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Association between viral loads of different oncogenic human papillomavirus types and the degree of cervical lesions in the progression of cervical Cancer

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ABSTRACT

Objective: In this study we determined the frequency of the most prevalent human papillomavirus (HPV) types in China and evaluated the association between viral loads of different oncogenic HPV types and the severity of disease.

Methods: We enrolled 15,518 women for this study and 3199 of them (20.61%) were identified as positive by a PCR assay, that can simultaneously quantify and genotype HPV.

Results: The viral loads of HPV 16, 31, 35, 52, 58, 39, and 56 were lower for women with normal cytology compared to those with disease progression; viral loads were not appreciable for HPV 33, 18, 45, 59, 68, 53, 66, and 51. The viral load of species 9 appeared significantly higher for women with cervical intraepithelial neoplasia (CIN) 2/CIN 3 relative to women with normal/low grade squamous intraepithelial lesion (LSIL)/CIN1 ($P < 0.001$), and significantly lower compared to those with cervical cancer ($P < 0.001$). The viral load of HPV species 6 was slightly higher for women with CIN2/CIN 3 compared to women with normal/LSIL/CIN1 ($P = 0.002$), and not significantly different from women with cervical cancer ($P = 0.548$). In addition, no statistically significant difference was found in HPV species 5 or species 7 ($P = 0.898$; $P = 0.136$).

Conclusions: The HPV viral load-associated risk for developing into CIN and cervical cancer is likely to be species-dependent and primarily restricted to species 9 (types phylogenetically close to HPV16).

1. Introduction

Cervical cancer is a major gynaecologic malignancy that has led to the deaths of approximately 266,000 women worldwide each year [1]. About 61,700 new cervical cancer cases are diagnosed and about 29,600 cervical cancer deaths occur annually in China. Cervical cancer is now the 3rd leading cause of cancer deaths in Chinese women aged 15 to 44 years [2]. Infection with oncogenic human papillomavirus (HPV) is known to be a precondition for cervical intraepithelial neoplasia (CIN) and cervical cancer [3]. HPV belongs to the papillomaviridae family, which is an ancient taxonomic family of non-enveloped DNA viruses, and is classified into genus, species and types based on nucleotide sequence comparison. To date, > 200 HPV genotypes have been identified and are divided into 5 genera (Alpha-, Beta- and Gamma papillomaviruses represent the largest groups). HPV genotypes from the Alpha papillomavirus genus are classified as low-risk human papillomaviruses (LR-HPVs) and high-risk human papillomaviruses (HR-HPVs) according to their carcinogenicity. Twelve types of HR-HPVs in 4 species groups of the Alpha papillomavirus genus are confirmed as oncogenic types via epidemiologic data, including HPV51 (species 5), HPV56 (species 6), HPV 18, 39, 45, and 59 (species 7) and HPV 16, 31, 33, 35, 52, and 58 (species 9). The remaining types of the HR-HPVs are “probable” or “possible” carcinogens [4–6].

The association between HR-HPVs and cervical cancer is now well established. HPV infection can be found in > 98.5% of patients with cervical cancer, and this provides evidence for the adoption of HPV DNA testing in the screening of cervical cancers [7]. Compared with cytologic and pathologic diagnoses, the detection of HR-HPVs by molecular biologic techniques is more convenient and objective, and can effectively avoid false-negative results. Therefore, HR-HPV testing has been included in the current cervical cancer screening approaches of the American clinical guidelines since 2015 [8].

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For certain types of HR-HPVs, the role of viral persistence in the carcinogenic process has been well documented, but a role for viral load as a risk marker for CIN and cervical cancer remains controversial. The inconsistencies in previous studies may be due to the detection of viral loads without identification of the genotypes of HPV [9], as well as the lack of standardization in the number of cervical epithelial cells [10,11]. There are relatively few studies that simultaneously analyze the type-specific viral loads of the most prevalent HR-HPVs. There is therefore great interest in determining whether phylogenetically closely related oncogenic HPV types behave similarly with respect to the viral load-associated risk for developing into CIN and cervical cancer.

In the current study we analyzed the association between type-specific viral load and cervical lesions in cancerous and precancerous cervical swab samples. Normalized viral load was determined with a quantitative measure for individual types and a single-copy gene encoding DNA topoisomerase III as a control [12]. With the availability of numerous clinical data, we investigated the prevalence of HPV types and observed the associations between HPV viral load and cervical lesions.

2. Materials and methods

2.1. Subjects and study design

We enrolled 15,518 women (aged 18-88 with a mean of 46.8 ± 18.5 years) from July 2016 to November 2017 for this cross-sectional study at the Shanxi Provincial People's Hospital. Women under 18 years of age or who had prior treatment with radiation or chemotherapy were excluded from this study. A real-time PCR assay was used to detect type-specific HPV infection. Three thousand one hundred ninety-nine women (aged 18–85 with a mean of 43.9 ± 10.7 years) tested positive for HPV in this study. ThinPrep liquid-based cytology was performed on all women with HPV infection during our clinical investigation, and the cases were defined cytologically as normal, atypical squamous cells of undetermined significance (ASCUS), low grade squamous intraepithelial lesion (LSIL), high grade squamous intraepithelial lesion (HSIL), or atypical squamous cells, cannot exclude high grade squamous intraepithelial lesion (ASC-H). Biopsies were performed on the suspected cervical areas in women with cytologic abnormalities (ASCUS, LSIL, HSIL, or ASC-H). The pathologic diagnosis of cervical lesions was confirmed by a panel of expert pathologists. For analysis in the present study, the grades of cervical lesions were grouped into 3 categories: normal/LSIL/CIN 1, CIN 2/CIN 3, and cervical cancer.

Our study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Shanxi Provincial People's Hospital Ethical Committee. Written informed consent was obtained from each patient enrolled in this study.

2.2. HPV genotyping and determination of viral loads by real-time quantitative PCR

A direct endocervical sample for cytology and a HPV DNA test was obtained by a physician using a vaginal speculum. Total DNA was extracted from samples using a commercially available extraction kit (QIAamp DNA Mini Kit; Qiagen, Hilden, Germany) according to the manufacturer's instructions. The concentration of DNA was determined with a spectrophotometer (SIA4000, Amoy Diagnostics).

Quantification of HPV viral load and normalization for input cellular DNA copies were performed as described previously [12]. In the real-time quantitative PCR assay, PCR primers and probes were designed for the most prevalent HR-HPV genotypes, and to account for the discrepancy in cell number collected in a given sample, levels of a single-copy gene encoding DNA topoisomerase III were also determined in the reaction.

PCR amplification was performed in a 20 μl reaction mixture containing 10 μL of Platinum Quantitative PCR SuperMix-UDG(Invitrogen), 10 pmol of each primer, 1–5 pmol of each probe, 2 μl of template (up to 50 ng), and RNase-free water for a total volume of 20 μl. The PCR reaction was executed at an initial denaturation at 95 °C for 10 min, 45 cycles at 95 °C for 10 s, and 58 °C for 40 s. Reactions were performed on an ABI Prism 7300 Detection System (Applied Biosystems). The normalized HPV viral loads were expressed as the number of viral copies/10,000 host cells.

2.3. Statistical analyses

HPV DNA viral load was $\log_{10}$ transformed prior to analysis. The different prevalence of HPV genotypes among groups were analyzed by χ^2 test. The correlation between infection rate of multiple HPV types and the grade of cervical lesion was evaluated by Spearman correlation analysis. The differences in viral loads of different HPV types among groups were analyzed by Kruskal-Wallis H test and Mann-Whitney U test. Statistical analyses were performed using SPSS software version 22.0 (IBM Company, Chicago, IL), and $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Prevalence of HPV genotypes

With real-time PCR we were able to observe the frequency of type-specific HPV infection. Three thousand one hundred ninety-nine of 15,518 women (20.61%) tested positive in our study, with 2479 infected with a single type, accounting for 77.50% (Table 1). In all cases infected with single types, the overall prevalence of HPVl6 infection was the highest, accounting for 23.36%; followed by HPV52 (13.19%), HPV58 (10.45%), and HPV53 (7.83%). The most common HPV type in the normal/LSIL/CIN1 group was HPV16 (16.88%), followed by HPV52 (14.45%), HPV58 (9.79%), and HPV53 (9.05%). The most common HPV type in the CIN2/CIN3 group was HPV16 (32.04%), followed by HPV52 (11.31%), HPV58 (11.31%), and HPV53 (6.12%). In addition, the most common types in the cervical cancer group were HPVl6, HPV58, HPV52, and HPV18, accounting for 38.26%, 12.08%, 11.41%, and 7.38% respectively (Table 1).

3.2. HPV infections with multiple types

We used PCR to observe the frequency of multiple infections, which were detected in 720 of 3199 women with HPV infection, accounting for 22.50%. The prevalences of multiple HPV types in the normal/LSIL/CIN1 group, CIN2/CIN3 group, and cervical cancer group were 19.95%,27.68%,14.86% respectively, and did not increase with the progression of cervical cancer. Moreover, there was no significant correlation between multiple-type infections and the grade of cervical lesion ($r = -0.063$, $P < 0.05$).

3.3. Association between type-specific or species-specific HPV viral load and the degree of cervical lesion

We analyzed the relationships between HPV DNA load ($\log_{10}$/10,000 cells) and the degree of cervical lesion in the patients. Irrespective of type of HPV, the viral loads of the normal/LSIL/CIN1 group, CIN 2/CIN 3 group, and cervical cancer group were 3.67(2.71–4.71), 4.61 (3.92–5.44) and 5.17 (4.21–5.72) $\log_{10}$/10,000 cells respectively (Fig. 1), which were statistically different from each other ($H = 273.674$, $P < 0.001$). These data showed that the viral load was lower for women with CIN 2/CIN 3 compared to those with cervical cancer ($Z = -3.434$, $P = 0.001$), and higher compared to those with normal/LSIL/CIN1 ($Z = -14.719$, $P < 0.001$). Irrespective of type of HPV, the viral load appears to be lower for women with a normal cytologic diagnosis compared to those with disease progression.