J Infect Chemother xxx (2017) 1–6

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Original Article

Influence of co-infection complicated with human papillomavirus on cervical intraepithelial neoplasia development in patients with atypical squamous cells of undetermined significance

Hisami Kiseki [a, *], Yutaka Tsukahara [a], Natsumi Tajima [b], Ayako Tanaka [b], Aya Horimoto [b], Naohiko Hashimura [a]

[a] Department of Obstetrics and Gynecology, Kohseichuo General Hospital, Tokyo, Japan
[b] Department of Clinical Laboratory, Kohseichuo General Hospital, Tokyo, Japan

ARTICLE INFO

Article history:
Received 7 April 2017
Received in revised form
26 July 2017
Accepted 14 August 2017
Available online xxx

Keywords:
Atypical squamous cells of undetermined significance
Cervical intraepithelial neoplasia
Human papillomavirus
Vaginal infection

ABSTRACT

Aim: Human papillomaviruses (HPV) infection is a primary cause of the development of cervical precancerous lesions and cervical cancer. However, the influence of other infections on intraepithelial neoplasia (CIN) development has not been fully elucidated. We evaluated the association between co-infection and CIN development in subjects with atypical squamous cells of undetermined significance (ASCUS).

Method: Data for ASCUS subjects who had undergone testing for high risk HPV (HR-HPV) and pathological diagnosis were analyzed. From the CIN grade, HR-HPV and vaginal infection (VI) data, both the relationship between HPV infection and CIN development and the influence of co-infection on CIN were retrospectively evaluated.

Results: Data for 56 ASCUS subjects who had undergone HR-HPV testing and cytological diagnosis were analyzed. Positive rates were HPV (73.2%), HPV16 (21.4%), HPV18 (7.1%), and HPV16 and/or 18 (26.8%). Seventeen of the subjects were diagnosed as having one or more VI pathogen; the major pathogens found were Candida spp., Gardnerella vaginalis, group B streptococcus, coagulase negative Staphylococcus, and *Chlamydia trachomatis*. The rate of CIN 2 or worse (≥CIN 2) was significantly higher in subjects positive for HPV16 compared with HPV negative subjects, and was significantly higher in subjects with a VI complicated with HPV compared to those without a VI. Univariate and multivariate logistic regression analysis identified positive for HPV16 and/or 18 and positive for VI to be significant variables for ≥ CIN 2.

Conclusion: Our results indicate that having a vaginal infection complicated with HR-HPV affects the development of CIN in subjects with ASCUS cytology.

1. Introduction

High-risk human papillomaviruses (HR-HPV) infection is a primary cause of the development of cervical precancerous lesions and cervical cancer [1–3]. According to the 2001 Bethesda System, the current standard diagnostic criteria for cervical cytology that is followed in Japan, the United States, and Europe, HR-HPV testing is recommended for cases of atypical squamous cells of undetermined significance (ASCUS) for cervical cancer screening [4–8]. The ATHENA (Addressing the Need for Advanced HPV Diagnostics) study conducted in the United States reported that the absolute risk (AR) for high grade cervical intraepithelial neoplasia (≥CIN2) were 14.0% and 31.5% in ASCUS subjects testing positive for HR-HPV or HPV 16, respectively [9]. On the other hand, zur Hausen et al. and others have reported that HR-HPV infection is an important factor but not the sole cause of cervical cancer [10,11]. Several reports have indicated that other infections complicated with HPV associate with CIN development via inflammatory response, immune alteration, or genomic alteration [11–16]. However, the relationship between CIN development and co-

* Corresponding author. Department of Obstetrics and Gynecology, Kohseichuo General Hospital, 1-11-7 Mita, Meguro-ku, Tokyo, Japan.
E-mail address: chamy0503@jcom.home.ne.jp (H. Kiseki).

http://dx.doi.org/10.1016/j.jiac.2017.08.008
1341-321X/

infection has not been fully elucidated. In this study, we retrospectively investigated the association between co-infection complicated with HPV and CIN grade in subjects with ASCUS cytology who had undergone HR-HPV testing.

2. Patients and methods

2.1. Patients

Data for ASCUS subjects who had undergone HR-HPV testing and pathological diagnosis from January 2014 to July 2015 at Kohseichuo General Hospital in Tokyo were collected. Data for subjects who had suffered a total hysterectomy were excluded. This study was conducted in accordance with the ethical principle of the Declaration of Helsinki and was approved by the institutional review board of Kohseichuo General Hospital.

2.2. Methods

From the data of the study population, CIN grade, HR-HPV and vaginal infection (VI) were examined. The relationship between HPV infection and CIN development and the influence of co-infection on CIN were retrospectively evaluated.

2.2.1. HPV infection and CIN

To evaluate the relationship between HPV infection and CIN, the absolute risks (ARs) for CIN 2 and CIN 3 were calculated according to HR-HPV genotypes, and the composition ratios of HPV genotypes according to CIN grade were determined. In addition, we compared the rates of subjects with <CIN 2 and ≥ CIN 2 between subjects with HR-HPV negative (HPV−) and those with HPV positive (HVP+) according to genotype: HPV16, HPV18, 12 other HPV genotypes (HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) (HPV other), and HPV16 and/or 18.

2.2.2. Influence of co-infection on CIN development

The rates of ≥CIN 2 were compared between the presence and absence of a VI complicated with HPV infection. To evaluate the influence of a VI on CIN development, the odds ratios of age, HPV, and VI for ≥ CIN 2 were calculated using univariate and multivariate logistic regression analysis. A positive VI (VI+) designation included testing positive for Gardnerella vaginalis (GV), Group B streptococcus (GBS), coagulase negative staphylococcus (CNS), *Corynebacterium* sp. (Corynebacterium), *Escherichia coli* (*E. coli*), *Enterococcus* sp. (Enterococcus), *Chlamydia trachomatis* (chlamydia), *Neisseria gonorrhoeae* (gonorrhoeae), *Candida* spp. (candida), or genital herpes (herpes).

2.2.3. Diagnostic methodology

The cytological results were classified according to the 2001 Bethesda System [4]. HR-HPV testing and pathological diagnosis were performed according to the Clinical Guidelines for Obstetrical Practice in Japan, revised in 2017 [5]. Cytology samples from cervical and vaginal tissue and endocervical material using a sterilized cyto-pick were collected and stained using the Papanicolaou (PAP) staining method after fixing with alcohol (conventional method). Definitive cytological diagnosis was performed by three independent cytologists using samples screened by two independent cytoscreeners.

HR-HPV infection including HPV16, HPV18, and HPV other was examined using the cobas 4800 HPV test® (Roche Diagnostics) using cervical samples collected using the same method as for cytology in subjects with ASCUS cytology within two weeks of their diagnosis. A punch biopsy was performed within four weeks of the initial visit using colposcopy in HPV + subjects. Pathological samples were stained using hematoxylin-eosin (HE), and a definitive diagnosis was performed by two independent pathologists. Samples from subjects who had increased vaginal discharge, discomfort in external genitalia, or other symptoms of infection were tested. All specimens were first cultured on blood agar and chocolate agar and tested using ID test HN-20 Nissui (Nissui Pharmaceutical Co., Ltd. to identify Corynebacterium, *E. coli*, and Enterococcus. GV, GBS, and CNS were identified by an additional culture test using Gardnerella agar® (SYSMEX bioMérieux Co., Ltd.), Seroiden Strepto Kit Eiken® (Eiken Chemical Co., Ltd.), and USAGI plasma Eiken® (Eiken Chemical Co., Ltd.), respectively. *Chlamydia trachomatis* was identified by PCR using cobas® 4800 system CT/NG (Roche Diagnostics). Gonorrhoeae was identified by using the Thayer-Martin agar HN-20 Nissui culture test. Candida was identified by culture test using CHROM agar® (Kanto Kagaku). Herpes was identified by EIA using HSV IgG (SRL).

2.3. Statistics

Descriptive statistics were expressed as n (%), median [interquartile range]. A chi-square test or Fisher's exact test was performed for comparisons of the categorical variables. Factors influencing ≥ CIN 2 were evaluated using univariate and multivariate logistic analysis; age, HPV16/18 infection, HPV other infections, and VI were used as independent variables. The alpha was set at 0.05 and all p values were two-sided. Statistics was

Table 1
Patient characteristics.

n	56
Age, median [inter quartile range]	33.5 [28.25, 41.0]
Age group, n (%)	
≤ 30	19 (33.9%)
31–40	22 (39.3%)
≥ 40	15 (26.8%)
HR-HPV[a] screening, n (%)	
HPV−	15 (26.8%)
HPV+	41 (73.2%)
i. HPV16+/18−/other−	5 (8.9%)
ii. HPV16+/18+/other−	1 (1.8%)
iii. HPV16+/18−/other+	6 (10.7%)
iv. HPV16−/18+/other−	1 (1.8%)
v. HPV16−/18+/other+	2 (3.6%)
vi. HPV16−/18−/other+	26 (46.4%)
HPV16+: i, ii, iii	12 (21.4%)
HPV18+: ii, iv, v	4 (7.1%)
HPV16 + and/or 18+: i, ii, iii, v, vi	15 (26.8%)
HPV other +: iii, v, vi	34 (60.7%)
VI[b] infection, n (%)	
VI−	39 (69.6%)
VI+	17 (30.4%)
Bacterial infection	
Bacteria−	46 (82.1%)
Bacteria+	10 (17.9%)
GV+	5 (8.9%)
GBS+	4 (7.1%)
CNS+	3 (5.4%)
Corynebacterium+	2 (3.6%)
E.coli+	1 (1.8%)
Enterococcus+	1 (1.8%)
STD[c] infection	
STD−	52 (92.9%)
STD+	4 (7.1%)
Chlamydia+	3 (5.4%)
Gonorrhoeae+	1 (1.8%)
Other infection	
Candida+	8 (14.3%)
Herpes+	1 (1.8%)

[a] High-risk human papillomavirus.
[b] Vaginal infections (bacterial infection, STD, or other infections).
[c] Sexually transmitted disease.

Please cite this article in press as: Kiseki H, et al., Influence of co-infection complicated with human papillomavirus on cervical intraepithelial neoplasia development in patients with atypical squamous cells of undetermined significance, J Infect Chemother (2017), http://dx.doi.org/10.1016/j.jiac.2017.08.008