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Analysis of Human Papillomavirus Infection in Cervical Exfoliated Cells

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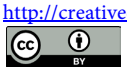

Abstract

To screen patients with early cervical lesions by analyzing the infection of high-risk Human papillomavirus (HR-HPV). **Research Methods:** The cervical exfoliated cell specimens and their clinical data were collected. The HPV infection types of the collected specimens were detected by fluorescence quantitative PCR, and the correlation between HPV infection and clinicopathological features was analyzed statistically. **Results:** 725 cases were HR-HPV positive from 2605 cases, including 15 high-risk types of HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68. Different histological types ranged from NILM to HSIL, and the positive rate of HPV showed an increasing trend with the aggravation of cervical lesions. **Conclusion:** The positive rate of 15 high-risk HPV types in the collected specimens was 27.8%. Patients with early cervical lesions could be screened for 15 high-risk HPV infection types.

Keywords

Cervical Exfoliating Cells, Human Papillomavirus, Infection, Cervical Lesions, Clinicopathological Features

1. Introduction

There are 604,000 new cases and 342,000 deaths of cervical cancer each year worldwide. These are serious threats to women's health [1]. High-risk HPV infection is the main pathogenic factor of cervical lesions [2] [3] [4]. Worldwide, As an HPV preventive vaccine, Gardasil-9 will protect against HPV types associated with 90% of cervical cancer cases in women and 80% - 95% of other HPV-associated anogenital cancers in both men and women. However, due to variations in HPV-type specific prevalence and distribution, the vaccine will offer different percentages of protection in different geographical regions [5].

Various types of HPV infections persist, almost every cervical cancer patient is associated with high-risk HPV infection, and HPV infection is also associated with various other types of malignant cancers, including vulvar, vaginal, anal and penile cancers, as well as genital warts [6]. Cervical cancer remains a major public health problem, ranking as the fourth most common cause of cancer incidence and mortality in women worldwide. Persistent infection with high-risk HPV types is the major risk factor for cervical cancer [7]; the pre-cancerous lesions were detected through the participation of high-risk HPV screening, the goal of HPV screening is to detect the cervical lesions early in order to be treated before cancer is developed [8]. Therefore, it is very necessary to screen for cervical lesions by high-risk HPV infection status in cervical exfoliated cells.

2. Materials and Methods

2.1. Materials

2605 samples of female cervical exfoliated cells were collected from January 2019 to December 2020 in the gynecological outpatient department of the Affiliated Hospital of North China University of Science and Technology. The ages of the patients are at 20 - 68 years (mean 45.0 ± 5.0), and they are all from Tangshan, Hebei Province. They are no history of cervical vertebra resection or hysterectomy, and no HPV testing has been done before, no vaccination against HPV, no drugs were used within 3 days, and no menstruating. These patients included: normal physical examination women and patients with cervical lesions. The collected specimens were divided into two parts and placed in two sterile preservation tubes for marking. The first specimen was made for pathological diagnosis by pathologists. The second sample was used to detect HPV infection types.

Main instruments and reagents: SLAN-96P Real-Time PCR System made by Shanghai Hongshi Medical Technology Company; Real-time fluorescence quantitative PCR kit developed by Shengxiang Biotechnology Company.

2.2. Pathological Cytological Diagnosis

The cell smears were prepared by centrifugal precipitation liquid-based thin laminate technique that is as follows: The exfoliated cervical cells were removed using cervical cell brush and gently shake the cells on the brush head into the cell liquid in the numbered bottle containing the preservation solution; 2 ml cell liquid was taken into a centrifuge tube (which had been placed into slides), and then centrifuged at 1500 RPM for 3 min, and then the cell smears were prepared, the centrifuge tube was conducted out and the supernatant was discard, the slides were removed and dry, pap staining was performed.

The staining results were interpreted as follows: the nuclei were dyed dark blue and the nucleoli were dyed red. The cytoplasmic color of squamous epithelial cells was also significantly different with different degrees of differentiation and maturation: the dark pink cells were keratinocytes, the orange cells

were incomplete keratinocytes, and the pre-keratinocytes were light emerald green or light azure blue. The pathological cytological analysis and diagnosis were made according to the grading system of the American TBS (the 2014 Bethesda system) through an optical microscope.

2.3. Analysis of HPV Infection Types in Specimens

• Specimen treatment: 1 ml specimen was put into 1.5 ml EP tube, remove the supernatant after instantaneous centrifugation, and take 50 μl samples into another sterile EP tube, add 50 μl nucleic acid lysate, and mix it upside down, which is used as the templates of the samples (including negative and positive control 50 μl each).

• Specimen Quality detection β-Actin was used as an internal reference to detect the quality of the specimen. The β-actin Primers are as follows: Forward TCACCCACACTGTGCCCATCT, Reverse GAACCGCTCATTGCCAATGG, the PCR product size 290 bp. The 293-cell DNA containing the home-guarding gene was used as a positive control to detect whether these samples could meet the requirements of further experiments.

• PCR amplification detected HPV types: HPV types were detected using PCR amplification through the real-time PCR kit for quantitative fluorescence analysis developed by Shengxiang Biotechnology Group, which could simultaneously test 15 high-risk HPV types including HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68. All PCR components were added into 200 ml sterile EP tube according to kit instructions, and 10 ml above sample templates (including control group) was added; PCR reaction cycle parameters: Reaction with UNG enzyme at 50˚C for about 2 min; Taq enzyme was activated at 94˚C for 5 min. 40 cycles: 94˚C denaturation 15 sec, 57˚C annealing, extension and fluorescence acquisition 30 sec; Run at 25˚C for 10 sec.

2.4. Statistical Data Processing

Statistical analysis was conducted using the SPSS17.0 software system based on the Excel database. Counting data between groups was used X2 test. There is a significant difference based on $P < 0.05$, and there is an extremely significant difference based on $P < 0.01$. The correlation between HPV infection status and clinicopathologic features in exfoliated cervical cells was analyzed.

3. Results

3.1. DNA Quality Verification of Specimens

The specimen DNA quality verification was conducted using β-Actin as an internal reference for extracted specimen DNA quality to detect the nucleic acid quality by PCR amplification. The results showed that the positive control was 290 bp amplified product, and the PCR product of the specimen DNA template was consistent with that of the positive control, these results indicated that the extracted DNA met the requirements for further experiments.

3.2. The Sample Histological Types

According to the Bethesda grading system, the collected specimens were diagnosed by pathological cytology classification analysis according to the Bethesda grading system, they are including negative for intraepithelial lesions or malignant lesions (NILM) (Figure 1A), atypical-squamous cells of undetermined significance (ASCUS) (Figure 1B), atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesion (ASCH) (Figure 1C), Low-grade squamous intraepithelial lesion (LSIL) (Figure 1D), and High-grade squamous intraepithelial lesion (LSIL) (Figure 1E).

3.3. Infected HPV Types in the Specimens

HPV types were analyzed by PCR amplification in 2605 cases of cervical shedding cell specimens, and 725 cases were HPV positive (positive rate 27.8%) (Figure 2). Infected HPV types in the specimens included HPV16 (82 cases), 18 (19 cases), 31 (26 cases), 33 (28 cases), 35 (15 cases), 39 (50 cases), 45 (11 cases), 51 (69 cases), 52 (144 cases), 53 (65 cases), 56 (40 cases), 58 (75 cases), 59 (19 cases), 66 (28 cases), 68 (54 cases), among which the top five highest infection rates were as follows: first, HPV52, with 5.5% infection rate; Second, the infection rate of HPV16 was about 3.1%. Third, the infection rate of HPV58 was 2.8%. Fourth, the infection rate of HPV51 was 2.6%. Fifth, the infection rate of HPV53 is 2.5%.

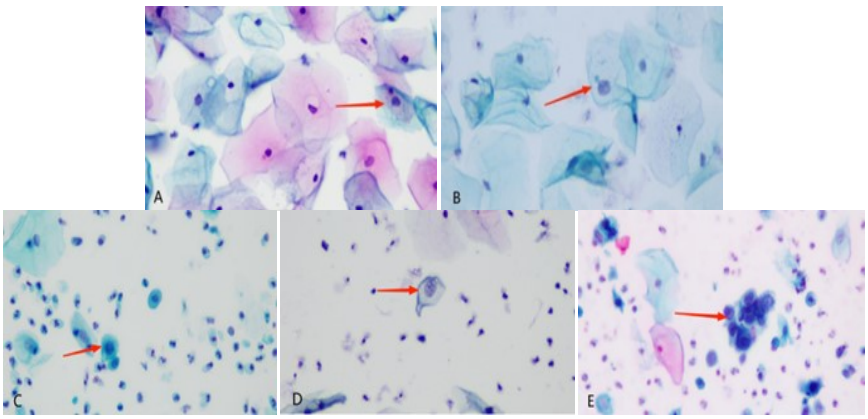

Figure 1. The results of different pathologic cytological diagnoses (Pap staining ×400). A: NILM, cell morphology is clear, and cell nucleus is centralization; B: ASC-US, the nucleus of the diseased cells was enlarged; the nuclear area of the diseased cell was 2.5 to 3 times as large as that of the normal intermediate cell nucleus; the nucleo-plasmic ratio of the diseased cell was slightly increased, and the chromatin distribution of the diseased cell was uniform; C: ASC-H, the nucleus of the diseased cells was enlarged; and the chromatin of the diseased cell was slightly darker, the nucleo-plasmic ratio of the diseased cell was increased; D: LSIL, the nucleus of the diseased cells was typical of hollowed out cells and non-hollowed out cells, is a polygon of superficial cells in the cervix; The nucleus was significantly enlarged, and the nuclear membrane was slightly irregular and deeply stained; E: HSIL, The cell morphology was changed obviously, the nucleus was significantly enlarged, chromatin was deeply stained, the nuclear membrane was uneven, and the nucleo-plasmic ratio was significantly increased.

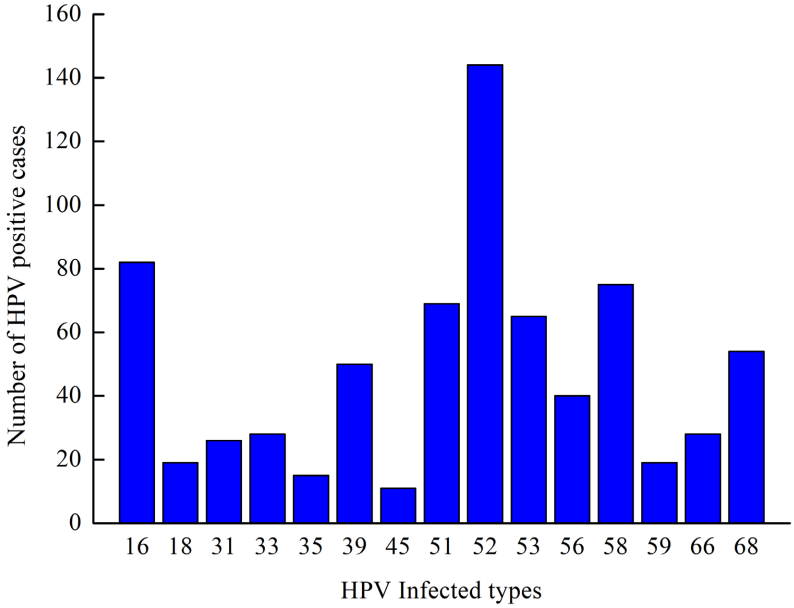

Figure 2. The results of high-risk HPV infection in the specimens.

3.4. Relationship between Histopathological Types with HPV Infection

The collected specimens were statistically analyzed according to histological types and HPV infection. 305 HPV positive in NILM group included 37 cases HPV6 (12.1%), 6 cases HPV18 (2.0%), 5 cases HPV31 (1.6%), 5 cases HPV33 (1.0%), 5 cases HPV35 (1.0%), 30 cases HPV39 (9.8%), 25 cases HPV51 (8.2%), 69 cases HPV52 (22.6%), 23 cases HPV53 (7.5%), 18 cases HPV56 (5.9%), 39 cases HPV58 (12.8%), 4 cases HPV59 (1.3%), 7 cases HPV66 (2.3%), 32 cases HPV68 (10.5%); 172 HPV positive in ASC-US group were 26 cases HPV16 (15.1%), 2 cases HPV18 (1.2%), 5 cases HPV31 (2.9%), 7 cases HPV33 (4.1%), 3 cases HPV35 (1.7%), 18 cases HPV39 (10.5%), 3 cases HPV45 (1.7%), 13 cases HPV51 (7.6%), 37 cases HPV52 (21.5%), 15 cases HPV53 (8.7%), 7 cases HPV56 (4.1%), 21 cases HPV58 (12.2%), 15 cases HPV68 (8.7%); 105 HPV positive in ASC-H group were 5 cases HPV16 (4.8%), 3 cases HPV18 (2.9%), 8 cases HPV31 (7.6%), 9 cases HPV33 (8.6%), 3 cases HPV35 (2.9%), 6 cases HPV45 (5.7%), 12 cases HPV51 (11.4%), 7 cases HPV52 (6.7%), 21 cases HPV53 (20.0%), 5 cases HPV56 (4.8%), 3 cases HPV58 (2.9%), 5 cases HPV59 (4.8%), 11 cases HPV66 (10.5%), 7 cases HPV68 (6.7%); 91 HPV positive in LSIL group were 8 cases HPV16 (8.8%), 5 cases HPV18 (5.5%), 7 cases HPV31 (7.7%), 5 cases HPV33 (5.5%), 3 cases HPV35 (3.3%), 2 cases HPV39 (2.2%), 2 cases HPV45 (2.2%), 11 cases HPV51 (12.1%), 16 cases HPV52 (17.6%), 3 cases HPV53 (3.3%), 9 cases HPV56 (9.9%), 5 cases HPV58 (5.5%), 7 cases HPV59 (7.7%), 8 cases HPV66 (8.8%); 52 HPV positive in HSIL group were 6 cases HPV16 (11.5%), 3 cases HPV18 (5.8%), 1case HPV31 (1.9%), 2 cases HPV33 (3.8%), 1 case HPV35 (1.9%), 8 cases HPV51 (15.4%), 15 cases HPV52 (28.8%), 3 cases HPV53 (5.8%), 1 case HPV56 (1.9%), 7 cases HPV58(13.5%), 3 cases

HPV59 (5.8), 2 cases HPV66 (3.8%).These HPV infection rates are irregular for different histological types ranging from NILM to HSIL. HPV positive rate in NILM group was 15.19% (105/2008), in ASC-US group was 62.55% (172/275), in the ASC-H group was 66.88% (105/157), and in the LSIL group was 81.25% (91/112), in HSIL group was 98.11% (52/53). The results of HPV infection in different pathological types shown in Table 1.

Statistical analysis showed that HPV infection of different histological types had statistical significance ($P < 0.01$), and the positive rate of HPV infection showed an increasing trend with the aggravation of cervical lesions.

3.5. Analysis of HPV Infection in Different Age Group Patients

The collected specimens were grouped according to their age as follows (Table 2): below 30 years old group, 31 - 40 years old group, 41 - 50 years old group, 51 - 60 years old group and over 61 years old group. Statistical analysis showed that there were different HPV-positive rates among different age groups, in which the highest HPV-positive rate was 33.33% (239/717) in the 41 - 50 age group. Chi-square test indicated that the difference was statistically significant ($P < 0.01$).

Table 1. HPV infection in different pathological types.

Pathological Types	HPV positive Numbers (cases)	HPV negative Number (cases)	Detected samples (cases)	Positive Rates (%)
NILM	305	1703	2008	15.19
ASC-US	172	103	275	62.55
ASC-H	105	52	157	66.88
LSIL	91	21	112	81.25
HSIL	52	1	53	98.11
Total	725	1880	2605	27.8

$\chi^2 = 733.415$, $P < 0.01$.

Table 2. HPV infection in different age groups.

Ages (Years old)	HPV positive Numbers (cases)	Detected Samples (cases)	Positive Rates (%)
≤30	98	459	21.35
31-	215	720	29.86
41-	239	717	33.33
51-	131	490	26.73
61-	42	219	19.18
Total	725	2605	27.8

$\chi^2 = 30.339$, $P < 0.01$.

4. Discussion

Cancer-related diseases represent the second overall cause of death worldwide. HPV is an infectious agent which is mainly sexually transmitted and may lead to HPV-associated cancers in both men and women [9]. HPVE6 and E7 genes are the main genes of transformation in cervical lesions. HPV infection status is tested by the HPV E6 and E7 genes to screen patients in the early stages of cervical disease [10].

In this study, 2605 cases of exfoliated cervical cells were collected, and HPV infection status was analyzed through high-risk HPV detection. The results showed that there were 725 cases of HPV positive, among which, the top 3 types of infection rate were HPV52, 16, 58. The severity of cervical lesions in patients with the positive rate of HPV infection increased ($P < 0.01$) according to the results of pathological cytology test. Therefore, early screening of patients with cervical lesions can be conducted by using high-risk HPV infection tests.

According to the age of patients, HPV infection status was statistically analyzed in different age groups, and the positive rate of HPV was the highest in 41 - 50 years old group. The results of this study are inconsistent with the report of Pandey [11] that young women (20 - 35 years old) are considered to be high-risk groups for HPV infection. Therefore, the sample size will be further increased in the future for in-depth study.

The results of this study suggest that cervical disease patients can be early detected through HPV tests and pathological cytology diagnosis screening regularly. In order to improve the quality of life of patients and improve the level of social health, early detection and treatment should be achieved to reduce the pain of patients.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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