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Lower genital tract infection and other factors associated with cervial intraepithelial neoplasia: a hospital-base case-control study

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Summary

Aim: To investigate clinical factors, especially pathogenic in the lower genital tract associated with cervical intraepithelial neoplasia (CIN) among women participating in a cervical cancer (CC) screening program in Liuzhou, south of China. *Materials and Methods:* A hospital-base case-control study for four years was designed. A total of 12,644 outpatients were involved in this study. Clinical characters were recorded and samples of the cervical secretion were obtained to detect Chlamydia trachomatis (CT), high-risk human papillomavirus (HR-HPV), low-risk HPV (LR-HPV), Neisseria gonorrhoeae (NG), and Ureaplasma urealyticum (UU). Logistic regression models were conducted to investigate the association between the factors and CIN. *Results:* There were 260 CIN patients of all included cases (2.06%). HR-HPV (OR=18.27, 95%CI 13.89-24.04), CT infection (OR=2.31 95%CI 1.70-3.13), numbers of pregnancy (OR=1.42, 95%CI 1.11-1.83), and older age (OR=1.59, 95%CI 1.16-2.19) were associated with CIN. The prevalence of CIN in patient group HR-HPV(+), CT(+), HR-HPV(+), CT(-); HR-HPV(-), CT(+); HR-HPV(-), and CT(-) was 18.00%, 10.26%, 1.97%, and 0.48%, respectively. Women both HR-HPV and CT positive had a significantly higher susceptibility to CIN ($p < 0.001$). *Conclusion:* Age and gravity might be risk factors of CIN. HR-HPV and CT play important roles in cervical carcinogenesis. Co-infection of HR-HPV, and CT might increase the risk of CIN.

Key words: Cervical intraepithelial neoplasia (CIN); Chlamydia trachomatis (CT); High risk human-papillomavirus (HR-HPV); Risk factor.

Introduction

Cervical cancer (CC) is the third most common cancer, ranking after breast and colorectal cancer. According to IARC report, more than 500,000 women were diagnosed with CC in 2008, which have caused 274,000 deaths [1]. This figure was similar to the 287,000 deaths estimated in 2010 by the World Health Organization (WHO). CC, which is known as a chronic infectious disease, is considered to be induced by specific pre-invasive lesions. The lesions are generally graded as cervical intraepithelial neoplasia 1, 2 and 3 (CIN1, 2, and 3). About 65-75% CIN3 and 35-45% CIN2 will progress to malignant lesions without treatment [2]. Effective local treatment of CIN prevents the progress of squamous cell carcinoma (SCC) of the cervix [3]. Now it is well known that the existence of the human papillomavirus (HPV) infection, especially types 16 and 18, is a key factor for the development of CC and cervical pre-invasive lesion. In fact, approximately 291 million women are HPV carriers worldwide, among them only a portion would develop cervical cancer and cervical pre-invasive lesions [4]. The increased frequency of other co-factors such as smoking, oral contraception, and sexually transmittable infections, such as human immunodeficiency virus (HIV) [5] and bacterial vaginosis [6], may contribute to the rise of cervical cancer incidences as well [7, 8].

Chlamydia Trachomatis (CT) is the most prevalent infection of the genital tract in women worldwide, which recently accounts for 40% of sexually transmitted diseases (STDs) [9]. Previous researches have implied that CT infections were highly associated with harmful and serious complications, such as pelvic inflammatory disease (PID), infertility, and ectopic pregnancy, therefore, it was considered as the co-factors of CC and CIN in the last decade [10].

To the best of the present authors' knowledge, there was no research assessing CT infections in CIN in China. The authors performed a hospital-base cross-section study of the CC screening program conducted for four years to investigate the potential risk factors of CIN and to estimate the frequency of co-infection of HPV and CT associated CIN in the People's Hospital of Liuzhou, South China.

Materials and Methods

This study was conducted from January 2010 to May 2014, which was a CC screening program for outpatients in the People's Hospital of Liuzhou. All women aged 20–72 years who were willing to participate in the screening program were included. The exclusion criteria were those patients who were pregnant, had taken oral contraception (for birth control or therapy) or antibiotics in the past three months, and patients who had never been married or experienced hysterectomy. As this study focused only on the risk factors of CIN, the samples from women who were diagnosed with CC were also excluded (Figure 1). All women were informed

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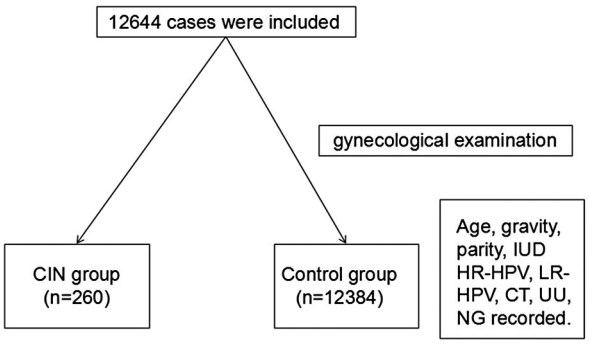

Figure 1. — Flow chart of participants in the study.

of the purpose of the screening program and signed the informed consent prior to enrolment. The study was approved by the Ethics Committee of People's Hospital of Liuzhou. Clinical information including age, gravidity, parity, and history of contraception were collected from all the cases.

All enrolled cases accepted gynecological examination and the cervical appearance was recorded. At the same time to obtain cervical exfoliated cells for Pap smear (TCT), ThinPrep Papanicolaou smear was employed and four cytology categories were used (normal, ASCUS/AGUS, LSIL, and HSIL).

Analysis of HR-HPV, LR-HPV DNA (type 6, 11, 16, 18, 31, 33, 35, 39, 45, and 51), CT, UU, and NG were also performed. Specimens were collected from the endocervical OS or the cervical canal. HR-HPV, LR-HPV, UU, NG, and CT DNA were detected using the commercial kits of real-time fluorescence probe nucleic acid test.

Women who were HR-HPV positive, or had abnormal cervical appearance or abnormal cytological outcome, were treated with colposcopy and guided biopsies. Suspicious lesions were graded using Reid Colposcopic Index 17. Endocervical curettage was performed if lesions were invisible. Final diagnosis was made based on histopathology, which was classified into negative, CIN1, CIN2, and CIN3.

Statistical analysis was performed using the SPSS 17.0 statistics software. The association between the selected variables and risk for CIN was investigated using multi-variant logistic regression analyses and the odds ratio (OR) at the 95% confidence interval (CI) was calculated. The frequencies between CIN cases and control cases among the different HR-HPV and CT infection status were compared using the Kruskal-Wallis test. $P < 0.05$ was considered as statistically significant.

Results

A total of 12,644 women from outpatient of People's hospital of Liuzhou participated in the screening program from January 2010 to May 2014. Of these participants, there were 260 patients diagnosed with CIN and its prevalence was 2.06%. Clinical characteristics of CIN patients and control cases are presented in Table 1.

Age, gravity, parity, intrauterine device (IUD) for contraceptive, infection factor including HR-HPV, LR-HPV, CT, UU, and NG were recorded. The prevalence of HR-HPV, LR-HPV, CT, UU, and NG were 12.99%, 1.57%, 13.21%, 40.89%, and 3.58%, respectively. With the increase of age, the risk of CIN also increased (OR=1.023, 95%CI, 1.163-2.185). The risk of CIN in the women whose cervix was exposed to HR-HPV (including type 16, 18, 31, 33, 35, 39, 45, and 51) was much higher than those who did not have HR-HPV infection (OR=18.463; 95% CI, 14.198-24.427). More than twice of the risk was observed in the women who were CT-DNA positive (OR=2.588, 95%CI, 1.911-3.541). There was also a weakly increased risk with the increase of gravity, (OR=1.526, 95%CI, 1.108-1.825). In this study, parity (OR=1.108, 95%CI, 0.866-1.417) and IUD for contraception (OR=0.989, 95%CI, 0.776-1.262) were not associated with the present of CIN. Meanwhile, there were no significant differences of LR-HPV (OR=1.611; 95%CI, 0.918-2.828), UU (OR= 0.997, 95%CI, 0.771-1.289) and NG (OR=0.656, 95%CI, 0.390-1.104) between CIN patients and control cases.

Since HR-HPV and CT infection played key roles in CIN, the authors divided all the study population into four groups according to the different infection status of HR-HPV and CT: HR-HPV (+), CT (+); HR-HPV (+), CT (-); HR-HPV (-), CT (+); HR-HPV (-), CT (-). Table 2 shows the frequencies of CIN in the different states of CT and HR-HPV. The frequencies of CIN were 18% (36/200) in HR-HPV(+), CT(+) group; 10.28% (148/1443) in HR-HPV(+), CT(-) group; 1.97% (29/1470) in HR-HPV(-), CT(+) group, and 0.48% (47/9531) in HR-HPV(-) and CT(-) groups, respectively. There were significant differences between the different aforementioned groups and the highest prevalence of CIN were in groups HR-HPV (+) and CT (+). (Table 2)

Discussion

A large-scale study to investigate the association between CIN and risk factors, especially other lower genital tract infection in Liuzhou, South China was designed. In this study, the authors found that the risk factors for CIN were age, gravity, HR-HPV, and CT, while parity, IUD, LR-HPV, NG, and UU did not increase the risk of CIN. The prevalence of HR-HPV and CT were similar, which was higher than the prevalence of LR-HPV and NG. However, the rate of positive of UU was the highest.

Older age and high gravidity were identified as risk factors for the development of CIN in this study, which support previous findings, including the community-based screening programs in India, Sudan, and Brazil, which have also revealed that older age and high gravidity increased the risk of cervical abnormalities [11-13]. On the contrary, parity and IUD were found not to be the risk factors for CIN, which differ from other research [14, 15]. It was probably related to the study population which mainly originated from urban areas and was influenced by the family planning policy in China.

NG was one of the classic STDs, which infected the cer-

Table 1. — *Association of Chlamydia trachomatis and HR-HPV and other risk factors for CIN in the multiple factors logistic models.*

	Cases (%) n=260	Controls (%) n=12384	B	S.E.	Wald	*p*	OR	95%CI
Age (years)								
20-29	23.84	36.43						
30-39	39.62	40.51						
≥ 40	36.54	23.05	0.467	0.161	8.421	0.004	1.594	1.163-2.185
Gravity								
0	6.92	18.48						
1	17.69	23.47						
≥ 2	75.38	58.05	0.352	0.127	7.642	0.006	1.422	1.108-1.825
Parity								
0	30.00	37.19						
1	52.31	50.17						
≥ 2	17.31	12.65	0.102	0.126	0.664	0.415	1.108	0.866-1.417
IUD								
No	57.30	57.03						
Yes	42.69	42.97	0.097	0.124	0.651	0.931	0.989	0.776-1.262
CT-DNA								
Negative	75.00	87.20						
Positive	25.00	12.96	0.959	0.158	37.007	0.000	2.602	1.911-3.541
HR-HPV								
Negative	29.23	88.22						
Positive	70.77	11.78	2.924	0.138	446.343	0.000	18.516	14.198-24.427
LR-HPV								
Negative	95.77	97.27						
Positive	4.23	2.73	0.477	0.287	2.757	0.097	1.611	0.918-2.828
NG								
Negative	93.70	96.48						
Positive	6.30	3.52	-0.421	0.265	2.520	0.112	0.656	0.390-1.104
UU								
Negative	60.37	59.08						
Positive	39.63	40.92	-0.003	0.131	0.001	0.981	0.997	0.771-1.289

Table 2. — *The incidence of CIN in different groups according to the Kruskal-Wallis test.*

	CIN1	CIN2+	(%)	Mean rank	*p*
HR-HPV (+), CT (+)	7	29	18.00	6220.04	<0.001
HR-HPV (+), CT (-)	60	88	10.26	6307.23	
HR-HPV (-), CT (+)	15	14	1.97	6875.14	
HR-HPV (-), CT (-)	28	19	0.48	7298.66	

vical columnar epithelial cells in women. It was widely considered to be one of causes of mucus purulent cervicitis and pelvic inflammatory disease. Whether it was a risk factor for CIN still lacks evidence. In this present study, NG seemed to have no obvious correlation with CIN because of the lower prevalence (3.58%). Moscicki *et al.* [16] reported that NG infection was associated with CIN2 regression because the intense inflammatory response induced by NG infections may have serendipitously assisted in viral clearance.

The prevalence of UU was higher than CT, NG, and even HPV (including LR-HPV and HR-HPV). Whether UU causes disease in the urogenital tract is still unknown [17, 18]. Although the prevalence of UU was highest in the present study, cases with UU were not found to have higher prevalence of CIN than those without. The present authors thus speculated that UU might not be associated with CIN. However, Zheng *et al.* [19] observed that high-density of cervical UU colonization was associated with CIN because it might change the local micro-environment.

Cervical HR-HPV positive was strongly associated with CIN, which was in accordance with other case-control research [20-23]. However LR-HPV (HPV6, 11) DNA positive was not associated with CIN in the present study. There was a research reporting the antagonistic interaction between HPV16 and HPV6 in cervical carcinogenesis, but the mechanism of this antagonism is not quite clear [24].

CT is the lead cause of sexual transmitted infection [19, 25]. CT had been considered as a potential co-factor of HPV or risk factor of CIN, due to similar form of transmission, intercellular growth, asymptomatic nature, and persistence if untreated [26]. According to the report of the World Health Organization (WHO), almost 100 million chlamydial cases occur every year. It was estimated that in 2008, there were 106 million new cases of CT in adults

[27]. In 2011, 1.4 million cases of CT were reported to the Centers for Disease Control and Prevention (CDC) and this was notable as being: "the largest number of cases ever reported to CDC for any condition". [28]. The present authors observed that CT was positive associated with CIN. The rate of HR-HPV and CT co-infection in the present study was 1.58%, and the prevalence of CIN was the highest among the four different HR-HPV and CT infection. In the cases without the presence of HR-HPV, CT infection was observed to increase the risk of CIN. In cervical dysplasia and neoplasia CT has been highly suspected to cause epithelial damage by inducing HPV infection and inflammation, leading to high levels of reactive oxidative metabolites, initiation of cell division and metaplasia, reduction of host cell mediated immunity, direct induction of squamous metaplasia, and anti-apoptosis function [29].

There are some limitations in the present study. First, life style factors, which include smoking, alcohol consumption, number of sexual partners, and age of first intercourse may be potentially associated with the development of CIN, are not recorded in this study. Second, some other pathogen (NG, Herpes simplex virus, and mycoplasma), which may be possibly associated with female reproductive tracts infection, were not investigated because of budget limitations. In conclusion, the current study provides valuable information for the assessment of risk factors CIN. HR-HPV plays a key role in cervical carcinogenesis and CT infection may also contribute to CIN development, which implies the importance of CT monitors in terms of CC screening.

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