, miR-3676 in NR vs CR. 7 miRNAs signature associated with DFS	Response evaluated through the RECIST criteria and computed axial tomography scans
Fan et al., 2016 (27)	To study the relationship between miR-125a and resistance in CC	n = 43 CCs: n = 23 responders vs. n = 20 non-responders	n = 21: stage I/II, n = 22: stage III/IV	NE	Taxol and platinum-based chemo	T	Microarray and RT-PCR	Y - In pts - In cell models - animal models	↓ miR-125a in non-responders vs. responders ↓ miR-125a: ↓ PFS, OS, Response Rate	Response defined according the RECIST criteria
Chen et al., 2014 (28)	To clarify the role of miR-181a in regulating the chemoresistance of CC	n = 18 SCCs: n = 7 resistant vs. n = 11 sensitive pts	n = 18: stage IIIB	NE	Platinum-based chemo + radio	T	RT-PCR	Y - In cell models - In animal models	↑ miR-181a in resistant vs. in sensitive pts	Resistance defined as described by Ke et al. (29)
Ke et al., 2013 (29)	To define the roles of miR-181a in determining sensitivity of CC to radiation therapy	n = 18 SCCs: n = 7 resistant vs. n = 11 sensitive pts	n = 18: stage IIIB	NE	Platinum-based chemo + radio	T	Microarray and RT-PCR	Y - In the same cohort - In cell models - In animal models	↑ miR-181a in resistant vs. in sensitive pts	Resistance defined based on histological finding of residual tumor cells in the cervical biopsies sampled 6 months after completion of radiotherapy
miRNAs expression in cervical cancer and radiotherapy										
Wei et al., 2020 (30)	To understand the role of miR-411 in radiotherapy response	n = 141 CCs: n = 92 responders vs. 49 non responders	n = 55: stage I, n = 62: stage II,	E	Radio	T/PB	RT-PCR	Y In cell models	↑ miR-411 in responders vs. in non-responders in both tissue and blood	Efficacy defined according to the RECIST criteria

(Continued)

TABLE 1 | Continued

Author, year, [ref]	Aim of the study	Number of patients	Stage	HPV genotype	Therapy	Biological matrix	Technique/s used	Validation of the results	Most important findings	Response definition
			n = 24: stage III						↑ miR-411 associated with higher OS and PFS	
Gao et al., 2019 (31)	To investigate the biological role of GAS5 in the radiosensitivity	n = 20 CCs: n = 9 resistant vs. n = 11 sensitive pts	IIb to IVb	NE	Radio	T	RT-PCR	Y -In cell models -In animal models	↑ miR-106b in resistant vs. in sensitive pts	Response defined according to the histological results of residual tumor cells in cervical biopsy samples 6 months after completion of radiotherapy
Wei et al., 2017 (32)	To evaluate miR-145 in CCs and investigate its biomarker potential	n = 120 CCs: n = 68 CR vs. n = 52 IR	n = 77: stage I–II; n = 43: stage III	E	Radio	P	RT-PCR	N	↑ miR-145 in CR than in IR pts	Response defined at 6 months after radical radiotherapy
Liu et al., 2015 (33)	To examine the role of miR-18a in regulating the radiosensitivity of CC	n = 48 CCs: n = 20 resistant vs. n = 28 sensitive pts	n = 34: stage I–IIb, n = 14: stage IIIa–Iv	NE	Radio	T	RT-PCR	Y In cell models	↑ miR-18a in sensitive vs. resistant	Response defined at 6 months after radical radiotherapy
Song et al., 2015 (34)	To explore the association between miR-375 and radioresistance in HR-HPV (+) CC	n = 22 CCs: n = 13 resistant vs. n = 9 sensitive pts	Ia/Ia2	E	Radio	T/S	RT-PCR	Y In cell models	↓ miR-375 in resistant vs. in sensitive	Resistance assessed by histological examination of residual tumor tissues 6 months after completion of radiotherapy
			miRNA expression in cervical cancer and neoadjuvant treatment							
Chen et al., 2014 (35)	To investigate the role of miR-143 expression in cervical SCC	n = 24 SCCs with and without NAC therapy (from a total cohort of 77 CCs and 20 normal cervix tissue)	n = 13. Stage Ib2, n = 9: stage IIa, n = 2: stage IIb	E	Taxol and platinum-based chemo	T	RT-PCR	/	↑ miR-143 after NAC	According to the WHO criteria *
Sun et al., 2013 (36)	To examine the hypothesis that NAC improves prognosis and outcomes after LRH	n = 21 CCs: n = 10 LHR vs. n = 11 NAC+LHR	IIb	E	Taxol and platinum-based chemo	T	RT-PCR	Y In cell models	↑ miR-34a, miR-605 in NAC treated vs. NAC non-treated treated pts	Response defined according to the WHO criteria *

**Complete remission (tumor completely disappeared); partial remission (tumor size decreased more than 50%); stable or no change (tumor size increased or decreased no more than 25%), progression (new lesions or tumor size increased more than 25% during the treatment).*

CC, cervical cancer; chemo, chemotherapy; CR, complete response; E, evaluated; HPV, human papillomavirus; HR, high-risk; IR, incomplete response; LRH, laparoscopical radical hysterectomy; NAC, neoadjuvant chemotherapy; NR, no response; Y, yes; N, no; NE, not evaluated; P, plasma; PB, peripheral blood; pts, patients; S, serum; SCC, squamous cell carcinoma; radio, radiotherapy; T, tissue; ↑, higher; ↓, lower; +, positive.

RESULTS

We included in the final review a total of 13 papers. The list of papers is reported in **Table 1**, while **Table 2** reports all of the miRNAs evaluated with the suggested targets. The majority of the studies analyzed miRNAs in tumor tissue specimens, a small portion (n = 2) investigated tissue and circulating miRNAs on the same study cohorts (30, 34), and one analyzed plasmatic miRNAs only (32). Overall, most of the studies were based on a comparison between treatment-resistant and non-resistant CC patients (**Figure 2**); in particular, 6 of the 13 papers evaluated miRNAs in patients treated with chemotherapy and radiotherapy, 5 in radioresistant or non-resistant CC patients, and 2 analyzed miRNAs in association with NAC. With regard to the studies performed in tumor tissue, the analysis was carried out starting from tissue preserved in formalin-fixed paraffin-

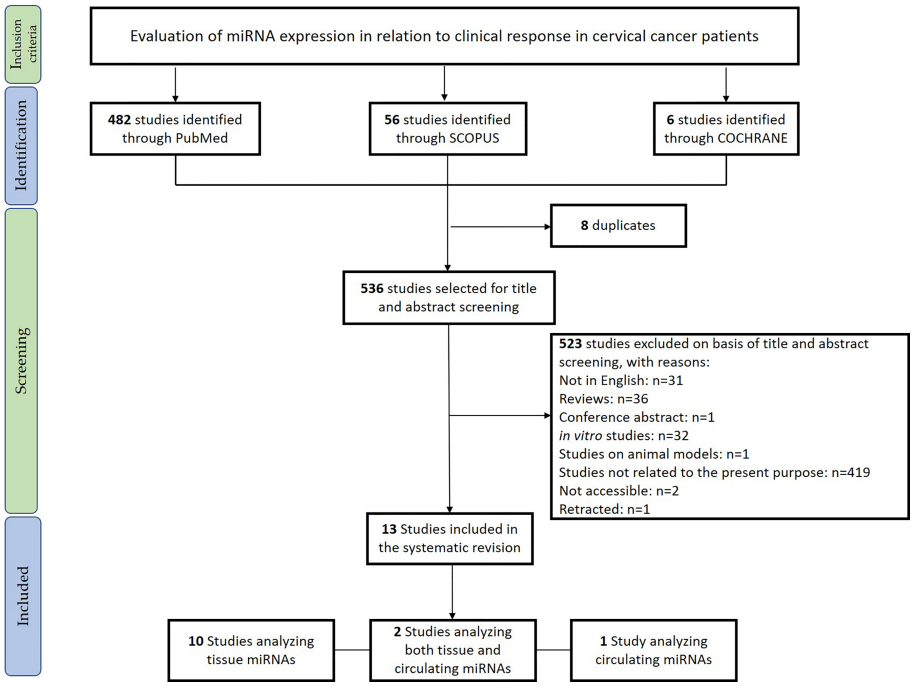

FIGURE 1 | Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart for the studies included in the systematic review.

embedded (FFPE) or frozen tumor stored at -80°C until use and collected before any type of treatment. Quality evaluation is summarized in **Table 3**. No study was rated as having good quality; however, four of the 14 criteria were non-applicable to these studies, while one was applicable to one study only. The most common biases were the absence of sample size justification and adjustment of statistical analysis for potential confounding variables.

miRNA Expression in Cervical Cancer and Chemotherapy and Radiotherapy

Among the six papers, five included profiling of miRNAs, whereas one analyzed single miRNAs based on literature evidence. In particular, four papers investigated miRNAs through microarray or Taqman array, while one paper used Genomic Data Commons (GDC) data portal (https://portal.gdc.cancer.gov/) to retrieve and analyze miRNAseq data on CC patients under treatment. The first miRNA profiling in CC dates back to 2013, when Ke et al. (29) investigated SCC frozen tumor samples from 18 patients, of which 7 were resistant and 11 were sensitive to radiotherapy in association with cisplatin; therapeutic resistance or sensitivity was defined based on histological findings on cervical biopsies that were sampled 6 months after completion of radiotherapy. Eight miRNAs were significantly deregulated (miR-16–2*, miR-18a, miR-21, miR-23a, miR-30*, miR-181a, miR-221, and miR-378), and 6 were selected to be further validated in cell models, showing that miR-181a had the most important role in CC radiosensitivity. The same study cohort was used by Chen et al. (28) to further investigate the role of miR-181a. However, in this case, the main goal was to understand the contribution of miR-181a in platinum therapy rather than radiotherapy. To do that, the authors carried out *in vitro* and *in vivo* experiments and corroborated that miR-181a acts as an oncogene to enhance the chemoresistance through the pro-apoptotic protein kinase PRKCD. A second miRNA profiling was carried out by Fan et al. (27) with the aim of shedding light on the relationship between miRNAs and paclitaxel sensitivity. The work started by analyzing the miRNA expression profiles in two CC cell lines and their paclitaxel-resistant counterparts; 18 deregulated miRNAs were detected in paclitaxel-resistant cells compared with paclitaxel-sensitive cells, and 6 of those were randomly selected to be further tested by real-time PCR (RT-PCR). The results were consistent with the array results, and miR-125a was the most deregulated miRNA, with a significant downregulation in resistant cells. After careful *in vitro* and *in vivo* analyses and the identification of Signal transducer and activator of transcription 3 (STAT3) as a potential miR-125a target, the authors evaluated miR-125a in 43 CC tissue samples that were collected before any type of treatment. The Response Evaluation Criteria in Solid Tumors (RECIST) (38) were adopted to assess the effect of chemotherapy on progression-free survival (PFS) and overall survival (OS), and CC patients were grouped based on high or low miR-125a expression. Based on that, low miR-125a expression was significantly correlated with poorer PFS, OS, and response rate compared with the high miR-125a expression group. Moreover, miR-125a expression was significantly downregulated in non-response patients. In the same way,

TABLE 2 | Summary of the miRNAs analyzed.

Tissue miRNAs		
miRNA ID	**Reference describing the miRNA**	**Potential targets of miRNAs**
let-7g	Fekete et al. (24)	/
miR-100-5p	Pedroza-Torres et al. (26)	/
miR-106b	Gao et al. (31)	IER3, GAS5
miR-125a	Fan et al. (27)	STAT3, ERBB2 and ERBB3, VEGF-A
miR-125a-5p	Pedroza-Torres et al. (26)	STAT3
miR-125b-5p	Pedroza-Torres et al. (26)	BAK1
miR-143	Chen et al. (35)	BCL2, KRAS, MACC1
miR-150	Fekete et al. (24)	/
miR-155	Fekete et al. (24)	/
miR-18a	Liu et al. (33)	ATM, PARP
miR-181a	Chen et al. (28) Ke et al. (29)	PRKCD, RalA
miR-200a-5p	Pedroza-Torres et al. (26)	NRAS, NR4A1, MAPK8, PDGFA, TCF4, DKK2, PSEN1, FZD1, NOTCH2, NOTCH4
miR-31-3p	Pedroza-Torres et al. (26)	
miR-34a	Sun et al. (36)	E2F1
miR-342	Pedroza-Torres et al. (26) Fekete et al. (24)	/
miR-3676	Pedroza-Torres et al. (26)	/
miR-375	Song et al. * (34)	UBE3A, SP1, role in EMT
miR-378a	Fekete et al. (24)	/
miR-378c	Fekete et al. (24)	/
miR-378d-2	Fekete et al. (24)	/
miR-411	Wei et al. + (30)	STK38L, STK17A
miR-492	Liu et al. (25)	ADAMTS1, CD44, TIMP2, MZF-1, CD147, PTEN, SOX7
miR-502	Fekete et al. (24)	/
miR-5586	Fekete et al. (24)	FOS, GNB1, CREB1, GNAQ, GRIN2A, GRIN2B, FOS, GSK3B, PPARGC1A, FOXO1
miR-605	Sun et al. (36)	MDM2
miR-7702	Fekete et al. (24)	/
Circulating miRNAs		
miRNA ID	**Reference describing the miRNA**	**Potential targets of miRNAs**
miR-145	Wei et al. (32)	HLTF
miR-375	Song et al. * (34)	UBE3A, SP1, role in EMT
miR-411	Wei et al. + (30)	STK38L, STK17A

*EMT, epithelial–mesenchymal transition; + and * indicate the same study in tissue and circulating miRNAs.*

Pedroza-Torres et al. (26) analyzed a total of 41 CC samples. Specifically, 10 of those were used for miRNA profiling, while 31 samples represented the validation cohort. Therapeutic responses were evaluated through the RECIST criteria and computed axial tomography scans, and patients were classified as having a complete response (CR) in case of disappearance of all signs of cancer in response to treatment or having no response (NR) if showing partial, progressive, or stable disease. The miRNA profiling on the discovery set displayed 101 differentially expressed miRNAs between the 5 CR and 5 NR patients. A subset of 7 miRNAs (miR-31-3p, miR-3676, miR-125a-5p, miR-100-5p, miR-125b-5p, miR-200a-5p, and miR-342) was assessed in the independent group of 31 samples by single-miRNA assay showing consistency with the global profile. Moreover, CC patients were dichotomized into two groups (i.e., low and high expression levels), and disease-free survival (DFS) was assessed showing that low expression was a significant predictor of non-response to standard treatment. Similarly, Liu et al. (25) performed miRNA profiling on a small study cohort of 6 CC patients, of whom 3 were resistant and 3 were sensitive to concomitant chemoradiotherapy. In this work, patients with recurrent disease within 12 months after completion of first-line therapy were defined resistant, while the ones with no recurrence were termed sensitive. Twenty miRNAs showed a significant differential expression between the two sample groups, with miR-492 as the most deregulated. miR-492 was further validated in 104 CC samples, confirming a lower expression of miR-492 in treatment-resistant tumors. A higher miR-492 expression was also associated with pelvic lymph node metastasis (LNM), and *in vitro* experiments demonstrated that miR-492 overexpression promotes cell proliferation and migration and enhances the sensitivity of CC cells to irradiation by apoptosis.

A different approach was used by Fekete et al. (24), who retrieved miRNA expression data through the GDC data portal. The aim of the work was to identify miRNA predictive biomarkers in platinum-treated SCCs, regardless of the tumor site; for this reason, they included CC and lung and head and neck SCC (HNSC) for a total of 266 patients. Of the 94 CC patients, 16 were non-responders and 78 were responders, defined based on the presence of disease progression at 18 months. In the CC subgroup, 16 miRNAs that were

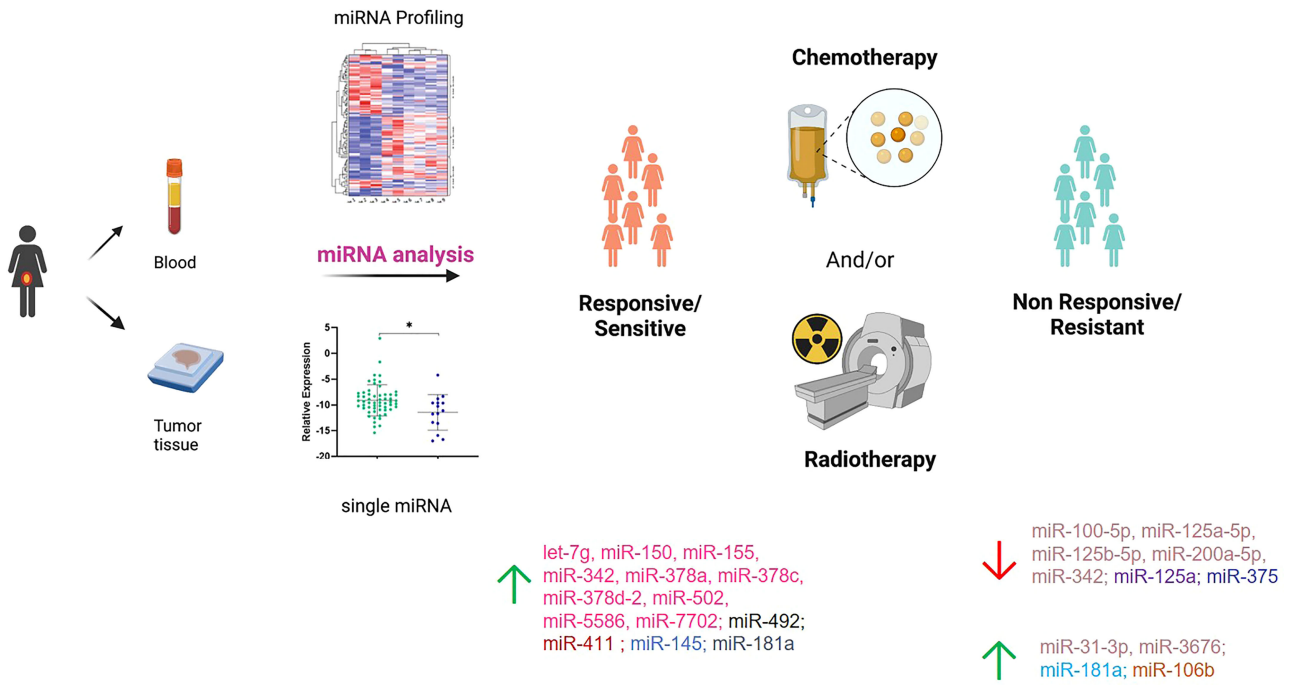

FIGURE 2 | Summary of the miRNAs investigated in responsive/sensitive vs. non-responsive/resistant cervical cancer patients. miRNAs highlighted with the same color derive from the same paper. Created in part with BioRender.com.

differentially expressed between responder and non-responder patients were retrieved. Based on a miRNA similarity score, CC and HNSC were combined (for a total of 199 cases), and a logistic regression model including 6 miRNAs (miR-101-2, miR-632, miR-642a, miR-2355, miR-5586, miR-6728) was established; the model was generated by randomly dividing samples in the training set and the test set and was able to predict chemotherapy resistance with an area under the curve (AUC) of 0.897. Unfortunately, given the small sample size, the authors did not apply the model in the CC group alone and we cannot speculate on its performance in this specific type of tumor.

miRNA Expression in Cervical Cancer and Radiotherapy

Five works evaluated miRNAs with regard to response to radiotherapy. None of these evaluated miRNAs by large profiling, but single-miRNA analysis was preferred. As mentioned, three studies analyzed circulating miRNAs, of which one in plasma and two conducted a parallel evaluation on tumor tissue and blood. In particular, in 2015, Song et al. (34) investigated a specific miRNA (miR-375) in both CC tumor and blood serum samples; in our knowledge, this was the first study to evaluate "liquid" miRNAs. In this case, the study cohort included 22 CC patients who were positive for high-risk (HR) HPV. miR-375, chosen based on previous literature evidence, showed lower levels in radioresistant patients compared with radiosensitive patients in both biological matrixes. Moreover, the role of miR-375 on radiosensitivity was further explored in cell line models. The results indicated a potential network between miR-375 and UBE3A, highlighting that miR-375 may promote radiosensitivity of HR HPV-positive patients, *via* p53 degradation. Similarly, Wei et al. (30) investigated miR-411 in 141 CC patients, in both CC tumor and blood samples. In this case, the cohort included 92 patients responding (complete and partial response) and 49 not responding (stable and progressive disease) to radiotherapy (30). miR-411 was increased in the radioresponsive group vs. the non-responsive patients, regardless of the type of sample (i.e., blood or tissue). Receiver operating characteristic (ROC) curves were used to assess the predictive value of miR-411 for radiotherapy efficacy, suggesting that miR-411 had good predictive value in tissues and peripheral blood in CC. Moreover, miR-411 was significantly higher in patients with longer 3-year OS and PFS rates compared with those with a lower miR-411 expression. Another interesting work is presented by Wei et al. who performed an evaluation of plasmatic miR-145 on 120 CC patients as a potential biomarker of the radiotherapy response (32). Indeed, from a previous report (39), a correlation between low levels of miR-145 in CC tissues and lymph node metastases and advanced clinical stage was observed, but its correlation with radiotherapy response had not been investigated. Among the 120 CCs, of which 68 were complete and 52 were incomplete responders, patients achieving a complete response presented higher levels of plasmatic miR-145 compared with the others; even in this case, ROC analysis confirmed the predictive value of miR-145 in differentiating complete from incomplete responders. Unfortunately, no validations of these interesting results in independent cohorts neither in cell models were carried out.

Two additional papers focused on tumor tissue miRNAs and radiotherapy response were also retrieved in our literature

TABLE 3 | Quality assessment of the studies included in the present review.

Criteria *	Fekete et al. (24)	Liu et al. (25)	Pedroza-Torres et al. (26)	Fan et al. (27)	Chen et al. (28)	Ke et al. (29)	Gao et al. (31)	Wei et al. (30)	Wei et al. (32)	Liu et al. (33)	Song et al. (34)	Chen et al. (35)	Sun et al. (36)
1.													
2.													
3.	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
4.													
5.													
6.	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		NA
7.	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
8.	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
9.													
10.													
11.													
12.	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
13.	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
14.													

**Criteria: 1) Was the research question or objective in this paper clearly stated? 2) Was the study population clearly specified and defined? 3) Was the participation rate of eligible persons at least 50%? 4) Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants? Including period and place of recruitment (setting and geographic location) adequately described 5) Were a sample size justification, power description, or variance and effect estimates provided? 6) For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured? 7) Was the time frame sufficient so that one could reasonably expect to observe an association between exposure and outcome if it existed? 8) For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure or exposure measured as a continuous variable)? 9) Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants? (Assessment of miRNA analysis and validation in an independent cohort) 10) Was the exposure(s) assessed more than once over time? 11) Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants? (Assessment of response) 12) Were the outcome assessors blinded to the exposure status of participants? 13) Was loss to follow-up after baseline 20% or less? 14) Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)? Highlighted in red criteria adapted to the papers analyzed.*

Quality was rated as poor (0–4 out of 14 questions), fair (5–10 out of 14 questions), or good (11–14 out of 14 questions).

Green, yes; Red, no; Orange, partial (i.e., validation on the same cohort but with different technique); NA, not applicable.

analysis (31, 33). Specifically, the one from Liu et al. (33) explored the role of miR-18a in regulating the radiosensitivity in CC. Indeed, the involvement of miR-18a has been reported in several cancer types, including but not limited to bladder cancer, hepatocellular carcinoma, and colon cancer (40–42), but its role in CC was unknown. The expression of miR-18a was investigated in 48 CC samples showing that it was significantly higher in radiosensitive patients compared with radioresistant patients.

Gao et al. (31) aimed to evaluate the role of a long non-coding RNA (lncRNA), GAS5, and miR-106 in CC. GAS5 is a known tumor suppressor that acts as a sponge of miR-106b. The analysis was performed on 20 CC samples of which 11 were from radiosensitive and 9 were from radioresistant patients; the RT-PCR analysis highlighted that GAS5 levels were significantly decreased, while miR-106b expression was increased in radioresistant tissues compared with radiosensitive tissues. Further *in vitro* studies from the same authors allowed to establish that miR-106b negatively regulated Immediate Early Response 3 (IER3), an important player in modulating sensitivity to chemotherapeutic drugs.

miRNA Expression in Cervical Cancer and Neoadjuvant Treatment

Two papers explored miRNA levels in relation to the efficacy of NAC before radical hysterectomy in comparison with patients not treated before resection. The goal of the works was to assess the efficacy of NAC rather than to evaluate miRNAs. Sun et al. (36) analyzed miR-34 and miR-605 in 21 CC patients, of whom 11 were treated with the neoadjuvant protocol. miR-34 and miR-605 were chosen due to their belonging to two protein networks (p53-miR34-E2F1 and p53-miR-605-Mdm2) related to aggressive oncogenic signaling cascades in different tumors. The specimens were collected during surgery, and miRNA levels were analyzed. Both miR-34a and miR-605 were higher in patients treated with NAC compared with the non-treated ones. As mentioned, this paper aimed to evaluate NAC efficacy, so the authors reported a smaller tumor size in NAC patients and lower metastasis rate and better DFS and OS. However, the value of miRNAs in discriminating responsive vs. non-responsive patients was not evaluated, and the paper evaluated only the difference in miRNA levels at the time of surgery between NAC-treated and non-treated patients.

In the same way, Chen et al. (35) intended to analyze miR-143 in relation to NAC. miR-143 was selected because literature reported its involvement in CC (43, 44). The total cohort included 77 CC samples; however, only 34 patients were treated with NAC. For each patient, the tumor material was collected before and after NAC and a comparison in terms of miR-143 levels was carried out in 24 cases. No significant differences at the two time points were recorded, suggesting that miR-143 does not contribute to mediate taxol sensitivity. However, this study is of particular interest because it is the sole to compare miRNA levels before and after treatment on the same patients, allowing to evaluate NAC effects on a selected miRNA.

Even in this case, the type of response (i.e., responsive or not) was not considered (35).

DISCUSSION

CC is one of the most common cancer types among women of developing countries, being the fourth most common female cancer worldwide (45).

While screening programs and HPV vaccines have led to a reduction of CC in developed countries, in developing countries CC remains an important issue, with ~80%–90% of patients at stages III–IV (36, 45).

Concomitant chemoradiotherapy as definitive approach remains the gold standard for locally advanced tumors, while surgery alone, or followed by radiotherapy, is the standard for early stages; NAC is offered to patients who wish to reduce cancer before surgical intervention, but this clinical approach is not considered a standard therapy. Unfortunately, a certain percentage of patients do not respond to the therapeutic plan with poor prognosis, and the prediction of response represents an important clinical issue. In this context, the identification of predictive biomarkers of chemotherapy and radiation sensitivity denotes an unmet clinical need.

In the last decade, the clinical value of miRNAs has been widely explored in cancer due to their recognized role in tumor development, progression, and response to therapy. Increasing evidence has shown their importance in mediating several biological processes in CC, while the number of reports investigating miRNAs in the therapeutic response is limited; this appears to be particularly relevant if compared with other cancers, including but not limited to ovarian, lung, or breast cancer (46–48), where the literature body is quickly increasing. In the present review, we aimed to provide an overview of the current literature on tumor tissue and circulating miRNAs significantly associated with therapeutic response in CC. In our analysis, we retrieved only 13 papers falling in our scope, of which 6 works evaluated miRNAs in patients treated with both chemotherapy and radiotherapy and 5 in radioresistant or non-resistant CC patients. In general, the analyses were heterogeneous in terms of type of miRNAs (i.e., tumor or circulating miRNAs) and techniques. In particular, 3 out of 13 papers analyzed circulating miRNAs, however one in peripheral blood, one in plasma, and one in serum; four of 13 used large profilings to simultaneously screen multiple miRNAs, one retrieved the miRNA levels from an available omics database, whereas the remaining papers adopted RT-PCR as the main technique to evaluate a limited number of miRNAs. Another source of heterogeneity was related to the assessment of therapeutic response. As summarized in **Table 1**, in a few cases, the cut-off to judge the responsiveness was 6 months, while in other cases, it was 12 or 18 months. Six works analyzed HPV together with miRNAs, as it is recognized as a risk factor for CC development, although the remaining works did not mention that.

Overall, as previously mentioned, the studies on miRNAs and therapeutic response available in the literature can be grossly divided in three groups according to the type of therapeutic plan (i.e., concomitant chemoradiotherapy and radiotherapy or radiotherapy alone as adjuvant setting or NAC); however, even considering this aspect, the consensus among the studies appears very limited; **Table 2** offers a good perspective of that, showing the large number of significant miRNAs but the concomitant lack of agreement. Moreover, our review highlights a wide range of treatments reported in CC patients that would require a global centralization to provide uniformity of care, at least in high-income countries where the disease is rarer. With all of these considerations in mind, it is comprehensible that no clinical translation has happened yet, and further research will be needed to outline reliable miRNA candidate biomarkers. In addition, the lack of standardized protocols, including sample collection, RNA extraction, and techniques, as well as definition of therapeutic response assessment, hampers the comparison of results between independent studies. On the other hand, identification of one or a few miRNAs able, by themselves, to accurately discriminate responsive/non-responsive patients seems unrealistic, while the best approach would be combining multiple variables (including, but not limited to miRNAs and clinical parameters). Another interesting observation arises from the limited research on circulating miRNAs. Indeed, if the number of works adopting this type of analysis is limited to three studies comparing responsive vs. non-responsive patients, "liquid" miRNAs have not been employed to monitor the response over specific treatment with a wide knowledge gap to fill. As a consequence, the research on circulating miRNAs in CC is still in its embryonal phase, and no reliable miRNA candidates to accurately follow the treatment response "in real time" have been explored, leaving space for additional studies.

In conclusion, to the best of our knowledge, this is the first systematic revision dealing with the role of miRNAs in the therapeutic response in CC. The potential application of miRNAs in CC remains to be elucidated given the inconsistent conclusions reported by different studies. This could be in part due to the limited number of investigations, the small sample size, the lack of standardized protocols to appropriately assess the miRNA contribution, and the heterogeneity of therapeutic schemes.

Further studies with standardized procedures and larger cohorts of patients should be warranted to foster the identification of miRNAs of potential clinical significance in CC.

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

Conceptualization: GR and AMP. Data curation: GR and FG. Writing—original draft preparation: GR, FG, GD, MT, EC, AM, and AP. Writing—review and editing: GR, FG, GD, MT, AM, PI, PH, SA, and AMP. All authors have read and agreed to the published version of the article.

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