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Analysis of the dose-response relationship between high-risk human papillomavirus viral load and cervical lesions

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Summary The aims of this study were to explore the dose-response relationship between high-risk human papillomavirus (hrHPV) load and cervical lesions; the relationship between hrHPV viral load and the severity of cervical lesions; and the clinical application of the hybrid capture II (HC-II) system in the secondary prevention of cervical cancer. HrHPV viral load was detected by the HC-II system and cervical lesions were diagnosed from biopsied tissue. Curve estimation and Mantel trend analysis were used to explore the dose-response relationship between hrHPV viral load and cervical lesions. Spearman's rank correlation analysis and ordinal regression model were used for the analysis of hrHPV viral load and the severity of cervical lesions. Curve estimation showed good correlation between cervical lesion rates and hrHPV viral load ($r = 0.775$, $P = 0.008$); the rate of cervical lesions increased with hrHPV viral load ($\chi_{trend} = 8.000$, $P < 0.001$). Medium intensity rank correlation was found between hrHPV viral load grades and the severity of cervical lesions ($r_s = 0.321$, $P < 0.001$); a correlation appeared between hrHPV viral load and the severity of cervical lesions ($P < 0.001$). These results suggest a dose-response relationship between hrHPV viral load and the severity of cervical lesions. This dependence has important clinical applications and shows the potential value of the HC-II system in cervical cancer prevention.

1. Introduction

Worldwide, cervical cancer is the second most prevalent cancer among women. The primary predictor for precancerous cervical lesions to progress to cervical cancer is

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concomitant infection with human papillomavirus (HPV). Although HPV can be detected in over 99% of cases of cervical cancer, a dose-response relationship between viral load and the severity of cervical lesions has not been definitely recognized. Likewise, the predictive value of viral load with respect to the severity of the lesions has not been determined. The aims of this study were to measure the high-risk HPV (hrHPV) viral load in the cervix with the semi-quantitative Hybrid Capture II (HC-II) system (Digene, Gaithersburg, MD, USA) in order to analyze the dose-response relationship between hrHPV viral load and cervical lesions, and to explore whether the hrHPV viral load in the cervix can predict the severity of cervical lesions.

2. Materials and methods

2.1. Study subjects

Eight hundred and sixty-one subjects seen at the Guangzhou Kingmed Center for Clinical Laboratory between 1 October 2006 and 31 August 2007 were selected for the study. For each case, hrHPV DNA levels were determined and colposcopy was performed. All study subjects were sexually active and had not undergone hysterectomy, cervical surgery or had a previous diagnosis of cervical intraepithelial lesions. Recent pregnancy and a history of previous or current immunosuppressive therapy were considered exclusion criteria. Informed consent regarding the use of patients' DNA and clinical information was obtained from all the subjects before participation in this study.

2.2. Detection of hrHPV DNA

HrHPV DNA was detected by the second-generation HC-II system. The principle of the system is a nucleic acid hybridization assay with signal amplification based on the production of DNA/RNA hybrids by a chemiluminescent reporter system. The samples were analyzed for the presence of hrHPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. The basic experimental steps were as follows: degeneration: the degeneration reagents were added to the samples to induce HPV DNA degeneration, changing double-stranded DNA into single-stranded DNA that could be hybridized with the RNA probe; hybridization: the degenerated, single-stranded DNA was linked to the RNA probe, forming an RNA-DNA heterozygote; capture: the RNA-DNA heterozygote was transferred to a plate coated with an antibody specific to the RNA-DNA hybrid, thus capturing the heterozygote; coupling: the captured heterozygote was coupled with alkaline phosphatase-labelled antibodies, resulting in signal amplification because several alkaline phosphatase molecules are conjugated to each antibody and multiple antibodies bind to each captured RNA-DNA hybrid; interpretation: a chemiluminescent substrate that emits light when cleaved by alkaline phosphatase was added, the intensity of the light emitted being related to the quantity of RNA-DNA heterozygote.

The intensity of the light emitted from the samples was measured by the DML 2000 instrument (Digene) calibrated in relative light units (RLU). A solution in which the hrHPV concentration was 10 pg/ml was used as a positive control. The viral load of a sample was indicated by the ratio of the RLU of the sample to that of the cutoff (CO) value of the positive control. A RLU/CO $\geq$1.0 was judged as positive for the 13 hrHPV types and <1.0 as negative. There was a positive correlation between RLU/CO and the HPV DNA level in the samples: the higher the RLU/CO ratio, the higher the level of HPV DNA in the samples and the higher the viral load. In order to analyze the data, hrHPV viral load (expressed as RLU/CO) was divided into five groups: <1, $\geq$1—<10, $\geq$10—<100, $\geq$100—<1000, $\geq$1000—10 000.

2.3. Biopsy and histology

Colposcopic examination was performed on patients after obtaining their consent. Biopsy specimens were taken from the area of the cervical lesion or at the 3, 6, 9 and 12 o'clock positions of the cervical transformation zone, if no lesions were found.

According to the severity and range of the heteromorphic cells, the subjects were classified as negative or chronic inflammation, cervical intraepithelial neoplasia (CIN) 1, CIN2, CIN3 or carcinoma in situ, or cervical cancer (including squamous cell carcinoma and adenocarcinoma). Cervical pathologies were divided into cases (with cervical lesions, i.e. CIN2 or greater abnormality) and non-cases (without cervical lesions, i.e. negative or chronic inflammation and CIN1) in order to analyze the dose-response relationship between hrHPV viral load and the cervical lesions.

2.4. Statistical analysis

A database was established with SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Non-parametric data were analyzed with the Mann-Whitney test. The dose-response relationship was analyzed by Mantel trend analysis. Spearman's rank correlation and ordinal regression model were used to analyze orderly multi-categorical data. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Patient characteristics

A total of 861 cases were examined, with an age range of 16—62 years and an average age of 32.4 $\pm$ 7.9 years. The proportion of Han nationality people was 99.8%. Patients had been pregnant 0—9 times (mean = 2) with parity 0—7 (mean = 1). Four hundred and fifty-four cases were hrHPV positive, giving an infection rate of 52.7%. The hrHPV infection rate differed according to histology and grade. The lowest infection rate was found in the negative or chronic inflammation group and the highest in the cervical cancer group (Table 1).

3.2. Dose-response relationship between hrHPV viral load and cervical lesions

In order to explore the dose-response relationship and any trend between hrHPV viral load and cervical lesions, Figure 1