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Human papillomavirus viral load as a useful triage tool for non-16/18 high-risk human papillomavirus positive women: A prospective screening cohort study

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HIGHLIGHTS

- First comparison of risk triage of type-specific HPV by viral load, cytology or VIA
- HPV16/18 infection was at high risk, even with normal cytology or low viral load.
- Triage non-16/18 HPV infection by viral load is viable when cytology is unavailable.

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ABSTRACT

Objective. ASCCP cervical cancer screening guidelines recommend triaging high-risk human papillomavirus (hrHPV) positive women with cytology and genotyping, but cytology is often unavailable in resource-limited areas. We compared the long-term risk of cervical cancer and precancers among type-specific hrHPV-positive women triaged by viral load to cytology and visual inspection with acetic acid (VIA).

Methods. A cohort of 1742 Chinese women was screened with cytology, VIA, and Hybrid Capture 2 (HC2) test and followed for ten years. All HC2-positive samples were genotyped. Viral load was measured by HC2 relative light units/cutoff (RLU/CO). Ten-year cumulative incidence rate (CIR) of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) for type-specific hrHPV viral load was estimated using Kaplan-Meier methods.

Results. Baseline hrHPV viral load stratified by specific genotypes was positively correlated with prevalent cytological lesions. Ten-year CIR of CIN2+ was associated with cytological lesions and viral load. Among HPV 16/18-positive women, ten-year CIR of CIN2+ was high, even with normal cytology (15.3%), normal VIA (32.4%), viral load with RLU/CO < 10 (23.6%) or RLU/CO < 100 (33.8%). Among non-16/18 hrHPV positive women, ten-year CIR of CIN2+ was significantly stratified by cytology grade of atypical squamous cell of undetermined significance or higher (2.0% VS. 34.6%), viral load cutoffs at 10 RLU/CO (5.1% VS. 27.2%), at 100 RLU/CO (11.0% VS. 35.5%), but not by VIA (19.1% VS. 19.0%).

Conclusions. Our findings support the guidelines in referring all HPV16/18 positive women to colposcopy and suggest triaging non-16/18 hrHPV positive women using viral loads in resource-limited areas where cytology screening was inaccessible.

Abbreviations: HPV, Human papillomavirus; hr, High risk; CIN, Cervical intraepithelial neoplasia; NILM, Negative for intraepithelial lesion or malignancy; ASCUS, Atypical squamous cell of undetermined significance; LSIL, Low grade squamous intraepithelial lesion; HSIL, High grade squamous intraepithelial lesion; AC, Analytic cohort; FU, Follow-up; HC2, Hybrid Capture 2; LBC, Liquid-based cytology; VIA, Visual inspection with acetic acid; CIR, Cumulative incidence rate.

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1. Introduction

The well-recognized knowledge that high-risk human papillomavirus (hrHPV) is a necessary etiology for progression to cervical cancer is reinforcing HPV DNA testing incorporated into cervical cancer screening programs in many countries [1–3]. But potential risk of excessive colposcopy and overtreatment would occur in women with transient HPV infections who regressed spontaneously within one to two years [4,5]. Therefore how to best triage HPV-positive women through secondary screening to identify those women with true precancerous lesions remains a pending issue in cervical cancer screening.

Given the different carcinogenicity of HPV among genotypes, partial typing tests such as HPV 16/18 and HPV 16/18/45 are being integrated into cervical screening to improve the risk stratification of general population [6–8]. In addition, other triage techniques are also desirable. The updated guidelines released by American Society for Colposcopy and Cervical Pathology (ASCCP) recommended the cytology for triaging the non-16/18 hrHPV positive women [9]. It is no doubt of high specificity of cytology screening [10], nevertheless, high-quality cytology screening programs are often unavailable due to the lacking of trained cytopathologists, limited healthcare resources, or poor infrastructures in low-resource countries. VIA is another option for secondary screening of HPV-positive women in low and middle income settings but with a wide variation of sensitivity rate from 41% to 92% for CIN2+ detection between providers [11].

Viral load resulting from productive viral replication might predict the risk of viral persistence and the progression to high-grade CIN and cervical cancer [12–16]. The likelihood of the viral load related risk was reported to be dependent on specific HPV genotypes, as demonstrated by many large screening population studies from US, Belgium and Denmark [17–20]. However, few studies focused on the longitudinal comparison of risk stratification of HPV viral load in comparison to cytology or VIA to date.

Our previously prospective study of a six-year follow-up cervical cancer screening cohort of 1997 women demonstrated that baseline high hrHPV viral load was associated with the increased risk of progression to CIN2+ and potentially served as a biomarker to triage hrHPV-positive women for colposcopy [21], however, did not specify the risk of CIN2+ by individual hrHPV type. In this present study, with the data of a ten-year follow-up of the same cohort, we further compared the long-term risk stratification of CIN2+ by hrHPV viral loads against cytology and VIA among HPV 16/18 and non-16/18 hrHPV-positive women, so as to investigate the feasibility of viral load as an alternative triage method for hrHPV-positive women in lieu of cytology, especially in regions with high-quality cytology examination unavailable.

2. Methods

2.1. Study population

A cohort of 1997 women, aged 35–45 years., married, not pregnant, and without history of hysterectomies, were enrolled in Shanxi Province of Cervical Cancer Screening I (SPOCCS I) study in 1999 [22]. These participants were then followed up through three organized visits in 2005, 2010, and 2014, respectively [23,24]. The incidence and mortality of cervical cancer in this cohort were also recorded by a national cancer registry. This study was approved by the Institutional Review Board of Cancer Institute/Hospital, Chinese Academy of Medical Sciences (CICAMS).

2.2. Clinical examinations

Each participant was screened with liquid-based cytology (LBC), Hybrid Capture 2 (HC2) testing, visual inspection with acetic acid (VIA) in 1999, 2005, 2010, and 2014 (except for VIA in 2014). Women positive by any of three tests were referred for colposcopy, and lesions visible under colposcopy were directly biopsied. Cytology results were interpreted using the Bethesda classification system and histological diagnoses were made according to CIN classifications. Histological diagnoses of CIN grade 2, CIN grade 3, squamous cell carcinoma (SCC), and adenocarcinoma in situ (AIS), or adenocarcinoma were categorized as CIN2+. Women with histology-confirmed CIN2+ lesions were recommended for treatment as per local clinical guidelines.

2.3. HPV DNA testing

HC2 assay was conducted on cervical cytological samples to test the presence of HPV DNA within two weeks of specimen collection. This assay detects the DNA of 13 carcinogenic hrHPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) but unable to discriminate individual genotypes. Samples were deemed as hrHPV-positive if the signal strength in relative light units compared with standard positive control (RLU/CO) in HC2 assay was 1.0 (1 pg/mL, approximately 5000 viral copies) or higher. A semi-quantitative viral load of women positive for HPV were then categorized into three groups: low (1.0–9.9 RLU/CO), moderate (10.0–99.9 RLU/CO) and high viral load (≥100.0 RLU/CO), with the same criteria made by our previous study and other studies [12,21,25,26].

2.4. HPV genotyping

All HC2-positive cytological specimens were genotyped using PCR-based reverse hybridization line probe assay (INNO-LiPA Extra, Innogenetics, Belgium) with a SPF_{10} primers set (DDL diagnostic laboratory, Netherlands) (SPF_{10}-LiPA). This assay identifys 28 hrHPV types on a line strip, including 13 hrHPV types covered by the HC2 assay, three probable carcinogenic genotypes (26, 53, and 66), and 12 low-risk genotypes (6, 11, 40, 43, 44, 54, 69, 70, 71, 73, 74, and 82). We defined hrHPV infections as any positive indication of HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 or 68. Specimens were deemed as hrHPV-negative if HC2 assay result was negative or if SPF_{10}-LiPA assay result for these 13 hrHPV genotypes was negative.

2.5. Statistical analysis

A total of 1742 women were examined in 2005 after excluding 255 women due to loss to follow up, hysterectomy, or death during 1999–2005. Thereafter, 209 hrHPV-positive women in 2005 confirmed by HPV genotyping were taken as an analytic cohort (AC), as shown in Fig. 1.

The differences of age were compared among women with low, medium, or high viral loads using one-way ANOVA method, and other categorical variables using Pearson Chi-square method. We analyzed the prevalence of cytological lesions in relation to type-specific HPV viral load. Cumulative incidence rate (CIR) of CIN2+ over ten-year follow-up by type-specific hrHPV viral loads was estimated using Kaplan-Meier methods. Hazard ratios were estimated using Cox proportional hazard models. The CIR of CIN2+ stratified by various viral load cutoffs, cytology grades, and VIA results among HPV16/18 positive women and non-16/18 hrHPV positive women were compared using Log-rank test. All statistical tests were two-tailed with 0.05 as significance level and all analyses were performed using SAS 9.2.

3. Results

3.1. Demographic characteristics

Among 209 hrHPV-positive women at AC baseline, the average age was 45 years and sexual debut age was 21 years. The majority of