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Original Article

The correlation between high-risk HPV infection and precancerous lesions and cervical cancer

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Abstract: Objective: To analyze the correlation between high-risk human papillomavirus (HPV) infection and precancerous lesions and cervical cancer. Methods: Patients with cervicitis (N=100), cervical intraepithelial neoplasia grade I (CIN I) (N=100), cervical intraepithelial neoplasia grades II-III (CIN II-III) (N=100) and cervical cancer (N=100) were enrolled. The exfoliated cervical cells were collected with the same method, and the detection of the HPV types was carried out by PCR-reverse dot blot (RDB) assay. Results: The top 5 HPV types in stage I-II cervical cancer were 16, 18, 52, 58, and 53, with a HPV positivity rate of 83.61%, while top 5 HPV types in stage III-IV cervical cancer were 16, 18, 58, 52, and 33, with a HPV positivity rate of 82.05%. The rate of high-risk HPV positivity for cervicitis was 5%, with HPV types of 16, 18, 52, and 33, 12% for CIN I, with HPV types of 16, 58, 52, 33, 56, 66, and 68, and 42% for CIN II-III, with HPV types of 16, 18, 58, 52, 33, 66, and 68. The prevalence of single, dual, and multiple HPV infection was 8.00%, 1.00%, and 0.00% for CIN I, 24.00%, 7.00%, and 1.00% for CIN II-III, and 57.00%, 25.00%, and 3.00% for cervical cancer, respectively. The age of patients with CIN I was mainly ≤24 and 25-34 years while CIN II-III in 25-34 and 35-44 years, and cervical cancer in 35-44 and 45-54 years. Conclusion: The distribution of HPV subtypes in cervical cancer is closely related to the pathological types, lesion grades, and stages of cervical cancer. The incidence of cervical lesions varies with age, suggesting that high-risk groups should be well monitored and receive regular screening and timely HPV vaccination to effectively prevent cervical cancer.

Keywords: Human papillomavirus, high-risk type, infection, precancerous lesions, cervical cancer, correlation

Introduction

Cervical cancer has a high incidence among female malignancies and even among all malignancies, and is second only to breast cancer [1]. With the increase of people's health awareness and the advancement of diagnostic technology, the detection rate of cervical cancer at an early stage has been gradually improved, but no targeted therapy has been found regarding the treatment of cervical cancer. Therefore, clinical attention to cervical cancer is more about prevention than treatment [2].

High-risk human papillomavirus (HPV) vaccination protects against cancer-causing infections and precancerous lesions. However, the vaccination and screening coverage remain low in China [3]. Evidence has found that it takes years or even decades to progress from cervical precancerous lesions to cervical cancer [4]. Therefore, it is an ongoing medical effort to make good and accurate diagnosis of cervical precancerous lesions and prevent their worsening. It has been found that HPV infection has a significant impact on the incidence of cervical cancer, and is the most common sexually transmitted infection, while other routes include mother-to-child transmission and gastrointestinal infections [5, 6]. Studies have found that HPV-expressed E6 and E7 proteins increase interleukin 18 (IL-18) levels and decrease interferon (IFN) levels, which can promote consistent viral replication and also help malignant cells avoid attack by the immune system, leading to the slow accumulation of microscopic lesions that eventually lead to cervical cancer [7, 8]. Another study has classified HPV infec-

tion as monotypic and polytropic, both of which are strongly associated with the occurrence and progression of cervical cancer [9].

Due to the complex etiology of cervical cancer, HPV infections in different regions and different populations also show different symptoms, so the research on the correlation between cervical cancer and HPV infection has been lack of comprehensiveness. In this study, patients with cervicitis, cervical precancerous lesions and cervical cancer were enrolled to specifically investigate the distribution of HPV types to provide new data on the prevention of cervical cancer.

Materials and methods

General data

Patients with cervicitis, cervical intraepithelial neoplasia grade I (CIN I), cervical intraepithelial neoplasia grade II-III (CIN II-III), and cervical cancer diagnosed in the Department of Obstetrics and Gynecology of our hospital from January 2017 to January 2020 were enrolled (N=100 for each group). And the patients' cervical exfoliated cells were collected. Inclusion criteria: patients aged 18-60 years, married or had sexual partner for >1 year, and were not pregnant. Patients understood the purpose, benefits, risks and alternatives of the treatment, and signed the consent form. The study passed the ethical approval of General Hospital of North Theater Command, People's Liberation Army of China. Exclusion criteria: unmarried and sexually active for <1 year, previous cervical conization, hysterectomy, pregnant or lactating, comorbid with immune system disorders, and received immunosuppressive drugs prior to study participation.

Methods

Apparatus and reagents: amplifiers, rapid DNA hybridization device, centrifuges, monitoring and color development systems, HPV genotyping kits. The HPV genotyping test was performed by PCR-reverse dot blot (RDB) assay with HPV genotyping kit. The specific primers were designed according to the characteristics of HPV gene. The target fragments of each HPV genotype were amplified by PCR, accordingly, and the amplified products were hybridized with the HPV genotyping probes fixed on the membrane strips to determine the HPV genotype according to the signal density.

Outcome measurement

The distribution of HPV types in different pathological types of cervical cancer was analyzed.

The correlations between staging of cervical cancer and HPV infection, between cervical lesions and HPV genotypes were analyzed.

The relationships between cervical lesions and high-risk HPV infection, between cervical precancerous lesions, cervical cancer and multiple high-risk HPV infections, were explored.

The age distribution of patients with cervical lesions and cervical cancer was analyzed.

Statistical methods

Statistical analysis was performed with SPSS 23.0 (International Business Machines Corporation, IBM, Armonk, NY, USA). Count data were expressed as [n (%)] and examined by X^2 test. Multiple comparisons were analyzed with analysis of variance (ANVOA) with post hoc F test. $P<0.05$ was considered statistically significant.

Results

Pathological type of cervical cancer and HPV infection typing

Of the 100 cervical cancer patients, there were 70 cases of squamous carcinoma, 20 cases of adenocarcinoma, and 10 cases of adenosquamous carcinoma, and the top 10 HPV types were 16, 18, 58, 52, 33, 53, 45, 56, 66, and 68, respectively, with HPV types of 16, 18, 58, 52, 33, and 66 for squamous carcinoma, HPV types of 16, 18, 52, 33, and 53 for adenocarcinoma were, and HPV types of 16, 18, 58, and 52 for adenosquamous carcinoma (**Table 1**).

Cervical cancer staging and HPV infection typing

There were 61 patients with stage I-II cervical cancer and 39 patients with stage III-IV cervical cancer in 100 patients. The top 5 HPV types in stage I-II patients included 16, 18, 52, 58, and 53, and the top 5 HPV types in stage III-IV patients included 16, 18, 58, 52, and 33 (**Table 2**).

Table 1. HPV infection typing in cervical cancer (n, %)

HPV typing	Squamous carcinoma (n=70)	Adenocarcinoma (n=20)	Adenosquamous carcinoma (n=10)	X^2	*P*
Type 16	27 (38.57)	7 (35.00)	3 (30.00)	0.849	0.341
Type 18	16 (22.86)	5 (25.00)	2 (20.00)	0.598	0.172
Type 58	10 (14.29)	0 (0.00)	1 (10.00)	1.628	0.106
Type 52	5 (7.14)	2 (10.00)	1 (10.00)	0.934	0.119
Type 33	2 (2.86)	1 (5.00)	0 (0.00)	0.533	0.295
Type 53	0 (0.00)	1 (5.00)	0 (0.00)	0.752	0.341
Type 45	0 (0.00)	0 (0.00)	0 (0.00)	/	/
Type 56	0 (0.00)	0 (0.00)	0 (0.00)	/	/
Type 66	1 (1.43)	0 (0.00)	0 (0.00)	1.082	0.319
Type 68	0 (0.00)	0 (0.00)	0 (0.00)	/	/
Other types	9 (12.86)	4 (20.00)	3 (30.00)	2.968	0.062

Table 2. HPV infection typing for different pathological stages of cervical cancer (n, %)

Pathological stage	Number of cases	1	2	3	4	5
Stage I-II	61	30 (49.18)	16 (26.23)	7 (11.48)	3 (4.92)	1 (1.64)
Stage III-IV	39	20 (51.28)	4 (10.26)	3 (7.69)	2 (5.13)	1 (2.56)
X^2		1.259	2.857	1.637	0.857	0.639
P		0.137	0.084	0.129	0.134	0.182

Table 3. Cervical cancer stage and HPV infection rate (n, %)

Pathological stage	Number of cases	HPV-positive	HPV-negative
Stage I-II	61	51 (83.61)	10 (16.39)
Stage III-IV	39	32 (82.05)	7 (17.95)
Total	100	83 (83.00)	17 (17.00)
X^2		0.041	
P		0.840	

Cervical cancer staging and HPV infection

The HPV positivity rate was 83.00% and HPV negativity rate was 17.00% in 100 cervical cancer patients, including 83.61% HPV positivity and 16.39% negativity in patients with stage I-II cervical cancer and 82.05% HPV positivity and 17.95% negativity in patients with stage III-IV cervical cancer (**Table 3**).

Cervical lesions and HPV infection typing

Of the top 10 HPV subtypes detected in cervical cancer, the subtypes detected in cervicitis were 16, 18, 52, and 33, the subtypes detected in CIN I precancerous lesions were 16, 58, 52, 33, 56, 66, and 68, and the subtypes detected in CIN II-III precancerous lesions were 16, 18, 58, 52, 33, 66, and 68 (**Table 4**).

The cervical lesions and high-risk HPV infection

Among 100 patients with cervicitis, 5 cases were positive for high-risk HPV and 95 cases were negative for high-risk HPV, with a high-risk HPV-positive rate of 5%. Among 100 patients with CIN I precancerous lesions, 12 cases were positive for high-risk HPV and 88 cases were negative for high-risk HPV, with a high-risk HPV positivity rate of 12%. Among 100 patients with CIN II to III precancerous lesions, 42 cases were positive for high-risk HPV and 58 cases were negative for high-risk HPV, with a high-risk HPV positivity rate of 42%. There was a statistical difference in the high-risk HPV positivity rate between different type of cervical lesion ($P<0.05$) (**Table 5**).

Cervical precancerous lesions and cervical cancer with multiple high-risk HPV infections

The rates of single HPV infection, dual HPV infection, and multiple HPV infection for CIN I precancerous lesions were 8.00%, 1.00%, and 0.00%, respectively; those for CIN II-III precan-

Table 4. Distribution of HPV infection typing for three types of cervical lesions (n)

HPV typing	Cervicitis (n=100)	CIN I (n=100)	CIN II-III (n=100)	X^2	*P*
Type 16	2	3	21	5.185	0.016
Type 18	1	0	7	3.684	0.027
Type 58	0	1	6	3.581	0.031
Type 52	1	4	5	2.194	0.172
Type 33	1	1	1	0.000	1.000
Type 53	0	0	0	/	/
Type 45	0	0	0	/	/
Type 56	0	1	0	0.695	0.248
Type 66	0	1	1	0.524	0.152
Type 68	0	1	1	0.524	0.152

Table 5. Cervical lesions and high-risk HPV infection (cases)

Type of cervical lesion	Number of cases	High-risk HPV-positive	High-risk HPV-negative
Cervicitis	100	5	95
CIN I	100	12	88
CIN II-III	100	42	58
X^2			4.859
P			0.027

Table 6. The correlation between precancerous lesions and cervical cancer and high-risk HPV infection (n)

Type of lesion	Number of cases	Single HPV infection	Dual HPV infection	Multiple HPV infections
CIN I	100	8	1	0
CIN II-III	100	24	7	1
Cervical cancer	100	57	25	3
X^2		2.069	2.172	1.639
P		0.067	0.072	0.371

Table 7. Cervical lesions and cervical cancer and age (n)

Type of lesion	Number of cases	≤um years	25-34 years	35-44 years	45-54 years	55+ years
CIN I	100	46	38	12	3	1
CIN II-III	100	3	37	40	13	7
Cervical cancer	100	1	13	39	41	6
X^2		4.295	3.978	5.827	4.968	1.751
P		0.019	0.024	0.005	0.013	0.024

cerous lesions were 24.00%, 7.00%, and 1.00%, respectively; and those for cervical cancer were 57.00%, 25.00%, and 3.00%, respectively, and there were differences in HPV infection rates between cervical lesion types, showing no statistical significance between cervical precancerous lesions and cervical cancer ($P>0.05$) (**Table 6**).

Relationship between cervical lesions and cervical cancer and age

Patients with CIN I precancerous lesions were concentrated in two age groups: ≤24 years and 25-34 years; patients with CIN II-III precancerous lesions were concentrated in two age groups: 25-34 years and 35-44 years; patients with cervical cancer were concentrated in two age groups: 35-44 years and 45-54 years (**Table 7**).

Discussion

Humans are at very high risk of HPV infection [10]. It has been found that the incidence of HPV infection in sexually active population is more than 80% [11]. HPV infection has been found to occur in different ages including newborns due to mother-to-child transmission [12]. Since HPV infection and subtype distribution vary in different regions [11], it is of practical significance to conduct a correlation analysis between high-risk HPV infection and precancerous lesions and cervical cancer.

In this study, 100 cases of cervical cancer were analyzed, and the results showed that the top 5 HPV subtypes were 16, 18, 58, 52, 33, and 66 for squamous carcinoma, 16, 18, 52, 33, and 53 for adenocarcinoma, and 16, 18, 58, and 52 for adenosquamous carcinoma. Moreover, it was found that the top 5 subtypes of HPV infection in stage I-II cervical cancer were 16, 18, 52, 58, and 53, and the top 5 subtypes in stage III-IV were 16, 18, 58, 52, and 33. Although there are some differences in the major subtypes of HPV in patients with different pathological staging, all of them have types 16 and 18, confirming their importance. It was found that HPV positivity rates exceeded 80% in patients with cervical cancer [13], suggest-

ing that HPV infection is closely related to the development of cervical cancer.

This study showed that the HPV positivity rate in patients with stage I-II cervical cancer was 83.61% and that in patients with stage III-IV cervical cancer was 82.05%. Other evidence [14] showed that the HPV positivity rate for CIN I was about 59.73% with major subtypes of 16, 58, 6, 52, and 43. Among them, types 16, 52 and 58 were the same as in this study, and the differences in the detected subtypes may be related to the region where the study was conducted as well as the combined analysis of CIN I and CIN II. This study showed that the rate of high-risk HPV positivity in cervicitis was 5%, with HPV types of 16, 18, 52, and 33; the rate of high-risk HPV positivity in CIN I was 12%, with types of 16, 58, 52, 33, 56, 66, and 68; the rate of high-risk HPV positivity in CIN II-III was 42%, with types of 16, 18, suggesting that the HPV positivity rate increased with the increasing severity of lesions, which confirmed the correlation between HPV infection and the occurrence of cervical precancerous lesions and cervical cancer, which should be explored further.

There is no uniform conclusion on whether multiple HPV infections increase the incidence of cervical precancerous lesions and cervical cancer [15, 16]. In this study, the prevalence of single, dual, and multiple HPV infections was found to be 8.00%, 1.00%, and 0.00% for CIN I, 24.00%, 7.00%, and 1.00% for CIN II-III, and 57.00%, 25.00%, and 3.00% for cervical cancer, respectively. With the increase of the severity of cervical lesions, the proportion of HPV single, dual and multiple infections altered as well, but the results of this study did not show a statistical difference, which may be related to the small sample size. Another study [17] also found that the highest rate of multiple HPV infections was observed in CIN III lesions, but the correlation between grade of cervical lesion and multiple HPV infections was not explored. A follow-up study [18] showed that more HPV infection types were associated with a higher risk of developing high-grade cervical intraepithelial neoplasia. A previous study [19] has found that a subject with a confirmed multiple HPV infection has a significantly higher risk of developing cervical cancer afterwards, even if the pap smear results appear normal, whereas if that subject has only a single HPV infection, the risk of developing cervical cancer is significantly lower. However, other studies [20-22] have suggested that there is no direct relationship between the types of HPV infections and the grade of cervical lesions, and that multiple HPV infections are not a direct risk factor for the development of cervical cancer. Exactly how multiple HPV infections are associated with cervical precancerous lesions and cervical cancer requires more research. A global study showed that the age of onset of high-grade cervical lesions in South America, Europe, Africa, and Asia was concentrated between 25 and 40 years [23]. A large study in China showed that the incidence of cervical precancerous lesions was very low in people under 35 years old, and that high-grade cervical precancerous lesions occurred mostly in people aged 40-44 years [24]. The results of this study showed that the age of CIN I was concentrated below 34 years, 25-44 years for CIN II-III, and 35-54 years for cervical cancer. It is suggested that different degrees of prevention of lesions should be carried out for different age groups, and the earlier the prevention is initiated, the lower the risk.

In conclusion, the distribution of HPV subtypes in cervical cancer is closely related to pathological type, lesion grade, and cervical cancer stage, and the incidence of cervical lesions varies with age, suggesting that high-risk groups should be constantly monitored and receive HPV vaccination. The present study also has some shortcomings, as shown by the fact that the correlation between high-risk HPV infection and cervical precancerous lesion and cervical cancer in terms of pathological type, lesion grade and clinical stage was only tentatively proposed, without an in-depth analysis about the underlying mechanisms, which need to be further investigated.

Disclosure of conflict of interest

None.

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