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Original Research

Cervical human papillomavirus infection, genotyping and the relationship with the results of Thinprep Cytology Test in 9174 female physical examinees in Xi'an, China

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Objective: To investigate the infection status of human papillomavirus (HPV) in the cervix of women in Xi'an of China, the genotyping characteristics and the correlation with the results of TCT (Thinprep Cytology Test), in order to provide the basis for the prevention and treatment of cervical lesions in Xi'an of China. *Methods*: The data of 9174 women who were screened by HPV and TCT in the First Affiliated Hospital of Xi'an Jiaotong University from June 2017 to June 2019 were collected and analyzed statistically. *Results*: Among 9174 patients, 1045 (10.76%) patients were HPV positive, and the highest infection rate reached 12.26% (116/1045) over 60 years old. In HPV positive patients, the infection rate of single genotype (76.56%, 800/1045) was higher than that of multiple genotype (23.44%, 245/1045) ($P < 0.01$). The top four genotypes were 52, 53, 58 and 16, accounting for 16.24% (214/1318), 11.31% (149/1318), 11.08% (146/1318), and 11.00% (145/1318), respectively. The positive rate of TCT was 10.61% (26/345) in patients with multiple HPV infection and 9.25% (74/800) in patients with single HPV infection. Among the 100 patients with positive HPV and TCT, the top four genotypes were 58, 52, 53 and 16, which were consistent with the overall HPV infection. *Limitations and Conclusion*: The main subtype of HPV infection in physical examination of women in Xi'an is 52, 58, 53 and 16. This work is valuable for achieving early detection and treatment and thus reducing the incidence of cervical cancer in this area. The combined detection of HPV typing and TCT can improve the effectiveness of cervical disease screening.

Keywords

Human papillomavirus; Liquid based cytology; Genotype; Cervical intraepithelial lesions; Xi'an, China

1. Introduction

Cervical cancer always occurred at the confluence of squamous and columnar epithelial cells in the cervicovaginal or transitional zone [1–3]. Worldwide, each year, about 200,000 women die from the cervical cancer, while 500,000 new cases occur [4]. Human papillomavirus infection has been confirmed as a cause of cervical cancer [5]. A number of studies have shown that cervical lesions have a long period of precancerous lesions before transformed into malignant tumors [6], that is, there is a time sequence relationship between human papillomavirus (HPV) infection and cervical cancer. The time interval from the beginning of infection to the development of cervical cancer is about 10 to 15 years [7]. Therefore, the effective "three steps" (naked eye observation/HPV/TCT), early detection, diagnosis and treatment can prevent the occurrence of cervical cancer [8]. The detection of HPV infection can indicate the risk of cervical cancer, which is of great significance for early diagnosis and prevention of cervical cancer. There are many genotypes of HPV. The HPV virus family contains more than 200 genotypes; among them, more than 40 genotypes could spread through the genital tract easily. Fourteen HPV genotypes were considered as the pathogenic or "high-risk" for causing the development of the cervical cancer. Even though most sexually active females were infected with HPV for one time in their lifetime, while less than 10% of women become persistently infected. It is the 'persistent' infection with a high-risk genotype HPV that contributed to the cervical cancer development. The specific subtypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82 are related to the cervical cancer. In order to effectively avoid the risk of cervical cancer, accurate genotyping for HPV is of great significance. TCT is a common method for early screening of cervical cancer. HPV combined with TCT is critical for the early screening and treatment of cervical precancerous lesions.

2. Patients and methods

2.1 Patients

From June 2017 to June 2019, 9174 women who underwent pre pregnancy examination or routine physical examination in the First Affiliated Hospital of Xi'an Jiaotong University and simultaneously underwent HPV and TCT screening were selected. The inclusion criteria were: adult (>18 years old), pre pregnancy examination or routine physical examination, no cervical surgery and hysterectomy history. The tested group are in the 24–75 years age range. The 9174 female examinees were divided into five age groups according to their ages: 20–30 years old, 31–40 years old, 41–50 years old, 51–60 years old and >60 years old, respectively. Prior to the examination, the patient was told to refrain from vaginal

Table 1. Comparison of HPV positive rate and TCT positive rate of 9174 women of different ages in Xi'an.

Age distribution (years)	Number of examination cases (n)	HPV positive (n)	Positive rate (%)	χ^2 value	*P* value	TCT positive (n)	Positive rate (%)	χ^2 value	*P* value
20∼30	1060	97	9.15			3	0.28		
31∼40	2486	274	11.02			24	0.97		
41∼50	2889	269	9.31	18.008	0.001	26	0.9	12.626	0.013
51∼60	2345	289	12.32			31	1.32		
>60	934	116	12.42			16	1.71		
Total	9714	1045	10.76			100	1.03		

irrigation or medication for 3 days or no sexual intercourse for 24 h. The study has been approved by the ethics committee and the subjects gave their informed consent. The IRB Number is XITU1F2020LSK-056.

2.2 Test method

2.2.1 Instruments and reagents

The sedimentation TCT production instrument (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), the HPV detection kit and PCR amplification instrument are provided by Guangzhou Anbiping company. The code of the HPV detection kit is CP.008.022-01.

2.2.2 TCT

The specific method of collecting cervical cells of all the examinees was to insert the collecting brush into the cervical os of the examinees, then rotated for 5 circles clockwise evenly at the junction of the cervical mouth and the cervical column, and holded for 3–5 seconds slightly, then transferred the cervical cells collected by the cervical brush into the cell preservation solution, and used the full-automatic production staining instrument. Finally, the cytologist of Pathology Department of our hospital would issue the test report. According to the TBS (2001) classification standard recommended by the international cancer society, the diagnosis standard was divided into normal or inflammatory, low-level squamous intraepithelial lesions (LSIL), high-level squamous intraepithelial lesions (HSIL), atypical squamous cells of unknown significance (ASCUS), squamous cell carcinoma and adenocarcinoma (CA), respectively.

2.2.3 Detection of HPV subtypes

The method of sample collection was the same as TCT. PCR reverse dot hybridization was employed to detect cervical exfoliated cell samples. 15 high-risk types were 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82, respectively; 3 suspected high-risk types were 26, 53, and 66, respectively; 10 low-risk types were 6, 11, 40, 42, 43, 44, 54, 61, 81, and 83, respectively. The operation steps and result interpretation were carried out according to the requirements of the kit.

2.3 Statistical methods

SPSS 25.0 software (SPSS Inc., Chicago, IL, USA) was used to process and analyze the data. Chi-square test was used to compare the counting data, and the difference was statistically significant ($P < 0.05$).

3. Results

3.1 Distribution of HPV genotypes and multiple infections

In the 9174 screening samples, 1045 cases had HPV infection, the total infection rate was reached 10.76% (1045/9174) (Fig. 1). Among the 15 high-risk, 10 low-risk and 3 suspected high-risk HPV subtypes that could be detected by the kit, except for the low-risk 83, the rest of the genotypes have been detected; the top three high-risk types are 52, 58 and 16. The composition ratio of suspected high-risk types 53 and 66 is the second and eighth, respectively (Table 1). Among 9714 cases of HPV positive, single infection accounted for 76.56% (800/1045), double infection accounted for 17.32% (181/1045), triple infection accounted for 3.83% (40/1045), quadruple infection accounted for 1.72% (18/1045) and quintic infection accounted for 0.57% (6/1045). Most of them were single infection and double infection.

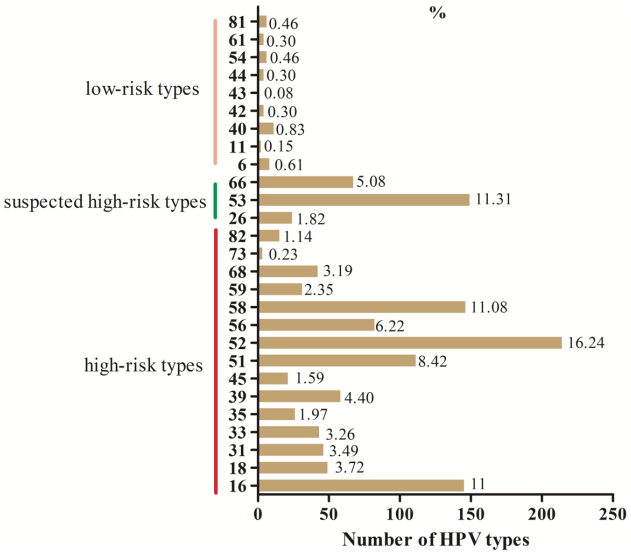

Fig. 1. Distribution of gene subtypes in 1318 cases of HPV positive in 9174 female physical examinees in Xi'an.

3.2 Age distribution and the difference of HPV infection

The age of 1045 HPV positive patients were ranged from 24–75 years old. The highest HPV infection rate was in the age group of >60 years old, and the lowest was in the age

Table 2. Number of TCT-positive cases in single and multiple HPV infection.

	HPV positive (n)	TCT positive (n) (%)	χ^2 value	P value
Single infection	800	74 (9.25)	0.330	0.566
Multiple infection	245	26 (10.61)		
Total	1045	100 (9.57)		

group of 20–30 years old. There was statistically significant difference in the positive rates of HPV and TCT among the age groups ($P < 0.05$) (Table 1).

3.3 TCT results

Among the 9714 patients, 110 were ASCUS, 20 were LSIL, 2 were HSIL, and 9582 were normal or mild inflammation. The total positive rate of ASCUS and above was 1.36% (132/9714). Among them, 100 cases were positive for HPV and TCT, the positive rate was 1.03%, 56 cases were single positive, the positive rate was 0.58%. Among the 32 negative HPV cases, their TCT results were all ASC-US.

3.4 HPV single infection, the relationship between multiple infection and TCT

The positive rate of TCT in multiple infection group was 10.61% (26/245), higher than that of the single infection group, which was 9.25% (74/800), but there was no statistical significance ($P > 0.05$) (Table 2).

4. Discussion

In recent years, the incidence of malignant tumors increased. Cervical cancer was one of the most common tumors in women, which seriously affected women's health. The persistent infection of high-risk HPV was one of the most important causes of cervical cancer and cervical intraepithelial lesions, which has been confirmed by researches. Moreover, the infection of high-risk HPV could promote the abnormal lesions or value-added of cervical epithelial cells, and increase the risk of epithelial malignant cells breaking via the basement membrane and epithelial tissue damage [11, 12]. Therefore, the HPV infection monitoring is an important link in the prevention and treatment of cervical cancer, which can be detected in advance via pre pregnancy examination or routine physical examination, so as to achieve the related early diagnosis and treatment.

The relationship between HPV infection and age was analyzed in this report. We found that the HPV infection is related to age. The infection rate of patients over 60 years old was highest, which reached 12.42%, and the second was 52–60 years old, which was 12.32%. The results showed that women who are in perimenopause and postmenopause were susceptible population. It was suggested that more attention should be paid to the detection of HPV in the higher age stage, especially in the population of over 60 years old. The relationship between age and HPV infection rate in this research showed slightly difference with the results in Jijings' results [13], which may be related to the deviation caused by population distribution. The population in this work selected was located in the city, while the population in Jijing's result was mainly from the village.

The infection rate of HPV subtype has been also studied. The results indicated that the infection rate of HPV was 1.40%~27.30%, usually about 10.00% [14, 17]. In addition, Boda also tested the cervical swab samples from 713 females with genital warts, and tested the samples for high- and low-risk genital HPV [18]. In this work, we found that the infection rate of HPV in Xi'an was 10.76%, which was consistent with the above results. In mainland China, HPV16 is the primary type of HPV, HPV18 the second major type, HPV58 and HPV52 represent the third and fourth type respectively [15]. In this research, we found that 15 high-risk types were detected in 9714 HPV positive samples, the top three types were 52 (16.24%), 58 (11.08%), 16 (11.00%), respectively. There were significant regional differences in HPV infection, and different subtypes of HPV had different carcinogenicity [16]. These results suggest that HPV52, 58 and 16 were more likely to be associated with cervical precancerous lesions in Xi'an. The HPV vaccine for cervical cancer prevention in Xi'an can mainly target specific types of 52, 58 and 16, respectively. However, since the observation and detection time of this study was only last for 2 years, the sample size will be further expanded for the further investigation to clarify the HPV typing. In addition, whether type 52 and 58 also exceed type 16 in other regions of mainland China remains to be further investigated. This work suggested that the prevention of cervical cancer and the research of HPV and vaccine in each region should be carried out according to the high pathogenic subtype in this region, which is beneficial to reduce the missed diagnosis rate of primary screening of cervical cancer. Moreover, the suspected high-risk type 53 infection rate was at a high level of 11.31%, ranked the second place, which may be related to cervical lesions. Therefore, we should pay great attention to it. In this study, single infection was significantly higher than that of multiple infection, however the positive rate of TCT in multiple infection was higher than that in single infection. It was consistent with the findings of Harper [19], which showed that multiple infection was more likely to cause cytological changes than single infection. Therefore, multiple HPV infection should be paid more attention to in clinical.

The appearance of TCT results and HPV infection subtype has improved the positive detection rate. The value of this method lies in the part of the region where HPV typing is not available. It could save all the collected cells in the cell preservation solution, and could timely fix to avoid cell degeneration. Through specific gravity centrifugation, removing the mucus, impurities and inflammatory cells in the specimen, and make a smear with clear field of vision and three-dimensional structure, which is detachable. The great improvement of cytology technology improved the quality of production, reduced the false-positive rate, and further enhanced the identification and sensitivity of pathological doc-

tors to abnormal cells. However, there was still a certain rate of missed examination, for the strong subjectivity of film reading physicians, and the unified diagnostic standards of each region. A single morphological cytology examination may lead to several false negatives. Meanwhile, the detection of HPV pathogens could effectively avoid the missed diagnosis. This work showed that in 9714 HPV patients, TCT results were mainly inflammatory. The results of TCT were as-cus and above, both of which were positive in 100 cases. The positive rate of TCT accounted for 9.57% of the HPV. The top three subtypes of TCT were the same as HPV. In this study, we found that the cytological abnormality of type 58 was higher than that of the other types, which may result in higher pathogenicity of type 58 in Xi'an area. This result suggested that in the screening of cervical lesions in this area, we should strengthen the follow-up management of type 58 infected ones. The results indicated that if there was no condition for processing HPV detection in this city or even in this province, if TCT results show as-cus or above, HPV may be combined with TCT. For type 58 infection, HPV detection can be recommended. Meanwhile, patients with type 52 or 16 infection and normal TCT at present, critical attention should be paid to follow-up to avoid further development of the disease. For persistent HPV infection of 58, 52 and 16 types, clinicians should be reminded to patients to screen TCT regularly to prevent the disease.

To summarize, this work analyzed the HPV infection status, genotyping characteristics, and their correlation with TCT in the physical examination of women in Xi'an area, provided basis for the prevention of different subtypes of HPV and the development of the related vaccines in this area. This work is valuable for achieving early detection and treatment and reducing the incidence of cervical cancer in this area.

Author contributions

Conceptualization, XFL, HYC, XL; Method, XFL, YYW; Software, YXS; Validation, GJZ; Formal Analysis, XFL; Investigation, XL; Resources, HYC; Data management, XFL; Writing, draft preparation, XFL; Project management, XL; Fund application, XFL. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the insititutional review board (CWO) of The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shanxi Province. The ethics committee's reference number is XITU1AF2020L5K-056. All patients provided written informed consent.

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Conflict of interest

The authors declare no conflicts of interest.

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