Original Article
Application value of different transformation zone types and its genetic relationship with high-risk HPV type in diagnosis and therapy of cervical disease

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Abstract: This study aims to discuss the influence of different types of transformation zone (TZ) on positive surgical margin of loop electrosurgical excision procedure (LEEP) and the significance of infection of different genetic high-risk HPV for cervical intraepithelial neoplasm. The clinical data of patients who had CIN2+ and received LEEP during January to December 2013 was investigated. The conditions of positive surgical margin of patients of different transformation zone (type I, II, III) were analyzed. The clinical high-risk types of HPV were divided into three groups, including A5/6, A7 and A9, compared with the pathological conditions of pre-operation and post-operation of the patients in respective group. The results indicated that type III transformation zone is more likely to cause positive cutting margin. For CIN2+ patients, sensitivity and specificity are 0.89% and 79.56% in group A5/6, and negative and positive predicted value (NPV, PPV) are 40% and 5%. The sensitivity, specificity, NPV, PPV in group A7 is 12.5%, 44.08%, 29.49% and 21.21%, respectively. The sensitivity, specificity, NPV, PPV in group A9 is 88.99%, 87.09%, 85.26%, 81.51%, respectively. Transformation zone type was correlated positively with positive cutting margin percentage (r = 0.8732, P < 0.05). Compared with type I, type II and III transformation zone is more likely to cause pathological upgrades. In conclusion, different types of transformation zone and high-risk HPV have clinical significance in causing positive cutting margin of surgery and disease extent.

Keywords: Cervical intraepithelial neoplasia, transformation zone, loop electrosurgical excision procedure, positive surgical margin

Introduction

Conization of cervix is one of the main treatment methods of cervical precancerous lesions. On the one hand it can excise lesion, on the other hand it can find disease that colposcope examination failed to detect, helping defining the degree of cervical disease. Therefore, this method has the dual role of diagnosis and therapy, playing an important role in the treatment of cervical disease. Loop electrosurgical excision procedure (LEEP) is widely applied in clinic because of its relatively simple operation, short operation time, less bleeding, and no need of hospitalization and less complication in patients [1, 2]. Although LEEP is more and more applied in clinic, the recurrence rate of postoperative CIN is 2% to 29.5% [3-6]. Some studies [7, 8] have proposed that the persistent infection of high-risk type HPV and the positive surgical margin are important factors causing relapse. How to deal with the positive surgical margin and relapse is a common problem puzzling physicians: to choose conservative treatment, they need to take the risk of worse disease progress; to carry out surgery, they need to concern the unnecessary trauma and complications. It is a dilemma to make a decision when the patients have fertility requirements. Therefore, it is of particular importance to decrease the positive surgical margin and reduce relapse after operation. The HPV test approved by the FDA-the Cervista high-risk HPV type test classifies patients into groups of A5/6, A7 and A9, basing on genetic distance. The A5/6 oligonucleotide probe mixture detects HPV type of 51, 56 and 66; the A7 oligonucleotide probe mixture detects HPV type
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of 18, 39, 45, 59 and 68; A9 oligonucleotide mixture type detects HPV type of 16, 31, 33, 35, 52 and 58. This study will divide the samples into three groups of A5/A6, A7 and A9 according to the theory, to examine the difference between different genetic relational groups. In clinic, different transformation area means that the degree of lesions aggravating into the cervix varies, and the pathogenicity of different genotype related high-risk HPV is different. This study retrospectively analyzes the clinical data of 215 cases of patients who were diagnosed as CIN2+ pathological changes and received LEEP in the cervical disease clinical center in our hospital during January to December 2013. We aim to explore the clinical value of different transformation zone of the cervical region and gene related high-risk HPV for predicting positive surgical margin and determining the operation range.

Materials and methods

Object of study

Clinical data of patients who had CIN2 or above before surgery and received LEEP in our center during January to December 2013 are selected. There are 215 patients, with average age of 46 ± 5 (25 to 68), average gestation times of 3.2 (0 to 10), average parturition times of 1.5 (0 to 5); 67.9% (146/215) of them had contactive bleeding or irregular vaginal bleeding or vaginal secretions increasing history, and 32.1% (69/215) of them do not show clinical symptoms. 29 of the patients have positive surgical margin. Postoperative pathological lesion degrees are as followed: 10 cases below CIN2, 93 cases of CIN2, and 108 cases of CIN3. Of the 29 patients, 15 have carcinoma in situ, and 4 have tiny infiltrating carcinoma (3 IA1 cases, 1 IA2 case). The distribution of the transformation zone type: 65 cases of type I, 77 cases of type II, and 63 of type III; distribution of high-risk type HPV genetic relationship groups: 20 cases of A5/A6 group, 76 cases of A7 group and 109 cases of A9 group.

Surgical methods

All patients had received transvaginal biopsies before operation (those did not satisfy with the imagines of transvaginal biopsy all received conventional endocervical curettage), and were diagnosed as CIN2, CIN3 (including carcinoma in situ) or tiny infiltrating carcinoma. As to the abnormal transformation area marked by iodine staining, surgeons selected the corresponding models of high frequency electricity knife (American Wallach Medical Instrument Company) according to the lesion area and the size of the cervix. They used Fish knife head, cutting vertically from 9 or 2 o’clock direction of

<table>
<thead>
<tr>
<th>Virus type</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>NPV</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>A5/6</td>
<td>0.89%</td>
<td>79.56%</td>
<td>40%</td>
<td>5%</td>
</tr>
<tr>
<td>A7</td>
<td>12.50%</td>
<td>44.08%</td>
<td>29.49%</td>
<td>21.21%</td>
</tr>
<tr>
<td>A9</td>
<td>88.99%</td>
<td>87.09%</td>
<td>85.26%</td>
<td>81.51%</td>
</tr>
</tbody>
</table>

Table 1. The sensitivity, specificity, negative predicted value and positive predicted value of each virus type

Figure 1. The relationship between classification of high-risk HPV type and their histology (CIN2+ cases). A. CIN2+ positive cases in HPV types. B. CIN2 negative cases in HPV types. The differences between two groups were illustrated in figures.
Set the cervical, rotated 360 degrees clockwise; the circular knife was used to cut from the 6 o'clock direction of the cervix and the diseased tissue was cut off in a coning up way; they were careful to cut the tissue 0.5 cm away out of the abnormal transformation zone. The samples were sent to the pathology department of our hospital for histopathological examination.

Collection of HPV sample

The special Kape HPV Brushes were completely inserted into the opening of the patients' cervix and rotated 3 to 5 circles in the same direction. The brush heads were then saved in the preserving liquid in small tightly capped bottles. After three steps of examination-total DNA extraction of cervical cells, gene amplification (PCR), and diversion hybridization (with Kape medical nucleic acid molecule hybridization instrument), fourteen kinds of high-risk type HPV were detected: HPV16, 18, 31, 33, 35; 39, 45, 51; 52; 56; 58, 59, 66, 68.

Statistical analysis

Using SPSS 13.0, we compared the positive surgical margin rate of specimens of different transformation zone types and different high-risk HPV types. χ² test was used and P < 0.05, showing a statistical significant.

Results

Relationship between different HPV gene type and degree of pathological change

There were 205 patients of high-risk HPV type and 112 of them suffered CIN2+ after operation, with 1 case of A5/A6, 14 cases of A7, and 97 cases of A9 (Figure 1). Ninety-three of the patients suffered CIN2, with 9 cases of A5/A6, 62 cases of A7, and 12 cases of A9 (Figure 1). There were 10 patients of high-risk HPV type did not suffered infection and they were CIN1 or did not showed cervical intraepithelial neoplasia, so their data were not included.

As showed in Table 1, for the post-operation CIN2 patients, the clinical sensitivity, specificity, negative and positive predicted value were also investigated.

Transformation zone types correlate with positive cutting margin percentage

The cervical transformation zone can be distinguished into type I, II, and III. As the transformation zone regressing into the uterine neck, the pathological change is more and more undetectable. In this study, 65 patients have type I transformation zone, and 2 of them have positive cutting margin. 77 patients have type II transformation zone, and 5 of them have positive cutting margin. 63 cases of patients have type III transformation zone, and 22 of them have positive surgical margin. The positive cutting margin percentage in type II was higher significantly compared to type I (Figure 2A, P < 0.05). The positive cutting margin percentage in type III was higher significantly compared to type II (Figure 2A, P < 0.01). The positive cutting margin percentage was higher significantly compared to type I (Figure 2A, P < 0.001). The correlation analysis showed that the transformation zone type was correlated positively with the positive cutting margin percentage (Figure 2B).
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Figure 2A and 2B indicated that type III transformation zone is more likely to cause positive cutting margin due to insufficient resection range compared with type I and type II respectively.

Relationship between different transformation zone types and surgical pathological change

As the transformation zone regressing into the uterine neck, the pathological change is harder to be detected, making it difficult to diagnose and carry out the vagina biopsy and affect the accuracy of diagnosis. In this study, 65 patients have type I transformation zone, 3 of them higher in pathological grade and 20 lower after operation. Seventy-seven patients have type II transformation zone, 5 of them higher and 5 lower (Figure 3A). Sixty-three patients have type III transformation zone, 20 of them higher and 7 lower (Figure 3A).

The correlation analysis showed that the transformation zone type was not correlated with the surgical pathological changes (Figure 3B, \( r = 0.8732, P < 0.05 \)). However, compared with type I, type II and III transformation zone is more likely to cause pathological upgrades, indicating that the pathological change may be neglected during diagnosing (Table 2). It is easier to find the important pathological site under mirror for type I transformation zone.

Table 2. The relationship between transformation zone type and post-operation pathological change

<table>
<thead>
<tr>
<th>Transformation zone type</th>
<th>Up-grade (%)</th>
<th>Down-grade (%)</th>
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<tbody>
<tr>
<td>I</td>
<td>40 (3/12)</td>
<td>60 (9/12)</td>
</tr>
<tr>
<td>II</td>
<td>71.4 (5/7)*</td>
<td>28.6 (2/7)*</td>
</tr>
<tr>
<td>III</td>
<td>74.1 (20/27)*</td>
<td>25.9 (7/27)*</td>
</tr>
</tbody>
</table>

*P < 0.05 represents the post-operation pathological changes in type III compared to type I. *P < 0.05 represents the post-operation pathological changes in type II compared to type I.

Discussion

LEEP treatment of cervical disease should reach the corresponding range and excise the lesion as far as possible. At present, it is commonly thought that the positive cutting margin is related with the LEEP operation itself, menopause, lesion level, lesion area and other factors [9]. As the lesion level rises, the depth of the lesion is increasing and the extension into the uterine neck is aggravating, leading to increasing difficulty of operation and residue of lesion. Histologic evidence shows that the average depth of involved glands of CIN III is 1 to 2 mm, with the deepest of 5.22 mm. Therefore, the UK National Health Service Cervical Screening Program (NHSCSP) guidelines suggest that removing tissue thickness should be up to 7 mm, and it is better to remove the whole piece of diseased tissue, to facilitate the accurate diagnosis of pathological examination, especially of the tiny infiltrating carcinoma clinical staging [10]. 8091 samples of the 35109 patients in the study of Ghaem-maghami etc. [4] show at least one side of cutting margin is positive, with a total positive cutting margin rate of 23%. The total positive cutting margin rate of our study is 13.48%, less than that of the above study. The reason for this may be
that there are less cases of carcinoma in situ and tiny invasive carcinoma. Also, our study shows the positive surgical margin rate of type III transformation is significantly higher than that of type I and that of type II, suggesting surgeons expanding the thickness and depth of excision for type III patients when it is not easy to estimate the range of diseased area.

The A9 group of HPV type has been proved to have a closer relationship with Asians’ HSIL. It is thought that the repeatability of CIN2 is not so good, and CIN3 or above deserves more focus. In this study, the clinical sensitivity, specificity, negative and positive predicted value of the A9 group are significantly higher than those the A5/6 and A7 group in the CIN2+ patients, implying that more tissue should be removed in the surgery in order to avoid insufficient resection caused by improper preoperative diagnosis and inadequate estimate for the patients of A9 group; meanwhile, it happens at a low rate that patients of other groups suffering CIN3 or above, so the range of operation should be appropriately controlled to reduce the incidence of complications after surgery. It is true that the Kape Classification method applied in this study has essential difference with Cervista classification method, and the data differ from that of Cervista method [11, 12]. However, some positive conclusions still can be reached and used to guide the clinical work. In the future, we will combine Kape and Cervista HPV classification methods to do further research.

There is statistical difference between patients of type I transformation zone and type III both in the pathological upgrade case and the downgrade case. It implies that as the transformation zone extend gradually into the deeper uterine neck, it is harder to detect the hidden pathology and the accuracy of biopsy under colposcope reduces. Satisfactory preoperative diagnostic accuracy can be achieved for type I transformation zone, while missed diagnosis and misdiagnosis often occurs for type III transformation zone. So LEEP can be taken into consideration to help improving the diagnostic accuracy when the biopsy under colposcope examination is not satisfied or obviously inconsistent with clinical examination.

To sum up, in order to improve the preoperative diagnostic accuracy and reduce inadequate resection range and suffering caused by misdiagnosis or poor estimate before surgery, surgeons should take comprehensive reference from cytology, colposcopy and type of high-risk HPV, and determine the specific range of operation, and makes every effort to excise the whole lesion.

Disclosure of conflict of interest

None.

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