Journal of Clinical Virology 59 (2014) 18–23

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

The relationship between the cervical and anal HPV infection in women with cervical intraepithelial neoplasia

Borek Sehnal[a,*], Ladislav Dusek[b], David Cibula[c], Tomas Zima[d], Michael Halaska[a], Daniel Driak[a], Jiri Slama[c]

[a] Hospital Na Bulovce and 1st School of Medicine, Charles University, Department of Gynaecology and Obstetrics, Budinova 2, Prague 8, 180 81, Czech Republic
[b] Institute of Biostatistics and Analyses, Masaryk University, Kamenice 3, Brno, 625 00, Czech Republic
[c] General University Hospital and 1st School of Medicine, Gynaecologic Oncology Centre, Charles University, Apolinarska 18, Prague 2, 128 51, Czech Republic
[d] General University Hospital and 1st School of Medicine, Institute of Medical Biochemistry and Laboratory Diagnostics, Charles University, U Nemocnice 2, Prague 2, 128 08, Czech Republic

ARTICLE INFO

Article history:
Received 13 August 2013
Received in revised form
11 November 2013
Accepted 16 November 2013

Keywords:
HPV
CIN
Cervical infection
Anal infection
Anal cancer

ABSTRACT

Background: More than 90% of cases of anal cancers are caused by high-risk human papillomavirus (HR HPV) infection and a history of cervical intraepithelial neoplasia (CIN) is established as possible risk factor.
Objectives: To demonstrate relationship between anal and cervical HPV infection in women with different grades of CIN and microinvasive cervical cancer.
Study design: A total of 272 women were enrolled in the study. The study group included 172 women who underwent conization for high-grade CIN or microinvasive cervical cancer. The control group consisted of 100 women with non-neoplastic gynecologic diseases or biopsy-confirmed CIN 1. All participants completed a questionnaire detailing their medical history and sexual risk factors and were subjected to anal and cervical HPV genotyping using Cobas and Lynear array HPV test.
Results: Cervical, anal, and concurrent cervical and anal HPV infections were detected in 82.6%, 48.3% and 42.4% of women in the study group, and in 28.0%, 26.0% and 8.0% of women in the control group, respectively. The prevalence of the HR HPV genotypes was higher in the study group and significantly increased with the severity of cervical lesion. Concurrent infections of the cervix and anus occurred 5.3-fold more often in the study group than in the control group. Any contact with the anus was the only significant risk factor for development of concurrent HPV infection.
Conclusions: Concurrent anal and cervical HR HPV infection was found in nearly half of women with CIN 2+. The dominant genotype found in both anatomical locations was HPV 16. Any frequency and any type of contact with the anus were shown as the most important risk factor for concurrent HPV infection.

1. Background

Anal cancer is uncommon disease, but its incidence is on the rise in most of the world's developed countries. More than 90% of cases are caused by high-risk human papillomavirus (HR HPV) infection combined with a predominant genotype HPV 16 [1,2]. Several studies have shown that persons at risk of developing anal cancer include HIV-infected subjects, males who have sex with males and transplant recipients [3–12]. Anal cancers are, however, detected in appreciable portion of the generally healthy and HIV-negative population [13–15]. Invasive anal squamous cell carcinoma rates increased by 1.7% per year among females in the U.S. between 1973 and 2005 [16]. Moreover the incidence of anal cancers is significantly higher in women than in men. In the U.S., there were an estimated 6230 new cases of anal cancer in 2012, with 3980 cases occurring in women and only 2250 cases in men [17]. This gender difference has not been clearly explained so far, although a history of cervical intraepithelial neoplasia (CIN) and cervical cancer, a history of anal intercourse and multiple sexual partners were established as possible risk factors [18–20].

Abbreviations: AIN, anal intraepithelial neoplasia; AIS, adenocarcinoma in situ; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; CIN, cervical intraepithelial neoplasia; CIN 2+, high-gradeCIN or microinvasive cervical cancer; COC, combined oral contraception; DNA, deoxyribonucleic acid; HIV, human immunodeficiency virus; HPV, human papillomavirus; HR, high-risk; HRT, hormone replacement therapy; HSIL, high-grade squamous intraepithelial lesion; IUD, intrauterine device; LR, low-risk; OR, odd ratio; PCR, polymerase chain reaction.

* Corresponding author at: Hospital Na Bulovce, Budinova 2, Prague 8, 180 81, Czech Republic. Tel.: +420 266 083 229; fax: +420 283 840 507.
E-mail addresses: boreksehnal@seznam.cz, borek.sehnal@bulovka.cz (B. Sehnal).

1386-6532/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jcv.2013.11.004

Based on data from the National Cancer Registry, the incidence of anal cancer in the Czech Republic has been stable over the last 30 years. Similar to the U.S., however, the prevalence of anal cancer is noticeably higher among women than in men. In 2010, there were 130 new cases of anal cancer, with 85 cases in women and 44 cases in men. Importantly, the proportion of HIV-positive women was less than 3%. This fact accentuated the need to identify the population of women with an increased risk of cancer, who could be candidates for more detailed or even screening examinations of the anus. We hypothesize that such a population would be particularly represented by women who were treated for CIN. All women with history of CIN and cervical cancer are at increased risk for developing anal cancer, presumably because of enhanced exposure to HR HPV infection.

2. Objectives

The aim of our study was to describe the association between anal HPV infection and cervical HPV infection in HIV-negative women suffering from CIN or microinvasive cervical cancer. To our best knowledge, this is the first study evaluating anal HPV infection in a strictly selected cohort of women with different grades of CIN and the very first data on the prevalence of anal HPV infection in Czech women.

3. Study design

3.1. Patients

Participants were recruited between September 2011 and June 2012 from women over 18 years of age attending two university-based colposcopy clinics in Prague. The study cohort included 172 "high-risk" patients in whom CIN 2, CIN 3, AIS or microinvasive cancer was confirmed by conization. The control group consisted of 100 "low-risk" patients with biopsy-confirmed CIN 1 and patients with diverse non-neoplastic gynecological diagnoses (irregular bleeding, endometrial polyp, missed abortion, induced abortion). Inclusion of patients with any STDs was carefully avoided. All participants completed an anonymous self-administered questionnaire on their medical histories, tobacco use, social status, and sexual behavior; they were familiarized with the study protocol and signed an informed consent. The study had been approved by the Local Ethical Committee.

3.2. HPV detection and genotyping

Trained clinicians obtained exfoliated cervical cell samples for HPV detection under general anesthesia. A brush was used to smear the entire ectocervix and endocervix, including the entire transformation zone and anal transformation zone. Following the cervical specimen collection, an exfoliated anal cell specimen was obtained using another brush, inserted ~1.5–2.0 cm into the anus and rotated 360° clockwise (3 times) and counter-clockwise (3 times). Each sample was dispersed separately in PreservCyt transport medium with maximal care being exercised to prevent contamination. The linear array genotyping HPV test used for all samples was as described by kit manufacturer (Roche Molecular Systems, Inc., Branchburg, NJ). The test was used to identify 37 HPV genotypes that included 13 high-risk (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and 24 low-risk types (HPV 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39 and CP6108). Subsequent hybridization and HPV genotyping were performed as described by the manufacturer (Roche Molecular Systems, Inc., Branchburg, NJ). The strips were manually interpreted using the linear array HPV reference guide, by reading the individual types down the length of the strip.

3.3. Histopathology

All biopsy specimens submitted for histological assessment were routinely examined in their entirety. Sections from the formalin-fixed and paraffin-embedded tissue fragments were stained with hematoxylin–eosin. Histological grading of dysplasia was based on the standard CIN 1, CIN 2 and CIN 3 criteria.

3.4. Statistical analysis

Standard robust summary statistics were applied to describe primary data, absolute and relative frequencies for categorical variables and median supplied with the 5th–95th percentile range for continuous variables. The statistical significance of differences between the control group and the group of CIN 2+ patients in categorical variables was tested using Fisher exact test; an exact Monte Carlo method with 100 000 samples was applied to estimate the significance of differences in variables with more than two categories. Mann–Whitney *U* test was applied to test the differences in continuous variables. Age-adjusted logistic regression analysis was used for the assessment of the association between various risk factors and defined end-points related to different types of HPV infection. The results are represented as estimates of odd ratios (OR, along with 95% confidence interval) with corresponding statistical significance (Wald's test). Concordance of HPV genotype profiles between cervical and anal infections was analyzed using Jaccard's coefficient which belongs to a group of asymmetric binary coefficients of similarity. All analyses were performed using SPSS 20.0.0. (IBM Corporation, 2011).

4. Results

Altogether 272 women were enrolled in the study, 172 "high-risk" women in the study group and 100 "low-risk" women in the control group. The characteristics of both groups are summarized in Table 1. The majority of evaluated social and medical aspects were similar in both groups. However, the patients in the control group reported significantly more pregnancies, childbirths, and the use of hormones ($p < 0.001$). There were 169 cervical samples for HPV testing and 163 anal samples available in the study group and 100 and 98 in the control group, respectively.

The prevalence, site and type of HPV infection were significantly different between the two groups (Table 2). Cervical infection was detected in 82.6% of women in the study group and 28.0% of controls ($p < 0.001$). Anal HPV infection was detected in 48.3% of women in the study group and 26.0% of controls ($p < 0.001$). No HPV infection in either the cervix or the anus was found in only 11.6% of women in the study group but in more than a half (54.0%) of the controls ($p < 0.001$).

Cervical and anal HPV infections were much more frequent in the study group than in controls ($p < 0.001$). HPV infection of the cervix only was diagnosed in 40.1% of the study group patients and in 20.0% of controls ($p < 0.001$). HPV infection of the anus only was detected in 18.0% of the controls and in 5.8% of the study group patients ($p = 0.001$). Concurrent cervical and anal infection was found in 42.4% of study group cases and in 8.0% of the controls ($p < 0.001$). The prevalence of concurrent infection significantly increased with the grade of cervical lesion (Table 3). Concurrent infection was found in 42.4% of CIN 2+ and in 49.0% of cases with CIN 3 and microinvasive cancer.

The presence of 13 high-risk (HR) and 18 low-risk (LR) HPV genotypes was confirmed; 13 HR and 18 LR genotypes were detected in the cervix and 11 HR and 16 LR genotypes in the