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The distribution of human papillomavirus genotypes in cervical cancer and intraepithelial neoplasia lesions among Chinese women in Yunnan Province

Li Yuanyue[a,b,1], Zulqarnain Baloch[c,*,1], Nafeesa Yasmeen[d], Yuan Tao[b], Xiaomei Wu[b], Xueshan Xia[a,*]

[a] Faculty of Environmental Science and Engineering & Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming 650500, People's Republic of China
[b] Department of Gynecology, The First People's Hospital of Yunnan Province Kunming, 630030, People's Republic of China
[c] College of Veterinary Medicine, South China Agricultural University, Guangzhou, 510642, People's Republic of China
[d] University of Veterinary and Animal Sciences, Lahore, Pakistan

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ABSTRACT

This study was designed to explore baseline data about the prevalence and distribution of human papillomavirus (HPV) genotypes among Chinese women who had cervical intraepithelial lesions and cervical cancer. A total of 511 patients were recruited, and biopsy samples were collected from these patients. Polymerase chain reaction was used to detect HPV-positive samples, and the HPV GenoArray kit was used for genotyping. A total of 23 genotypes were detected, including 13 that were high risk-HPV (HR-HPV), 3 that were potential high risk-HPV (PHR-HPV) and 7 that were low risk-HPV (LR-HPV). The prevalence rates of HPV infection in Han women diagnosed with cervical intraepithelial lesions (CIN) 1, 2, and 3 and squamous cell carcinoma (SCC) were 98.30%, 97.56, 100% and 90%, respectively. The HPV-positive cases in women of other ethnicities diagnosed with CIN1, CIN2, and CIN3 and SCC were 95%, 90.91%, 88.23% and 83.33%, respectively. The most frequent genotypes in both ethnic groups were HPV-16, 52, and 58. LR-HPV was detected in SCC lesions in the non-Han ethnic group. In the Han ethnic group, the LR-HPV genotype was mostly restricted to CIN1 lesions. Furthermore, we found a high prevalence of PHR-HPV-81 in SCC lesions among Han women. Ethnic background, smoking, sex at an early age, unprotected sex, use of contraceptives, and the withdrawal method were found to be significantly associated with HPV infection. In conclusion, this study explores epidemiological data regarding the prevalence of HPV and the genotype distribution in patients with SCC and CIN lesions in Yunnan Province, China.

Background

Human papillomavirus (HPV) infection is an established cause of cervical cancer [1,2]. In 2013, approximately 529,000 new cervical cancer cases were reported, and half of these patients died due to health complications [3]. HPV infection is most frequently transmitted through sexual contact. However, 54% of infections are cleared within the first year [4], and 91% of cases are resolved in two years [5]. The epidemiological characteristics of HPV infection throughout the world are quite different by geographic region [6,7]. The prevalence and genotype distribution of HPV vary substantially with respect to patient age and cytology stages [8]. Generally, HPV-16 is the predominant high-risk genotype worldwide, while other genotypes vary from region to region: HPV-52 is the most frequent genotype in the African continent, whereas HPV-52 and HPV-58 are more common in the Asian continent [9].

Molecular epidemiological data indicate that almost 40 HPV genotypes are the primary etiological agents of cervical cancer and cervical intraepithelial neoplasia (CIN) [10–13]. It is broadly accepted that the distribution of HPV genotypes in CIN lesions was generally the same, but not identical to, the distribution observed in cervical cancer (CC) [9]. Therefore, data on the prevalence of HPV and the distribution of its

* Corresponding authors.
E-mail addresses: zulqarnain@kmust.edu.cn, znbalooch@yahoo.com (Z. Baloch), oliverxia2000@aliyun.com (X. Xueshan).
[1] These authors equally contributed to the work and should be regarded as co-first authors.

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genotypes among females with CC and precursor lesions are important for determining the impact of the available HPV vaccine and establishing the importance of adding new HPV genotypes to the next generation vaccine.

Cervical cancer cases are increasing in China, and due to distinct topographical variations, the prevalence of HPV varies in different regions of China [14]. It is estimated that the high-risk HPV (HR-HPV) infection rate ranges from 15.0% to 20.8% and that the mortality rate due to cervical cancer surges by 4.1% per year [15]. Previous studies have also suggested that the HPV prevalence rate and its genotype circulation vary in women with abnormal cytology. HPV prevalence differed from 66.2% in HSIL to 61.3% in LSIL, respectively [16]. Throughout the world, 70% of CC patients are infected with HPV-16 and HPV-18. However, in China, the available data indicate that HPV-16 (24.5%), 52 (22.5%), 33 (21.6%), and CP8304 (HPV-81) (10.1%) are the most frequently identified CIN lesions [17], while HPV-16 and 18 are the leading genotypes [18] followed by HPV-31, 52 and 58 in squamous cell carcinomas (SCC) and adenocarcinomas [19].

Yunnan is a land of 56 state-certified ethnic minorities in China. Different ethnic minorities live in urban areas with racial admixture in Yunnan Province. However, most of them prefer to live in individual concentrated communities with distinctive socio-cultural practices. The Han population is a well-developed ethnic majority in China, particularly in Yunnan Province where the population is equally dispersed across all regions of the province. Several HPV-related epidemiological studies have been reported from different regions of China, and most of them recruited Han women and demonstrated an uneven HPV distribution in relation to geographic locations. To our best knowledge, no descriptions of the prevalence or genotype distributions of HPV in cervical cancer and intraepithelial lesions have been reported. This study was designed to determine the prevalence and distribution of HPV genotypes among Chinese women who were diagnosed histopathologically with cervical intraepithelial lesions or cervical cancer.

Materials and methods

Specimen collection

This was a hospital-based study which was approved by the Ethical Research Committees of Kunming University of Science and Technology and Yunnan First People's Hospital. Between November 2012 and December 2015, women with a diagnosis of cervical cancer or cervical intraepithelial neoplasia were recruited from Yunnan First People's Hospital located in Kunming, the largest city in Yunnan Province with approximately 44 million inhabitants. The Han are the predominant ethnic group in Yunnan Province, with a population of 29 million. In contrast, other ethnic groups are in the minority and are scattered throughout various parts of Yunnan Province with a combined population of 15 million. The Yunnan First People's Hospital is an advanced medical center attracting thousands of patients from all over Yunnan Province. All participants who consented to participate were required to answer questions about age, ethnic background, education level, profession, working hours, salary, sexual behavior, previous history of sexually transmitted infections (STI), smoking, alcohol intake, number of sexual partners and use of contraception techniques. Six hundred patients were initially recruited. Cervical and biopsy samples were collected for each participant: one for histopathological diagnosis and another for the HPV study. Eighty-nine patients were excluded due to the absence of histopathologically confirmed CIN or SCC lesions. Ultimately, the study investigated specimens from 511 patients between the ages of 21 and 86 years. The disease stage was coded following the criteria set forth in the guidelines of the International Federation of Gynecology and Obstetrics (FIGO) [20].

HPV genotyping

Biopsy samples underwent deparaffinization with xylene, followed by rehydration in graded ethanol. Subsequently, DNA was extracted from each sample using the QIAamp ® DNA mini Kit (Qiagen, Shanghai, China). The quality of the DNA was evaluated using PCR amplification of the housekeeping gene β-globin [21]. Samples which were positive for the β-globin amplicon were then used in the PCR amplification of HPV DNA. HPV-positive samples were confirmed by PCR using two consensus primers (MY09/11, GP5/6) [22]. DNA from HPV-positive cell lines (HeLa & Caski) was run as a positive control. HPV genotyping was completed using the HPV GenoArray test kit (Hybribio, Chaozhou, China), following the manufacturer's directions. The GenoArray test is an L1 universal primer-based PCR system that can amplify 13 HR-HPV [6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68], three PHR-HPV [53, 66, 81], and seven LR-HPV [6,11, 42, 43, 44, 55, and 61] genotypes. The assay was conducted according to the constructor's guidelines. PCR was performed in a reaction volume of 25 μl, containing 5 μl of DNA template, 19.25 μl of the provided master mix, and 0.75 μl DNA Taq polymerase, using a Perkin-Elmer GeneAmp PCR System 9700 (Applied Biosystems). The amplification procedure was performed as follows: 9 min of denaturation at 95 °C, followed by 40 cycles of 20 s of denaturation at 95 °C, 30 s of annealing at 55 °C, 30 s of elongation at 72 °C, and a final extension for 5 min at 72 °C.

Statistical analysis

The Chi-square test was used to compare the differences in HPV genotype distribution among the histopathologically different lesions and both ethnic groups. All statistical tests were two-sided; P values <0.05 were considered statistically significant.

Results

A total of 511 patients were recruited for simultaneous HPV genotyping and cytological diagnosis. The prevalence of HPV DNA was 77.3%, of which 56.9% were single infections and 20.3% were multiple-genotype infection. The HPV L1 gene-based genotyping results revealed 23 genotypes, including 13 HR-HPV (HPV-16, 52, 58, 18, 31, 33, 35, 39, 45, 51, 56, 59, and 68) genotypes, 3 PHR HPV (HPV-53, 66, 81) genotypes and 7 LR-HPV (HPV-42, 43, 44, 55, 11, 6 and 61) genotypes. HPV-16 (105/511, 20.5%) was the predominant HR-HPV genotype followed by HR-HPV-52 (96/511, 18.8%), 58 (367/511, 13.1%), 18 (31/511, 16.1%), and 33 (26/511, 5.1%) (Table 1). Overall, 81.4% of patients were HR-HPV-positive, 10.8% were PHR-HPV-positive, 5.7% were LR-HPV-positive, and 5.1% of cases were unclassified HPV genotypes.

Among the subjects with a baseline cytology analysis (n = 511), 205 women had abnormal histopathological results classified as CIN1. Of these, 153 women were classified as Han, and 52 women belonged to other ethnicities. A total of 129 women were classified as CIN2, of whom 27 belonged to other ethnicities and 102 were Han women. Similarly, 109 women were diagnosed with CIN3, of whom 42 were from other ethnic groups, and 67 were Han. The remaining 68 women had SCC; of these, 18 were of other ethnicities, and 50 were Han (Tables 1 and 2).

A total of 23 genotypes were detected in CIN1, and HPV-52 was the most frequent genotype (44/205, 21.5%) followed by HPV-16 (29/205, 14.2%), HPV-58 (21/205, 10.3%), HPV-53 (16/205, 7.8%), HPV-18 (13/205, 6.3%), HPV-68 (12/205, 5.8%), HPV-33 (11/205, 5.4%), HPV-39 (10/205, 4.9%), and HPV-51 (8/205, 3.9%). Almost 80% of CIN1 patients were positive for HR-HPV: three PHR-HPV

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