Twelve-month follow-up detection of high-risk human papillomavirus (HPV) DNA for 93 cases with cervical intraepithelial neoplasia grade 2 or 3 (CIN 2-3) after a loop electrosurgical excisional procedure (LEEP)

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Summary

Purpose: The purpose of this study was to follow-up cervical intraepithelial neoplasia grades 2 or 3 (CIN2/3) cases after a loop electrosurgical excisional procedure (LEEP) by liquid-based cytology (LBC) and detection of HPV DNA. *Methods:* LEEP was performed for the first diagnosed CIN2/3 cases. Six and 12 months after LEEP, LBC and HPV DNA detection were performed. *Results:* The number of cases with CIN2 accounted for 64.5% (60/93) of the total cases before LEEP. Six months post LEEP, the number of cases with normal LBC and negative HPV DNA accounted for 63.4% (59/93). Cases with abnormal LBC accounted for 17.2 (16/93), and cases with persistent positive HPV DNA accounted for 11.8% (7/93). Two cases had both persistent positive HPV DNA and abnormal LBC. A vaginal intraepithelial neoplasm (VAIN2) was found in one of the HPV DNA persistent positive cases. Twelve months post LEEP, 4.3% (8/93) of the cases were HPV DNA positive. Abnormal LBC was observed in four cases (of which 2 cases were HPV DNA positive) with normal LBC and negative HPV DNA at six months post LEEP. *Conclusion:* HPV DNA examination is instrumental for the detection of VAIN.

Key words: Loop electrosurgical excisional procedure; Human papillomavirus; Follow-up.

Introduction

Cervical cancer is the second malignant tumour threatening the health of women globally. Treatment of patients with cervical intraepithelial neoplasia grades 2 or 3 (CIN 2-3) is the most important approach to prevent the occurrence of late-stage cancer. The loop electrosurgical excisional procedure (LEEP) is the main operation for patients with CIN2/3 and previous studies showed that the curative rate of LEEP was up to 67-90% [1-4]. Follow-up examinations after operation include cytology and colposcopy. High-risk HPV has been recognized as a reason for cervical cancer [5]. With the development of the Hybrid Capture II (HCII) technique, detection of HPV DNA has been introduced in the follow-up examinations after surgery is performed for CIN patients. To evaluate the role of HPV DNA detection in follow-up examinations, we performed a 12-month follow-up examination with both liquid-based cytology (LBC) and HPV DNA detection for the patients who underwent surgery from November 2006 to January 2008.

Materials and Methods

Subjects

LEEP was performed for the first diagnosed CIN2/3 cases with the lesion areas being less than 50% of the cervix. All cases with LEEP were included during the study period. Patients living in different locations were excluded from the study because follow-up examinations could not be performed for these patients. A total of 132 cases were included in the study and 93 cases were completed with follow-up examinations. This study was approved by the Ethics Board of Beijing University Third Hospital and patients were informed about the aims of the study and gave their informed consents.

LEEP

Wallech electric knife with a power of 50W was used for LEEP. Lugol's solution was used to display the cervical transformation areas before LEEP. The complete region with cervical transformation was excised for pathological examination.

Follow-up examinations

Six months post LEEP, LBC and HPV DNA examinations were performed. Twelve months post LEEP, both LBC and HPV DNA examinations were performed for those patients with positive HPV DNA in the first follow-up examination. HPV DNA-negative patients in the first follow-up examination underwent LBC 12 months after LEEP. Biopsy was performed for those patients with either abnormal LBC or positive HPV DNA in the follow-up examination. LBC examination was performed using Surepap (TriPath Imaging Inc., Burlington, NC). HPV DNA examination was performed using the HCII method (Digene Orporation, Gaithersburg, MD).

Statistical analysis

Statistical analysis was performed with the Student's *t*-test and p values < 0.05 were considered statistically significant.

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Results

General information for the patients with CIN2/3 before LEEP

The average age of the patients included in this study was 37.3 ± 6.7 and the average time of giving birth was 0.9 ± 0.7. Diagnoses of CIN2/3 were made for 91 cases by abnormal cytological changes and the remaining two cases were diagnosed by HPV DNA positive results with a cytological report of "no intraepithelial lesion or malignant change". The number of the cases with CIN2 and CIN3 accounted for 64.5% (60/93) and 35.5% (33/93), respectively. The lesion area in the patients did not exceed 50% of the total cervical areas by colposcopy examination.

Pathological observation of patients after LEEP

After LEEP, 41.9% (39/93) of the patients had similar pathological changes compared to pre-LEEP pathology, while 10.8% (10/93) of the initially diagnosed CIN2 cases were upgraded to CIN3. In contrast, 47.3% (44/93) of the patients had lower grade pathology after LEEP. Approximately 12% (11/93) of the initially diagnosed CIN3 were downgraded to CIN2. There was no invasive cervical carcinoma detected in any of these 93 cases.

Follow-up examinations six months after LEEP

LBC and HPV DNA examinations were performed for the patients six months after LEEP. Biopsy was performed for some of the patients under the direction of a colposcope. All the patients were divided into four groups based on the LBC and HPV DNA examination results. The first group contained only patients with abnormal LBC; the second group contained only patients with positive HPV DNA; the third group contained patients with both abnormal LBC and positive HPV DNA; and patients in the fourth group were normal in both LBC and HPV DNA examinations. Pathological analyses for the patients in all these four groups are shown in Table 1. Colposcopy examination was not performed for six abnormal patients due to personal reasons (pregnant or leaving the country, etc.). These six patients were involved in the late-stage examination and no CIN2 or high grade was detected.

The number of cases with normal LBC and negative HPV DNA accounted for 63.4% (59/93) six months after LEEP. The number of cases with negative HPV DNA accounted for 80.6% (75/93) of the total cases. Two cases were detected with CIN2 and one case was detected with VAIN2. The percentage of the persistent CIN2/3 after LEEP was 2.2% six months after LEEP.

Both LBC and HPV DNA examination can detect patients with CIN2 or higher grade pathological changes. The sensitivity and specificity of a single HPV DNA examination was not lower than those of a single LBC examination or combination with both LBC and HPV DNA examinations (Table 2).

Table 1. — *LBC, HPV DNA and pathological examinations for the patients six months after LEEP.*

Biopsy analysis	No. of LBC Abnormal cases	No. of HPV DNA positive cases	No. of cases with abnormal LBC and positive HPV DNA	No. of cases with normal LBC and negative HPV DNA
Inflammation	8	3	2	10
CIN1/cuminatum	4	5	2	8
≥ CIN2	0	0	2*	0
VAIN	1 (VAIN1)	1 (VAIN2)	0	0
N/A	3	2	1	41
Total	16	11	7	59

Note: "*" indicates the two cases with persistent disease. One case was diagnosed with CIN3 before LEEP and the edge of the disease area was excised completely. One case was diagnosed with CIN2 before LEEP and pathological analysis showed CIN1 after LEEP. The edge area was positive. N/A indicates the number of cases that were not examined with biopsy analysis.

Table 2. — *Sensitivity and specificity of the follow-up examinations on the detection of CIN2 or higher grade.*

Follow-up examinations	Sensitivity	Specificity	Positive percentage	Negative percentage
LBC	100% (2/2)	76.9% (70/91)	8.7% (2/23)	100% (70/70)
HPV DNA test	100% (2/2)	82.4% (75/91)	11.1% (2/18)	100% (75/75)
Combination of LBC and HPV DNA	100% (2/2)	64.8% (59/91)	5.8% (2/34)	100% (59/59)

*One case was diagnosed as VAIN2 in follow-up and was included in the group with CIN2 or greater lesions.

Table 3. — *LBC, HPV DNA and pathological examinations 12 months after LEEP.*

	HPV DNA positive 6 months after LEEP				HPV DNA negative 6 months after LEEP	
	LBC+	HPV DNA+	Abnormal in both LBC and HPV DNA	Normal in both LBC and HPV DNA	LBC-	LBC+
< CIN2	1	6	2	0	4	0
≥ CIN2	0	0	0	0	0	0
N/A	0	2	0	7	12	59
Total	1	8	2	7	16	59

Follow-up examinations after 12 months of LEEP

All the patents were divided into two groups: the first group contained patients with positive HPV DNA six months after LEEP and the second group contained patients with negative HPV DNA six months after LEEP. Twelve months after LEEP, 4.3% (8/93) of the cases were HPV DNA positive. Abnormal LBC was observed in four cases (of which 2 cases were HPV DNA positive) with normal LBC and negative HPV DNA at six months post LEEP. CIN2 or higher grade was not detected 12 months post LEEP (Table 3).

Discussion

LEEP uses a thin wire loop electrode which is attached to an electrosurgical generator to excise tissue with abnormal pathological changes. During the LEEP procedure, the tip of the electrode produces super high-fre-

quency electric wave. Instant contact between the tissues and electric wave produces high heat due to absorption of the electric wave by the tissue, which achieves the purpose of incision and hemostasis. Recently, LEEP has been widely used for the treatment the cervical precancerous lesions [6-8]. The advantage of LEEP to treat CIN2/3 is that (1) LEEP can completely remove the cervical transformed regions; (2) the samples from LEEP can be used for pathological examinations; (3) the procedure produces less bleeding; (4) the anaesthesia procedure is simple; and (5) LEEP can be easily performed in an outpatient department. Therefore, LEEP has replaced to a large extent the procedure of cold knife conisation (CKC). Previous studies have shown that the curative rate of cervical lesions by operation is approximately 67-90% [1-4]. Our studies showed that the rate of disease persistence was 2.2% after CIN2/3 patients with lesion areas less than 50% of the cervical area underwent LEEP, which is consistent with the results in the literature.

Persistent infection with HPV is one of the critical factors affecting the occurrence of persistence or recurrence of disease. Application of HPV detection in the follow-up examinations after operation displays many advantages. Some studies have shown that the changes of HPV detection results between postoperation and preoperation can predict the prognosis of persistence or recurrence of disease. High loads of HPV before operation would indicate a high chance of operation failure [9]. If the HPV can be cleared immediately after operation, the chance of operation success would be increased, otherwise the chance of operation failure would be increased due to the persistent infection with the same type of HPV [10, 11]. Because the predicative value of HPV negative is approximately 100%, researchers suggest that HPV detection be used as a single follow-up examination. For those HPV-negative patients after surgery, the intervals between follow-up examinations can be longer [12]. However, due to the fact that the operation failed in a few cases, the value of the HPV loads on the prognosis of disease persistence or recurrence in our study could not be analysed.

Cytological analysis is also an important follow-up examination. Cytological examination has higher specificity, while HPV DNA examination has higher sensitivity for the prognosis of disease [13]. Combined cytological and HPV DNA examination decreases the examination times, which is also recommended for clinical applications [14]. In our study both LBC and HPV DNA testing had a sensitivity of 100%. HPV DNA testing had a higher specificity, but was not statistically different.

Studies have shown that persistence or recurrence of disease after surgery are related to the residual tissue around the edge of the lesion, pathological changes in the glands caused by the original lesions, and multiple distribution of the lesions [15-18]. Some studies showed that cases where the cutting edge had positive pathological changes after LEEP/CKC had a higher recurrence rate when lesions remained in the cervical canal [19, 20]. When complete hysterectomy was performed for those patients with positive pathological changes in the cutting edge, only a small number of patients still had high-grade pathological changes in the cervix. This might be due to the edge thermal effect of LEEP and heating haemostasis, which further disrupts the lesions. Therefore, in the guidelines of the American Society for Colposcopy and Cervical Pathology, individual treatment should be applied for patients with a positive cutting edge in the surgical samples. Firstly, cytological analysis can be used for the follow-up examinations. However, cytology should include a sample from the cervical tube because lesions may be deeper once the new transformation regions are developed after LEEP. Secondly, for those patients with positive cutting edges in the samples from operations, diagnostic conisation can be performed. Thirdly, if it is not appropriate to perform diagnostic conisation, complete hysterectomy can be performed [21]. Lastly, previous reports showed that persistent CIN occurred after conisation for those CIN patients with a failed LEEP operation. For patients who do not want to have complete hysterectomy, partial trachelectomy can be performed and satisfactory prognosis is achieved [22]. All patients undergoing the procedure should have ten years of follow-up examinations.

Among the 93 cases investigated in this study, two patients had persistent lesions but no infiltration carcinoma was detected. One of the reasons that the operation had a high rate of success was that the lesion areas were relatively small (< 50%). In the two patients with persistent lesions, one sample from the operation had positive pathological changes in the outer edge and the other one was negative. More cases are needed in this regard.

Close follow-up examination should be performed for patients undergoing the procedure. Combination examinations should be applied for patients with high-risk disease factors. Our two patients were diagnosed six months after the operation. Previous studies show that persistence or relapse of lesions normally occurred within two years post operation. Thus, follow-up examination in the early stage is most important to prevent and detect recurrence of disease [23, 24].

It is worth noting that infection with high-risk HPV is not only related to the high grade of CIN, but also closely related to the occurrence of VAIN. During the follow-up examinations, VAIN was detected in this study. Therefore, follow-up examination should be performed for the whole lower genital tract. We also found that LBC results were negative, while HPV DNA was positive for the VAIN2 patients, indicating that HPV DNA has a higher sensitivity. Statistical analysis was not performed because the number of these cases was low.

In conclusion, persistent lesions were observed in 2.2% (2/93) of the CIN2/3 cases with LEEP treatment during the one-year follow-up with HPV DNA and LBC examinations. Both HPV DNA and LBC analyses can efficiently detect CIN2 or higher grade for post surgical patients during follow-up examinations. In addition, HPV DNA examination has a high specificity for the detection of CIN2/3.

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