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Human papillomavirus infection among Uyghur women with cervical intraepithelial neoplasia in Xinjiang area

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Summary

Objective: To obtain the baseline data of Uyghur women for human papillomavirus (HPV) vaccination in Xinjiang. *Materials and Methods:* The authors analyzed the infection and distribution characteristics of HPV genotypes in genital tracts among Uyghur women with cervical intraepithelial neoplasia (CIN) in Urumqi of Xinjiang. A total of 1,431 eligible cases involved in this trial. All cervical samples from these patients were detected for HPV genotype. *Results:* High-risk HPV was identified in 24.7% of 979 histologically confirmed normal samples and 89.2% of 452 samples with CIN ($p < 0.05$). The prevalence of one single high-risk type, low-risk type, and multiple HPV types were 74.6%, 10.4%, and 4.2%, respectively. A single high risk HPV infection progressively increased with the severity of cervical lesions significantly ($\chi^2 = 31.53$, $p < 0.01$). While interestingly multiple infection and single low risk HPV infection were decreased with the severity of cervical lesions, and there was significant difference ($\chi^2 = 6.44$, $p < 0.05$; $\chi^2 = 4.85$, $p < 0.05$). The major prevalent high-risk HPV genotypes in 346 samples of CIN II-III were HPV-16, -58, -31, -33, -68,-18,-45, and -39. The comparison of HPV genotype distributions between normal cytology and CIN II-III was analyzed. The estimated risks for progression from viral infection to CIN II-III was highest in HPV-33 (prevalence ratio (PR), 2.62), followed by HPV-31 (2.27), HPV-16 (1.92), HPV-58 (1.62), HPV-18 (1.51), HPV-68 (1.05), and HPV-39 (1.05), suggesting that the six genotypes of HPV-31, -16, -58, -18, -68, and -39 (PR > 1) are higher-risk HPV types in Uyghur women with CIN in Urumqi of Xinjiang. There was no association between multiple infection and cervical lesion progression (0.31, PR < 1). *Conclusion:* Except for the common HPV-16, -58, -31, -33, -18 in Xinjiang, HPV-68 and HPV-39 may be the oncogenic subtypes to Uyghur female with CIN in Xinjiang. Distinguishing these HPV subtypes may have implications for future cervical screening strategies and vaccine implementation. Multiple infections were not association with an increased risk of high-grade cervical neoplasia.

Key words: Cervical intraepithelial neoplasia; Human papillomavirus; Genotypes; Infection characteristics; Uyghur.

Introduction

Invasive cervical carcinoma is the second common malignant tumors that threaten the health of female worldwide, with an estimated 493,000 new cases and 274,000 deaths in the year 2002. In general terms, it is much more common in developing countries, where 83% of cases occur and where cervical cancer accounts for 15% of female cancers, with a risk before age 65 of 1.5% [1]. Persistent infection of oncogenic human papillomavirus (HPV) is the pathogenesis of cervical cancer and precancerous lesion, cervical intraepithelial neoplasia (CIN). So far, there are about 100 subtypes of HPV have been identified, of which more than 40 types are known to infect female reproductive tract, according to their pathogenicity,they are classified as high-risk HPV includes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82, and so on, types 26, 53, and 66 should be considered as probably carcinogenic [2]. Some recent meta-analysis showed that HPV16 and 18 subtypes are the two common types in Asian women with invasive cervical carcinoma, and pointed out that HPV-52, 58, and 45, 33 subtypes are the common types of Southeast Asia and East Asia, South Asian women with cervical cancer and precancerous lesions [3, 4]. Therefore, oncogenic HPV testing combined with cytological testing was accepted as primary screening in the USA, because of sensitivity and effectiveness [5]. In addition, in view of the wide applications of HPV vaccines in the world, polyvalent vaccine containing HPV16, 18L1 virus-like particle (virus-like particle, VLP) has been licensed, which has been confirmed to be effective in the prevention of the CIN II/ III and the efficacy of the vaccine is sustainable for about 42 months since it has been approved [6, 7]. From evidence of clinical trials, these two targeting of HPV (detection and vaccine) are increasingly attractive for cervical cancer prevention worldwide. At present, in Xinjiang, HPV DNA testing has not yet been available in routine screening and HPV vaccine has not been licensed. Evaluating the type-specific data of HPV genotypes in Uyghur women with normal cytology and with CIN in Xinjiang is as important as evaluating the potential benefits of vaccination in cervical cancer prevention and the future role of HPV screening. For now, there are only limited data available from Xinjiang on HPV-type prevalence in normal cytology and in CIN.

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In order to provide the prevalence data of HPV genotypes infection in Xinjiang Province of China, this pilot study was performed to analyze the infection characteristics of HPV-type prevalence from 1431 Uyghur women in Xinjiang. This data may help provide effective models for HPV screening and vaccination among Uyghur women in Xinjiang.

Materials and Methods

Study population

The study subjects consisted of 1,431 Uyghur women in Xinjiang (979 normal cytology, 106 CIN I, 210 CIN II, and 136 CIN III). All of the eligible women enrolled have been living in Xinjiang for more than ten years. Inclusion criteria included the following: continuing irregular vaginal bleeding, bleeding after intercourse, those found on examination to have an unhealthy cervix, or those who visited the hospital for cervical cancer screening. All patients underwent cervical liquid-base cell smears detection and HPV genotyping chip detection. An enrolment questionnaire was completed. All cervical exfoliated cell samples were obtained for cervical liquid-based cytology examination and HPV detection. After the cervical scrapes, women with abnormal cervical smear results underwent colposcopic examination of cervix and cervical biopsy for histological verification was performed. Classification of each cytological diagnosis was based on the Bethesda System 2001.The histological analysis were obtained by two professional pathologists who did not know the HPV status. Final histological diagnosis of the specimens was based on the WHO classification of cervical neoplasia. At last histological diagnosis confirmed 106 as CIN I, 210 cases as CIN II, and 136 cases as CIN III.Then the infection distribution of the HPV types according to the histological diagnosis were evaluated.

HPV sample and genotyping: the collection of cervical cell suspensions for each patient was performed using a plastic cervical swab from ecto-and endocervix of uterus. Each plastic swab was well-mixed with one ml of specimen transport medium and stored immediately at 4°C . All specimens were finally sent to the laboratory for HPV genotyping analysis. The authors used gene amplification and flow-through hybridization technology as an HPV genotyping method, which concluded three processes of extraction of total DNA of cervical cells, gene amplification (PCR), and flow-through hybridization. The experimentation was performed strictly according to the manufacturer's instruction. The final results of the testing were achieved colourimetric change on the chip under direct visualization. The result was a single or mixed HPV infection. The three steps can detect 21 different HPV genotypes, which were classified as flows: high- risk genotypes: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, low-risk genotypes: HPV 6, 11, 42, 43, 44 genotypes and the common HPV genotypes in Chinese population as HPV 53, 66, CP8304.

Statistical analyses

The data were analyzed Using SPSS for Windows, standard version 17.0 statistical software. To estimate different HPV genotype risk for progression from viral infection to CIN II-III, prevalence ratios (PRs) were calculated. Statistical analysis was carried out with Chi-square (χ^2) test or Fisher's exact test. A p value of < 0.05 was considered to be statistically significant.

Results

This study included 1,431 Uyghur women (979 normal cytology,106 CIN, and 346 CIN II-III).The mean age of the study subjects was 36.9 years (range, 18-76) for women with normal cytology, 35.6 years (range, 18-69) for CIN I, 35.9 years (range,18-73 years)for CIN II-III. A total of 17 HPV types were detected in women with normal cytology. In CIN II, 15 different HPV genotypes were detected. In CIN I and III, 13 and 14 subtypes were found, respectively.

Table 1. — *The characteristics of HPV infection in different CINs.*

HPV	CIN I, n (%) n=106	CIN II-III, n (%) n=346
Negative	23 (21.7)	26 (7.5)
Positive	83 (78.3)	320 (92.4)
Single high-risk HPV infection	57 (53.8)	280 (80.9)
Multiple infection	18 (17.0)	29 (8.4)
Single low-risk HPV infection	8 (7.5)	11 (3.2)

The characteristics of HPV infection in CIN:

Of the 452 patients with CIN, HPV tested positive in 403 cases (89.2%, 403/452), 92.4% of HPV prevalence in CIN II-III. Single high-risk HPV genotypes infection were detected in 74.6% of the women (337/452), while single low-risk HPV genotypes were detected in 4.2% of the women (19/452). Multiple infections with two and three were found in 10.4% of the women (47/452). Dual infection was present in 37 women, triple infection in ten cases. The present study showed that high-risk HPV genotypes were major genotypes in CIN. A single high-risk HPV infection progressively increased with the severity of cervical lesions significantly ($\chi^2 = 31.53$, $p < 0.01$). While interestingly multiple infection and single low risk HPV infection were declined with the severity of cervical lesions. There was significant difference ($\chi^2 = 6.44$, $p < 0.05$; $\chi^2 = 4.85$, $p < 0.05$) (Table 1).

The distribution characteristics of HPV genotype in cervical intraepithelial neoplasia

The diversity of HPV types was widespread in women with CIN. Of the 21 detectable HPV types, 16 genotypes were identified. The frequencies of main types of HPV in CIN are summarized in Figure 1. The most prevalent high-risk HPV genotypes were HPV-16 (50.4%), followed by HPV-58 (10.7%), HPV-31 (8.4%), HPV-33 (7.9%), HPV-18 (5.7%), HPV-68 (5.2%), HPV-45 (4.7%), HPV-52 (4.2%), and HPV-39 (3.7%) Concerning the low-risk HPV, the distribution HPV-6 (3.7%), HPV-11 (3.0%), other types were much less common.

HPV genotype risk for CIN in Uyghur women in Xinjiang

To assess the progressive potential risk of each HPV genotype, HPV type distribution and prevalence in women with HPV-positive are shown in Table 2. Among Uyghur women with normal cytology, HPV was identified in 24.7% of 979

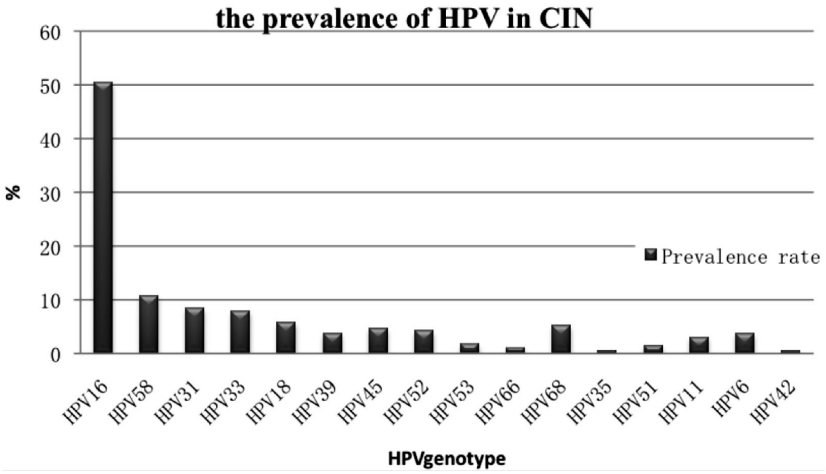

Figure 1. — The prelevance of HPV in CIN.

Table 2. — *The prevalence of HPV in different grades of CINs.*

HPV type	Normal cytology		CIN II-III		PRs	95% CI
	n	%*	n	%*		
HPV-16	63	26.0	160	50.0	1.92	1.51-2.43
HPV-58	13	5.4	28	8.7	1.62	0.86-3.08
HPV-31	8	3.3	24	7.5	2.27	1.04-4.96
HPV-33	5	2.1	16	5.0	2.62	0.97-7.05
HPV-18	5	2.1	10	3.1	1.51	0.52-4.37
HPV-39	5	2.1	7	2.2	1.05	0.34-3.29
HPV-45	8	3.3	8	2.5	0.76	0.28-1.98
HPV-52	6	2.5	5	1.6	0.63	0.19-2.04
HPV-53	4	1.6	2	0.6	0.38	0.07-2.05
HPV-66	4	1.6	2	0.6	0.38	0.07-2.05
HPV-68	10	4.1	14	4.4	1.05	0.47-2.34
HPV-51	3	1.2	3	0.9	0.75	0.15-3.71
HPV-59	0	0	0	0	0	0
HPV-56	1	0.4	0	0	0	0
HPV-11	11	4.5	4	1.3	0.28	0.09-0.85
HPV-6	13	5.4	6	1.9	0.35	0.14-0.91
HPV-42	10	4.1	1	0.3	0.07	0.01-0.59
HPV-43	2	0.8	0	0	0	0
Multiple	70	2.9	29	9.1	0.31	0.21-0.47

* Percentage among HPV-positive women.

cases and 89.2% of 452 samples with CIN, ($p < 0.05$). HPV-16 (26.0%), and HPV-58 (5.4%) were most frequent among Uyghur women with normal cytology, followed by HPV-68 (4.1%), HPV-31, 45(3.3%), HPV-52 (2.5%), HPV-18,-33,-39 (2.1%), HPV-53, -66 (1.6%), and HPV-51 (1.2%). In CIN II - III, HPV-16 was also the most prevalent genotype (50.0%), followed by HPV-58 (8.7%), HPV-31(7.5%), HPV-33 (5.0%), HPV-68 (4.4%), HPV-18 (3.1%), HPV-45 (2.5%), HPV-39 (2.2%), HPV-52 (1.6%), HPV-51 (0.9%), HPV-53 and HPV-66 (0.6%). When compared the HPV genotype distribution between normal cytology and CIN II - III, the authors found that estimated risks for progression from HPV infection to CIN II - III was highest in HPV-33 (PR 2.62), followed by HPV-31 (2.27), HPV-16 (1.92), HPV-58 (1.62), HPV-18 (1.51), HPV-68 (1.05), and HPV-39 (1.05), which suggesting that HPV-33, -31, -16, -58, -18, -68, and -39 (PR > 1) might be the high-risk HPV in Uyghur women in Xinjiang. Although the prevalence of multiple infection increased from normal cytology to CIN II - III, PR was 0.31, suggesting that there was no association between multiple infection and cervical lesion progression.

Discussion

This study represents the characteristics of the distribution and analysis of HPV genotypes in cervical intraepithelia among Uyghur women in Xinjiang region. In this study, the authors also have described the prevalence of a much

wider spectrum of genotypes among Uyghur patients with different grade CIN and normal cytology from Xinjiang region. The present study found that the prevalence of HPV genotype in patients with cervical cancer and precancerous lesions has ethnic and regional characteristics. At present, there are few studies addressing HPV distribution in patients with CIN. A meta-analysis in the Asian population found that the prevalence of HPV in patients with CIN was 76.4% [8]. At the same time, a meta-analysis from Asia suggested that the prevalence of high-risk HPV genotype in patients with CIN was 81.0% [9]. The present study showed that the prevalence of HPV in patients with CIN was 89.2%, which was higher than the two aforementioned studies from Asia, but slightly lower than the prevalence (98.5%) of HPV in patients with CIN in Europe [10]. Another current study which covered Northern to Southern regions in China revealed that HPV type-distribution prevalence in high-grade lesions was 82% [11]. However the present study found that the prevalence of HPV in high CIN was 92.4% in CIN II - III. Importantly variations in HPV prevalence could be caused by the following factors: specimens' quality and storage, as well as HPV type detectable method by different systems. Therefore due to this variability, it is difficult to compare HPV prevalence between studies. The assay used in the present study, may be a reason for the discrepancy.

The present study also revealed that the prevalence of HPV showed a significantly increased trend with increased grade of CIN. As expected, the increased proportion of single high-risk HPV infection was also significantly among CIN I - CIN II / III. While the comparable declined in proportion of single low-risk HPV infection. These findings were similar to those of Sandri *et al.* [12]. All facts support that high-risk HPV persistent infection was the necessary etiology factor in cervical carcinogenesis. On the other hand, given cervical lesion precede the development of cervical cancer by several years, the mean duration of low-risk and high-risk HPV carriage was four and eight months, respectively [13]. This phenomenon could be explained that the high-risk HPV may persist longer than low-risk HPV. Hence single low-risk HPV infection may be cleared by the body's immune system over time, with which it is not easy to cause persistent infection. From another point of view, it is not the high risk factors to cause the progression of CIN.

In the present study the multiple HPV infections among Uyghur women in Xinjiang area was found in 10.4% in CIN, of which double infection was detected in 8.2%, with triple infection in 2.2%, and quadruple infection had not been detected. Multiple HPV infection was significantly decreased with the severity of cervical lesions. The phenomena might indicate that Uyghur women in Xinjiang intermarry rarely with other nations and are not vulnerable to multiple HPV infection. Other causes may be their special background of life and living habits. While the present data suggested that there was no association between multiple infection and cervical disease progression in Uyghur women in Xinjiang (PR < 1), whether or not multiple infection may be a risk factor for the occurrence and development of CIN and cervical carcinoma have not been confirmed. Some scholars believe that the multiple HPV infections are the risk factors for development of CIN [14, 15], while other scholars suggested that multiple HPV infection was not associated with the progression of cervical lesions [16, 17]. The present results do not support an association of multiple infection with increased severity of CIN. Another study concluded that in all studies of invasive carcinoma, the risk linked to multiple HPV types does not vary significantly from the risk linked to single HPV types [13].

Worldwide studies show that the predominant HPV genotypes of the female with normal cervical cytology were HPV-16, -31, -18 in Europe, HPV-16, -18,-33 in Asia, and HPV-16 in South America [18]. The present study found that the prevalence HPV genotype of the Uyghur women with normal cytology were HPV-16, -68, -31, -45, -52. It is somewhat different because of different geographical areas. A worldwide meta-analysis shows that the distribution of high-risk HPV genotype was different by different geographical areas, it's noteworthy that HPV-31, -52, and -58 genotypes were more prevalent in other areas except for Europe [15]. A study from India showed that the prevalence of high-risk HPV genotype in patients with CIN was 87.5%; the most common HPV genotypes in decreasing order were: HPV-16, -18, -33, -39, -35. and -56 [19]. Another study collected specimens from 17 cities of Europe and discovered that the prevalence of HPV genotypes in patients with CIN II or worse were HPV-16, -33, and -31 [10]. The present study found that the prevalence of HPV genotypes in Uyghur female patients with CIN were in decreasing order: HPV-16, -58, -31, -33, -18, -68, -45, -39, and -52. The common HPV genotypes in CIN II - III were also in decreasing order: HPV-16, -58, -31, -33,- 68, and -18, respectively. The present study also found that HPV-16 was the main high-risk genotype in Uyghur women with CIN in Xinjiang. As previously reported, HPV-16 was the most frequent type associated with high-grade cervical lesions and cervical cancer, compared to all other high-risk HPV types [20, 21]. These conclusions may indicate that there is a more rapid progression to high cervical lesion in HPV-16 infected women. Similar to this result, another study had suggested that HPV-16 associated with CIN II could easily lead to progression than CIN II infected with other genotypes [22]. The present data also assessed risks for progression from HPV infection to CIN II-III by different HPV genotypes. The authors found that estimated risks was highest in HPV-33 (PR 2.62), followed by HPV-31 (2.27), HPV-16 (1.92), HPV-58 (1.62), HPV-18 (1.51), HPV-68 (1.05), and HPV-39 (1.05), which suggested that HPV-33, -31, -16, -58, -18, -68, and -39 (PR > 1) might be the high-risk HPV in Uyghur women in Xinjiang. The present authors inferred that in addition to HPV16, 58, 33, 31, and18, HPV-68 and HPV-39 may play important role in Uyghur women with CIN in Xinjiang. However further study is necessary to confirm the real risk related to these

genotypes. These observations suggest that testing a specific oncogenic HPV types may be useful for confirming female at increased or decreased risk during the follow-up.

If we assume a 100% efficacy and coverage of an HPV 16/18 vaccine, the present results suggest that the vaccine could prevent at least 50.0% of all CIN II - III lesions, but the current vaccine does not include HPV-58,-33,-31 or -68, -39 which need to establish a new vaccine specific to Xinjiang areas.

In conclusion, the findings presented in this study provide description of HPV genotype distribution among Uyghur women diagnosed with CIN and normal cytology in Xinjiang, China. The study found the risk of progression to CIN II - III varied by different HPV genotype. Except for the common subtypes (16, 58, 33, 31, and 18) in Asia, HPV-68 and HPV-39 may be the oncogenic subtypes to Uyghur female with CIN in Xinjiang. Distinguishing these subtypes may have implications for future cervical screening strategies. The investigation could contribute to the design of multivalent prophylactic vaccines against predominant HPV types in preventing CIN in Xinjiang. Finally, multiple infection was detected in 9.1% of patients with CIN II - III; however the presence with one or more high-risk HPV was not association with an increased risk of high-grade cervical neoplasia.

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