Submitted: 01 June, 2023 Accepted: 13 July, 2023 Published: 15 February, 2024 DOI:10.22514/ejgo.2023.071

ORIGINAL RESEARCH

European Journal of Gynaecological Oncology

Evaluation of colposcopy and LEEP results performed in gynecology and gynecological oncology surgery services

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Abstract

The diagnostic performances of colposcopy and Loop Electrosurgical Excision Procedure (LEEP) results in gynecology and gynecological oncology surgical services were evaluated. Their differences regarding biopsy numbers were investigated. The other objective was to examine factors associated with recurrence and residual lesions after LEEP. This study included the cytology results of 1217 women undergone colposcopy at our hospital colposcopy unit between 2012 and 2017. The colposcopicsensitivity, specificity, positive predictive value and negative predictive value were calculated based on LEEP results. The qualitative data were compared by employing Chi-square and Fisher's exact tests. χ^2 predicted the relation between age and number of involved margins with recurrent disease. Moreover, it predicted the link between age, cytology and number of relevant margins with residual disease. There was no significant difference regarding the diagnostic performance of two groups when LEEP was determined as the gold standard against colposcopy. The diagnostic accuracy rate was 1.83 times higher when more than 2 biopsies were taken compared to 2 or fewer. A significant increase was observed in the residual rate among women having pre-LEEP high-risk human papillomavirus (HR-HPV) positive tests compared to those with HR-HPV negative tests (48.0% *vs.* 15.4%, $p = 0.04$). Women with $\geq$ High grade squamous intraepithelial lesion (HSIL)-positive margins in the first conization exhibited higher residual rates compared to those with High grade squamous intraepithelial lesion (LSIL)-positive margins (50.7% *vs.* 9.5%, $p < 0.001$). Patients ofpositive surgical margins, residual lesions and cervical intraepithelial neoplasia (CIN) with HPV 16 had higher probability of persistent HPV infection after conization. There was no significant difference pertaining to the diagnostic performance of two groups. HPV 16+ and the positive surgical margin were the predictive of recurrence.

Keywords

LEEP; Cervical dysplasia; Cervical intraepithelial neoplasia; Conization

1. Introduction

Cervical cancer is one of the most prevalent cancers worldwide with ~600,000 new cases in 2020, and accounts for 3.3% of cancer-related deaths [1]. Cervical cytology and high-risk human papillomavirus (HR-HPV) screening contribute to the early detection of cervical cancer and precancers such as high-grade squamous intraepithelial lesion (HSIL) or cervical intraepithelial neoplasia 2/3 (CIN2/3) [2]. CIN is a precursor of cervical cancer. Untreated high-grade CIN increases the risk of invasive cervical cancer. The accurate and standardized treatment of high-grade CIN can prevent cervical cancer [3]. Loop electrosurgical excision procedure (LEEP) is a minimally invasive surgery for cervical conization and implemented in recent years to treat high-grade CIN. The patient's HPV status, age, smoking, hormonal contraception and immunosuppression are also considered to avoid the overtreatment prior to performing LEEP [4]. However, LEEP treatment causes some damage to the cervix and may influence fertility after the surgery. Strong correlation exists between the preterm birth and $\geq$0.5 cc volume of excised cervical tissue, regardless of CIN severity. Caution is taken during the excisional treatment in women of reproductive age as well as in case of multiple biopsies. Fertile women with a history of multiple biopsies or excisional treatment for CIN may benefit from close surveillance during pregnancy [5].

This study aims to evaluate the diagnostic performance of colposcopy and LEEP in the gynecology and gynecological oncology surgical services at our institution. The differences pertaining to biopsy numbers are investigated. The other objective is to examine factors linked with recurrence and residual diseases following the LEEP.

2. Materials and methods

This study included 1217 women with cytology results conducted in our hospital colposcopy unit between 2012 and 2017. The study group had patients of 18–70 years' age and were referred based on the national screening program and opportunistic screening. Patient information, pathology results, follow-up and examination reports were received from sources like national "e-nabız" portals, the outpatient colposcopy registry, and hospital electronic record system. The study exclusion criteria had the patients who followed up after treatment for invasive and pre-invasive cervical diseases, those undergone colposcopy because of vulvar and vaginal intraepithelial neoplasia, those undergone hysterectomy and the pregnant women. Zeiss OPM1F colposcope (Carl Zeiss, Jena, Germany) was used for the colposcopy by applying acetic acid and Lugol iodine solution. The cytological results were categorized according to the Bethesda 2014 classification prior to colposcopy. ThinPrep Cytologic Test (TCT, Hologic, USA) was conducted using liquid-based cervical cytology. Pap smear outcomes were categorized according to the Bethesda system, including classifications like "negative for malignancy and intraepithelial lesion", "atypical squamous cells of undetermined significance (ASC-US)", "low-grade squamous intraepithelial lesion (LSIL)" and "high-grade squamous intraepithelial lesion (HSIL)". Intraepithelial lesions during cervical biopsy were categorized as CIN I, II or III depending on dysplasia extent [6]. The abnormal Pap smear results were managed by adhering to The American Society for Colposcopy and Cervical Pathology (ASCCP) recommendations [6]. Colposcopic indications followed the ASCCP guidelines which included abnormal or inconclusive The Papanicolaou PAP smear results, abnormal findings in pelvic examination, abnormal genital tract bleeding, unexplained cervicovaginal discharge, and previous cytologic and/or pathologic abnormalities of the anogenital tract [7]. A biopsy was thus conducted if abnormal results were found in the cervical cytology and/or during colposcopic cervical examination [8]. In Atypical glandular cell (AGC) group, the routine endocervical curettage in addition to colposcopy was recommended for the patients of over 35 years, and endometrial sampling was suggested for the patients in risk groups of under 35 years [9]. Endocervical sampling was made according to the 2019 ASCCP management guidelines [9]. In our practice, the endocervical sampling was performed during colposcopy at the initial visit of non-pregnant patients. Like the ASCCP guidelines, our recommendation was to either conduct immediate LEEP for women with cytologic HSIL unless the patient was pregnant or had age of 21–24 years, or to perform colposcopy. For the cases where colposcopic examination was deemed inadequate, a diagnostic excisional procedure (LEEP) was conducted except during the pregnancy.

The objective of cervical excisional procedure was to remove the entire transformation zone. It was ensured that the excision was neither too small which might result in incomplete removal of lesion, nor too large which lead to immediate or delayed complication. The size and shape of excision should be tailored for each individual case by considering the preoperative colposcopy and the sound surgical judgment. Performing colposcopy in operating room just before the procedure may not always be practical, however it can be beneficial in many instances. Extending the colposcopic scope to include evaluation of upper vagina was valuable, particularly when dealing the large and high-grade ectocervical lesions. The LEEP procedure was performed under general anesthesia. The surgical excision was carried out using high-frequency electric generator after adjusting speculum and cervical exposure. The cutting diathermy was set to 45–55 Watts while coagulation set to 50 Watts. Electrodes diameter of 0.2 mm, and widths and depths of 25 and 10 mm, 20 and 8 mm or 10 and 10 mm were selected based on the lesion size. Ball diathermy for the hemostasis purposes was applied to lesion base at the end of procedure. Follow-up visits were scheduled every six months in the first three years of initial LEEP conization, and then annually. The follow-up duration of this study was from 6 to 48 months. Patients underwent a combination of HR-HPV testing (using the Hybrid Capture 2 test with the results as positive or negative for HR-HPV) and cervical cytology at every postoperative visit. LEEP was considered as the gold standard method in evaluating the diagnostic performance of CDB (colposcopy-directed biopsy). Two-step classification was created for both CDB and LEEP results: HSIL+ (CIN2–3, squamous cell carcinoma (SqCC), Adenocarcinoma in situ (AIS) and adenocarcinoma (AC)), and HSIL-(CIN1 or LSIL and chronic cervicitis). Repeat cervical conization was performed on 112 cases with positive margins for repeating the LEEP in three months of initial LEEP conization (procedure was like the initial LEEP). Out of 70 women, 31 underwent hysterectomy because of fertility completion. Radical hysterectomy with lymphadenectomy was conducted on 39 women due to invasive cervical cancer. Indications for simple hysterectomy included the cases diagnosed with HSIL as per the International Federation of Gynecology and Obstetrics (FIGO, 2018) stage IA1 without lymphovascular space invasion (LVSI) when fertility preservation was not required. Patients of FIGO stage IA1 having positive LVSI, IA2, IB1–IB2 and IIA1 underwent radical (or modified radical) hysterectomy alongside the pelvic lymphadenectomy with or without para-aortic lymph node biopsy. The hysterectomy indication in HSIL patients was: (1) positive margins after LEEP and no desire to preserve fertility, (2) combined with benign lesions such as uterine leiomyoma and adenomyosis, and (3) poor follow-up conditions. The radical hysterectomies were conducted using an open abdominal approach. The colposcopy was performed by experienced colposcopists (certified with colposcopy accreditation) who were the gynecologists and the gynecologic oncology surgical specialists with minimum 10 years of experience. LEEP procedures were performed by the surgeons designated by respective clinics. The biopsy specimens were examined and reported by certified gynecopathologist at tertiary-level specialized center.

In this study, statistical analyses were conducted using the Number Cruncher Statistical System (NCSS) 2007 Statistical Software package program (Kaysville, Utah, USA). The descriptive statistical methods such as mean, standard deviation, frequency and percentage distributions were used in data evaluation. Sensitivity, specificity, positive predictive value, negative predictive value, accuracy and Likelihood Ratio (LR) (+) values were calculated based on LEEP results. The chi-

square test and Fisher's exact test were employed to compare the categorical data. χ^2 test estimated the relation between age and number of involved margins with recurrent disease, and the relation between age, cytology and number of related margins with residual disease. Kaplan-Meier analysis calculated the disease-free survival time of the patients. The results were evaluated at significance level of $p < 0.05$.

3. Results

The patients mean age was 45.6 ± 12.5. Out of 1217 patients, 762 were nulliparous (62.61%) and 455 multiparous (37.39%). There were 300 patients who reported smoking (24.65%), and 336 had the history of oral contraceptive pill (OCP) usage (31.94%). Groups descriptive information is summarized in Table 1.

The cytology, colposcopy, LEEP results and number of biopsies for the patients are summarized in Table 2.

LEEP was identified as the gold standard for evaluating colposcopy results in entire patient group. The following values were obtained: Sensitivity 0.75, Specificity 0.53, Positive Predictive Value 0.69, Negative Predictive Value 0.60, Accuracy 0.66 and Likelihood Ratio LR (+) 1.60. The values evaluated by gynecologists were: Sensitivity 0.76, Specificity 0.50, Positive Predictive Value 0.59, Negative Predictive Value 0.59, Accuracy 0.65 and Likelihood Ratio LR (+) 1.52. The values by gyn-oncologist were: Sensitivity 0.75, Specificity 0.55, Positive Predictive Value 0.70, Negative Predictive Value 0.61, Accuracy 0.67 and Likelihood Ratio LR (+) 1.67. LEEP diagnostic accuracy was 2.34 times higher in women over 50 years compared to those under 50 years. When evaluated based on the number of biopsies, the diagnostic accuracy ratio was 1.83 times higher in cases of more than 2 biopsies compared 2 or fewer biopsies. The findings are summarized in Table 3.

Among 182 (14.95%) women having positive surgical margins in pathology results after LEEP, 112 underwent re-conization procedure in 3 months after the initial LEEP operation. Out of 112 re-conization patients, 80 (15.21%) had first LEEP operation in the gynecology clinic, while 32 (4.63%) in gynecologic oncology surgical services. Re-conization was performed in the clinic where LEEP operation was conducted. The pathology results of second surgery depicted chronic cervicitis in 50 cases (27.5%), CIN 1 in 50 cases (27.5%), CIN 2–3 in 70 cases (38.5%), and cervical cancer in 12 cases (6.5%). The findings are summarized in Fig. 1. Among 182 cases of second surgery, residual disease was reported in 82 cases (45.05%), with 12 (14.6%) being the residual cervical cancer. In our findings, a notable increase was observed in the residual rate among following groups: women with pre-LEEP HR-HPV positive tests compared to those with HR-HPV negative tests (48.0% *vs.* 15.4%, $p = 0.04$), and women with ≥HSIL-positive margins in first conization, as opposed to those with LSIL-positive margins (50.7% *vs.* 9.5%, $p < 0.001$).

In re-conization patients' group after positive surgical margin following LEEP, over 50 years' age and multiparity were the statistically significant risk factors ($p = 0.0001$). HPV 18 presence before LEEP was also statistically significant regarding risk in re-conization group ($p = 0.006$). In co-test performed after 1 year of LEEP, HPV-DNA was not detected in 910 patients (74.77%). HPV 16+ was detected in 266 patients (21.86%). Other HR+ HPV types were found in 41 patients (3.37%). HPV 16+ in the smear taken after 1 year of re-conization following the positive surgical margin was statistically significant ($p = 0.0001$). The findings are summarized in Table 4.

The estimated median disease-free survival time is 16.5 months for the patients as shown in Fig. 2. The persistent HPV infection probability after conization is 13.99 times higher (95% confidence interval (CI) 8.76–22.37) in CIN patients with positive surgical margins and residual lesions.

4. Discussion

The cytology-based screening has inherent simplicity, low cost and large knowledge base regarding cytological patterns of precancerous lesions. The cytology identifies women at risk of harboring high-grade cervical premalignant lesions or invasive cancer. A diagnostic test such as colposcopy is imperative for women having abnormal cytology pertaining to abnormality localization, diagnosis confirmation and appropriate management. It is not graded as an effective screening tool for cervical cancer [10]. The colposcopy effectiveness in detecting CIN and cervical cancer depends on expertise and training of colposcopist. A vital aspect includes colposcopist ability to accurately interpret colposcopic findings and appropriately

TABLE 1. Descriptive information by groups.

	Gynecologist	Gynecologic oncologist	Total patient group (n: 1217)
Number of patients	526 (43.22%)	691 (56.78%)	1217 (100%)
Age (Mean ± SD)	42.6 ± 9.9	43.5 ± 10.1	42.7 ± 9.9
Parity			
Nulliparous	103 (19.58%)	659 (95.37%)	762 (62.61%)
Multiparous	423 (80.42%)	32 (4.63%)	455 (37.39%)
OCP use	166 (31.56%)	170 (32.32%)	336 (31.94%)
Smoking	100 (19.01%)	200 (28.94%)	300 (24.65%)
Re-conization	80 (15.21%)	32 (4.63%)	112 (9.20%)

SD: standard deviation; OCP: oral contraceptive pill.

TABLE 2. Results of patients' cytology-CPD-LEEP.

		Gynecologist n: 526		Gynecologic oncologist n: 691		Total patient group n: 1217	
Smear Cytology							
	Negative	114	21.67%	146	21.13%	260	21.36%
	ASC-US	105	19.96%	126	18.23%	231	18.98%
	ASC-H	30	5.70%	56	8.10%	86	7.07%
	LSIL	123	23.38%	155	22.43%	278	22.84%
	HSIL	129	24.52%	182	26.34%	311	25.55%
	Not performed	25	4.75%	26	3.76%	51	4.19%
HPV DNA							
	Negative	35	6.65%	48	6.95%	83	6.82%
	HPV 16	230	43.73%	303	43.85%	533	43.80%
	HPV 18	62	11.79%	74	10.71%	136	11.18%
	Other HR	103	19.58%	135	19.54%	238	19.56%
	Unknown	96	18.25%	131	18.96%	227	18.65%
Colposcopy							
	Chronic Cervicitis/Benign	50	9.51%	81	11.72%	131	10.76%
	CIN 1	130	24.71%	169	24.46%	299	24.57%
	CIN 2	154	29.28%	188	27.21%	342	28.10%
	CIN 3	156	29.66%	203	29.38%	359	29.50%
	CIS	21	3.99%	27	3.91%	48	3.94%
	SCC	4	0.76%	5	0.72%	9	0.74%
	Insufficient	11	2.09%	18	0.026	29	2.38%
Number of Biopsies							
	0	1	0.19%	0	0.00%	1	0.08%
	1	129	24.52%	196	28.36%	325	26.71%
	2	266	50.57%	341	49.35%	607	49.88%
	3	88	16.73%	107	15.48%	195	16.02%
	4	35	6.65%	39	5.64%	74	6.08%
	5	7	1.33%	8	1.16%	15	1.23%
Number of Biopsies	Mean ± SD	2.09 ± 0.89		2.02 ± 0.88		2.05 ± 0.89	
LEEP							
	Benign	88	16.73%	118	17.08%	206	16.93%
	CIN 1	129	24.52%	171	24.75%	300	24.65%
	CIN 2	92	17.49%	118	17.08%	210	17.26%
	CIN 3	200	38.02%	262	37.92%	462	37.96%
	SCC	17	3.23%	22	3.18%	39	3.20%

Values are presented as N (%); ASC-US: atypical squamous cells of undetermined significance; LSIL: low grade squamous intraepithelial lesions; HSIL: high grade squamous intraepithelial lesions; ASC-H: atypical squamous cells cannot exclude HSIL; CIN: cervical intraepithelial neoplasia; LEEP: loop electrosurgical excision procedure; SCC: squamous cell carcinoma; HR: high-risk; HPV: human papillomavirus; CIS: carcinoma in situ; SD: standard deviation.

TABLE 3. Sensitivity and specificity of LEEP-colposcopy.

LEEP/Colposcopy	Sensitivity	Specificity	PPV	NPV	Accuracy	LR (+)
All Patient Groups	0.75	0.53	0.69	0.60	0.66	1.60
Specialty						
Gynecologist	0.76	0.50	0.69	0.59	0.65	1.52
Gyn-Oncologist	0.75	0.55	0.70	0.61	0.67	1.67
Age						
<50 yr	0.76	0.48	0.69	0.57	0.65	1.46
>50 yr	0.72	0.69	0.73	0.68	0.71	2.34
Number of Biopsies						
≤2	0.68	0.32	0.69	0.31	0.57	1.00
>2	0.78	0.57	0.70	0.68	0.69	1.83

LEEP: loop electrosurgical excision procedure; PPV: positive predictive values; NPV: positive and negative predictive values; LR: likelihood ratio.

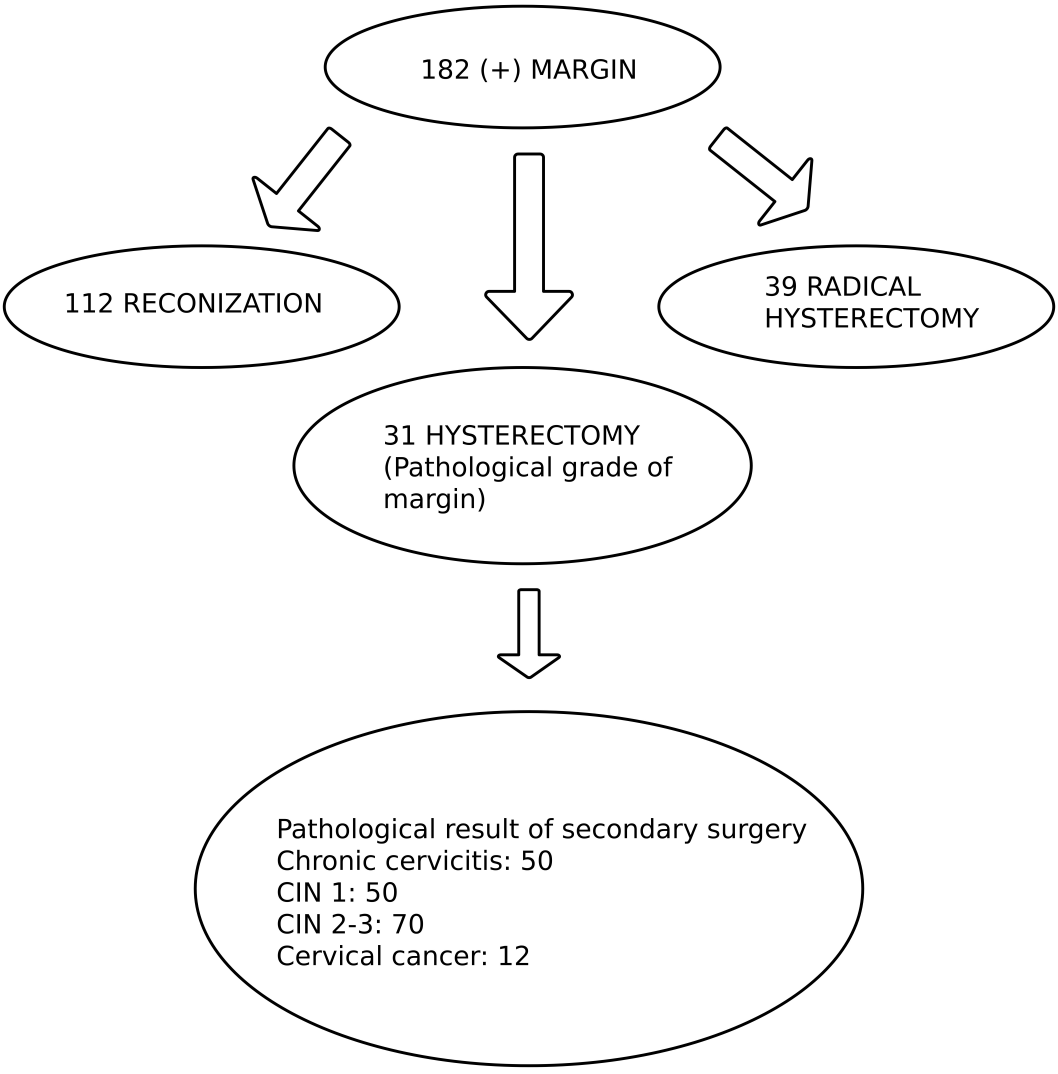

FIGURE 1. Management of patients with positive surgical margins. CIN: cervical intraepithelial neoplasia.

TABLE 4. Risk factors analysis for patients undergoing re-conization with positive surgical margins.

	Re-conization (−)		Re-conization (+)		*p*
Age					
<50 years	884	80.00%	71	63.39%	0.0001
>50 years	221	20.00%	41	36.61%	
Parity					
Nulliparous	742	67.15%	20	17.86%	0.0001
Multiparous	363	32.85%	92	82.14%	
HPV DNA					
Negative	81	7.33%	2	1.79%	0.0060
HPV 16	489	44.25%	44	39.29%	
HPV 18	117	10.59%	19	16.96%	
Other HR	221	20.00%	17	15.18%	
Unknown	197	17.83%	30	26.79%	
HPV DNA after 1 year LEEP					
Negative	885	80.09%	25	22.32%	0.0001
HPV 16	213	19.28%	53	47.32%	
Other HR	7	0.63%	34	30.36%	

HPV: human papillomavirus; LEEP: loop electrosurgical excision procedure; HR: high-risk.

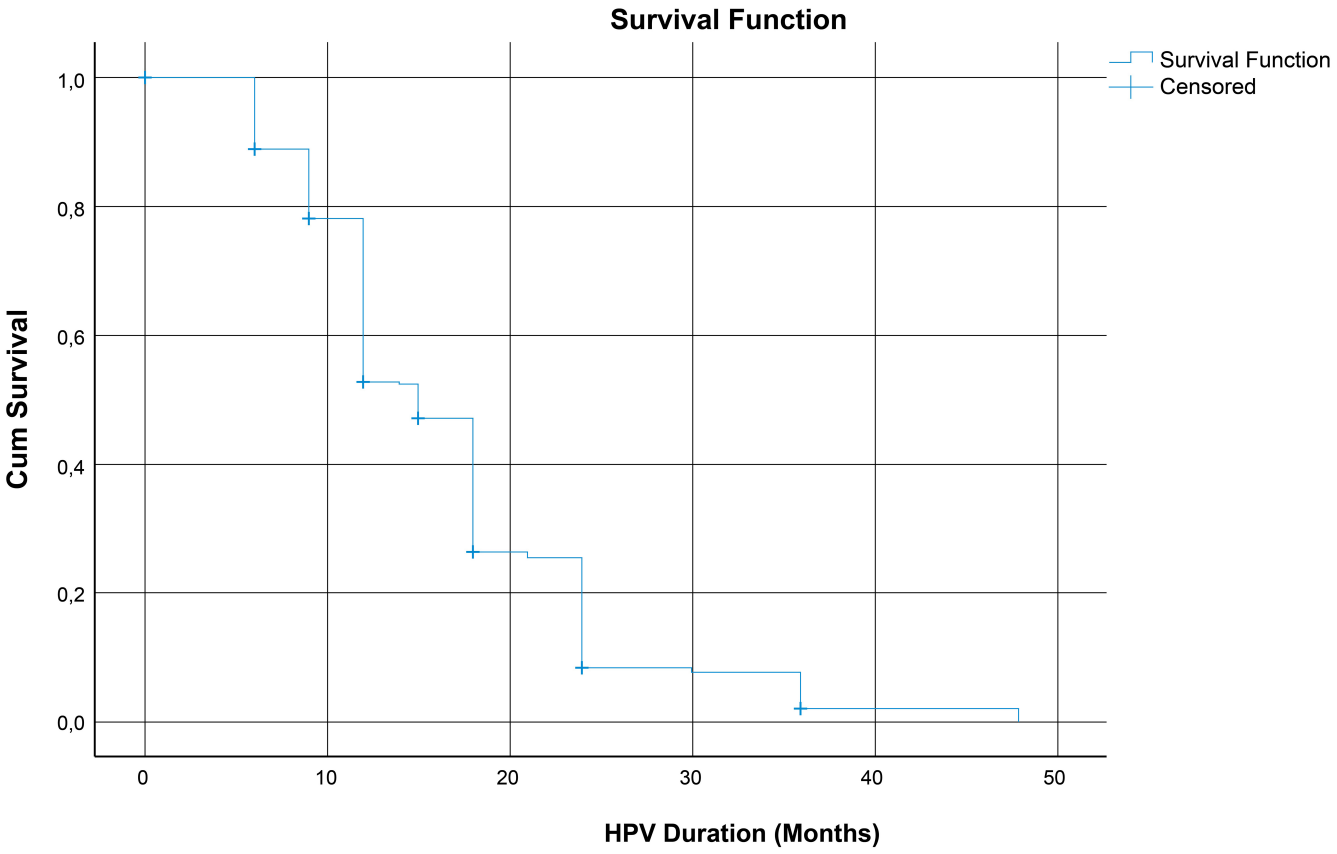

FIGURE 2. Kaplan-Meier analysis. HPV: human papillomavirus.

obtain the targeted biopsies. There is a high level of agreement among the experienced colposcopists regarding the assessment of normal epithelium, CIN 2–3 and invasive cancer [11]. CIN 1 diagnosis exhibits more interobserver variations among the colposcopists [12]. However, the lack of agreement extends to histopathologic diagnosis of these conditions. The colposcopy diagnostic performance in detecting cervical neoplasia was assessed through the meta-analysis of 32 studies involving ~8000 colposcopic punch biopsies. The patients included in this analysis underwent excisional biopsy (cone biopsy or loop electrosurgical excision), and the results were used as reference. Acolposcopic punch biopsy of CIN 1 or higher yielded 91% sensitivity and 25% specificity. Similarly, punch biopsy of CIN 2 or higher exhibited 80% sensitivity and 63% specificity [13]. In our study, LEEP was defined as the gold standard for evaluating colposcopy results. The results obtained were consistent with literature regarding sensitivity (75%), specificity (53%), positive predictive value (69%) and negative predictive value (60%). Increasing the sensitivity is seemingly linked with the performance of two or more biopsies [14]. Conducting random biopsies of normal-appearing cervixes are not justified as optimal approach has yet not been studied. This approach would result in numerous biopsies of healthy patients without considering the potential costs and risks [15]. In this study, similar variations were observed in biopsy numbers and diagnostic performance among the clinics being investigated.

In recent years, LEEP has emerged as standard strategy in treating cervical intraepithelial neoplasia, demonstrating superiority regarding effectiveness and minimally invasive nature. Poor prognostic factors following the excision include positive margin, HPV 16 positivity persisting for 6 months or longer, and endocervical gland involvement [16–18]. CIN shows higher cure rate with the complete excision of lesion, however limited long-term studies are available. It was found in a study that women undergoing primary LEEP for CIN 2–3, their dysplasia required further treatment in 3.2% of population at 12-months post-treatment, after having one negative 6-month colposcopic assessment [19]. Studies suggested that patients with positive margins after excision might have increased recurrent risk and residual disease compared to those with negative margins [20]. In the meta-analysis of 66 studies including large cohort of above 35,000 patients undergone excision for various CIN grades, the patients with positive margins had more than fivefold increased risk of developing any CIN grade after treatment compared to those with negative or uncertain margins (relative risk (RR) 5.47, 95% CI 4.37–6.83). This increased risk was also observed at the endpoint of post-treatment CIN 2–3 (18% versus 3%, RR 6.09, 95% CI 3.87–9.60) [16]. In this study, 182 women with positive margins had a rate of 14.95% which was consistent with the recent extensive systematic review and meta-analysis of 97 studies (2.8%–59.5%) [20]. Herein, the residual cervical lesions' rate in patients with positive margins was 45.05%, who underwent second surgery after initial LEEP. This was in accordance with the previously reported residual cervical lesions' rates (7.6%–53.7%) [21]. The prognosis raised questions where entire excisional specimen was negative and lesion might have been missed. Resultantly, the patients in this situation must be monitored similarly to those with positive margins. In a study examining over 670 LEEP specimens of patients with proven biopsy, and high-grade CIN, it was concluded that 14% patients showing no evidence of CIN in LEEP specimen experienced high recurrence rate of 24%. This recurrence rate (27%) was similar to that observed in patients with positive margins [22]. According to the meta-analysis of 128 studies, it was determined that HPV status was a more reliable predictor of recurrence compared to positive margins [20]. In this study, it was found that pre-LEEP HPV DNA positivity and surgical margins ≥HSIL increased the residual disease rate. In cases with positive surgical margins, it was observed that HPV 16 positivity was a recurrence predictor, being consistent with the literature [23].

This study had the access to cytology, HPV DNA records and pathology data of included patients. However, our study had the limitation pertaining to relatively short follow-up period. Moreover, the negative outcomes related to LEEP, and pregnancy were not investigated. In conclusion, this study encompassed the importance of close and vigilant monitoring of patients with positive margins after LEEP cervical conization. Finally, for the patients being candidates for HPV vaccination (11 to 26 years' age and selected patients aged 27 and older), a history of cervical dysplasia or genital warts did not prevent them from receiving vaccination. HPV vaccination did not have therapeutic effects on pre-existing HPV infection or cervical neoplasia. Studies indicated that it was linked to the reduced rate of CIN recurrence [24]. In our practice, HPV vaccination was incorporated as part of management strategy for CIN patients who had not received HPV vaccination series previously.

5. Conclusions

There was no significant difference regarding the diagnostic performance of two groups. HPV 16+ and positive surgical margin were the predictive of recurrence.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

FŞ—designed the research study; wrote the manuscript. EA, EUBÖ and SÖ—performed the research. EA, ÖA and AK—analyzed the data. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The retrospective study received ethical approval from the Istanbul Prof. Dr. CemilTaşcıoğlu City Hospital Clinical Research Ethics Committee, with approval NO: 333. Written permission has also been obtained from the institutions where the research was conducted, and informed consent has been

obtained from the patients. The study was conducted in accordance with the Principles of the Declaration of Helsinki.

ACKNOWLEDGMENT

Not applicable.

FUNDING

This research received no external funding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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How to cite this article: Fatih Şahin, Emine Aydın, Emine Ufuk Büyükkaya Öcal, Savaş Özdemir, Ali Murat Kasapoğlu, Özgür Akbayır. Evaluation of colposcopy and LEEP results performed in gynecology and gynecological oncology surgery services. European Journal of Gynaecological Oncology. 2024; 45(1): 116-123. doi: 10.22514/ejgo.2023.071.