Distribution of HPV genotypes in uterine cervical lesions among the Uighur women in Xinjiang province of China

A. Abudukadeer[1], Y. Ding[1], M. Niyazi[2], A. Ababaikeli[3], A. Abudula[4]

[1]Department of Gynecology of the First Affiliated Hospital, Medical University of Xinjiang;
[2]General Hospital of Xinjiang Uighur Autonomous Region; [3]Third Affiliated Cancer Hospital, Medical University of Xinjiang;
[4]Key Laboratory of Molecular Biology and Endemic Diseases of Xinjiang Uighur Autonomous Region, and Department of Biochemistry, Medical University of Xinjiang, Urumqi (P.R. China)

Summary

Objective: The aim of this study was to investigate the distribution of HPV genotypes in uterine cervical lesions of Uighur women in the Xinjiang province of China. *Methods:* A total of 223 formalin-fixed paraffin-embedded cervical tissue specimens from Uighur patients with squamous cell carcinoma (SCC) and cervical intraepithelial neoplasia (CIN) were analyzed with HPV specific general primer pairs MY09/11 by PCR amplification and HPV chip. *Results:* Among 223 cases, HPV-positive samples accounted for 58.7% (131/223). HPV infection rate increased along with the pathological grade of the specimens, with a clear tendency of normal < CIN 1 < CIN 2 < CIN 3 < SCC. HPV16 infection was the predominate one and reached the highest level in SCC with 96%. HPV18 and 58 were detected only in some specimens as a second infection in addition to HPV16. The infection rate and type of HPV was not closely associated with the histological differentiation of the cervical cancer. *Conclusion:* HPV16 was the most common type detected in Uighur women with SCC and CIN in the Xinjiang area of China. Together with the high infection rate, this may be the reason for the four-fold higher cervical cancer incidence in this province and in this population, when compared to total China. The prevalence of HPV18 and 58 was relatively low.

Key words: HPV-genotypes; Cervical cancer; CIN; Uighur population of Xinjiang.

Introduction

Cervical cancer continues to be one of the leading female genital cancers worldwide. About 80% of cases occur in developing countries [1-3]. In China, there is an annual incidence of about 46,000 cases, and cervical cancer presents a major health problem [4]. The Xinjiang province has one of the highest incidence (590/100,000) and mortality rate of cervical cancer in China, especially the south of Xinjiang. Thus the incidence of cervical cancer among the Uighur women is four times higher than the mean incidence of China (138/100,000) [5, 6]. Molecular epidemiologic evidence clearly indicates that certain types of HPV are the principal cause of invasive cervical cancer and cervical intraepithelial neoplasia (CIN). It is widely believed that persistent infection with high-risk human papillomavirus (HPV) represents the prime risk factor in cervical carcinogenesis [7, 8]. Of a total of approximately 40 mucosal HPV types [9], 15-18 types are currently considered 'high-risk' with variable oncogenic potential [10, 11]. Squamous cervical cancer (SCC) is strongly associated with HPV infections and the prevalence of HPV is about 95%, whereas HPV DNA cannot be identified in the same proportion of adenocarcinomas [12]. Since the distribution of HPV genotypes in various geographical areas and populations varies widely [13, 14], development of effective vaccines would require a comprehensive study of the HPV genotypes in different regions of the world.

With regard to these facts, the aim of the present study was to evaluate the frequency and distribution of high-risk HPV (HR-HPV) types in a series of paraffin-embedded tissues from SCC and CIN among the Uighur women in Xinjiang. This seems to us important for guiding the selection of vaccine candidates and studying the pathogenesis of cervical cancer.

Methods and Materials

Cervical tissue specimen

A total of 223 formalin-fixed paraffin-embedded (FFPE) cervical tissue specimens from Uighur patients who had been diagnosed or hospitalized at the Department of Gynecology of both the First Affiliated Hospital of Xinjiang Medical University and the Hospital of Xinjiang Uighur Autonomous Region between February 2006 and June 2007 were analyzed. Cervical tissue specimens were derived from punch biopsies, loop electrosurgical excisions, cone biopsies, and hysterectomies. The pathology slides were reviewed and original histological diagnoses of samples were confirmed by experienced pathologists. The diagnoses were as follows: non-neoplastic cervix, n = 38; CIN, n = 94 (CIN 1, n = 36; CIN 2, n = 28; CIN 3, n = 30); and squamous cell carcinoma (SCC), n = 91, of which 15 were well differentiated (grade 1), 49 were moderately differentiated (grade 2), and 27 were poorly differentiated (grade 3). Patient age ranged from 18 to 78 years, with a mean of 48 years.

DNA extraction

The paraffin-embedded tissue sections were deparaffinized with xylene, rehydrated by decreasing concentrations of ethanol and double distilled water (ddH_2O) followed by digestion with

Revised manuscript accepted for publication

100 mg/ml proteinase K. The genomic DNA was extracted by the standard phenol-chloroform (1:1) extraction and ethanol precipitation. Purified DNA was then quantified using a Gene Quant II spectrophotometer (GE) and stored at -20ºC until further use.

Screening and genotyping of HPVs in tissue specimens

To screen HPV-positive samples, the genomic DNA was analyzed with HPV specific general primer pairs MY09/11 (MY09: 5'-GTCCMARRGGAWACTGATC-3'; MY11: 5'-GCMCAGGGWCATAAYAATGG-3') by PCR amplification, followed by electrophoresis on 2% agarose gel labeled with ethidium bromide and ultraviolet visualization (Gel Doc XR, Biorad, Germany). The HPV genotyping of 23 common subtypes (HPV 6, 11, 16, 18, 31, 33, 35, 39, 42, 43, 45, 51, 52, 53, 56, 58, 44, 59, 66, 68, 73, 83 and MM4) was carried out using HPV chip kit (YANENG Biotech, PR China) according to the manufacturer's instruction.

Statistical analysis

Statistical analysis was performed by SPSS statistical software package version 11. The chi-square test was applied for comparing results of HPV and other parameters. Statistical significance was assumed at a value of < 0.01.

Results

HPV infection was highly associated with cervical cancer in Uighur women. Among 223 cases in this study, 58.7% (131/223) were positive for HPV, and the highest HPV positivity rate was observed in patients aged between 35 to 44 (data not shown). HPV infection increased along with the pathological grade of the specimens, with a clear tendency of normal < CIN 1 < CIN 2 < CIN 3 < SCC (Table 1).

Table 1. — *Incidence of positive HPV tests in different types of cervical lesions*.

Pathologic examination	HPV test	
	No.	Positive (%)
Normal	38	3 (7.9)
CIN 1	36	5 (14)
CIN 2	28	17 (61)
CIN 3	30	26 (87)
SCC	91	80 (88)

HPV typing of HPV-positive samples using HPV chips containing 23 common HPV types showed that the HPV16 infection predominated with an infection rate of 33% in normal individuals. The rate gradually increased with the pathological grade of CIN and reached the highest level in squamous cell cervical cancer with 96% (Table 2). Interestingly, we detected HPV 18 and 58 only in some specimens as a second infection in addition to HPV 16.

We further evaluated the association of histological differentiation (grade) of cervical cancer with the HPV infection. HPV 16 infection occurred in equally high rates in every grade of cancer. The infection with HPV 18 or 58 seems to be associated with cancer progression, as positivity rate of these high-risk HPVs increased gradually with cancer grade (Table 3). However this was not statistically significant.

Table 2. — *Distribution of HPV types in HPV-positive specimens [cases (%)].*

Groups	No.	HPV 16 (+)	HPV 18 (–)	HPV 58 (+)
Normal	3	1 (33)	0	0
CIN 1	5	2 (40)	1 (20)	0
CIN 2	17	14 (82)	2 (11)	2 (11)
CIN 3	26	25 (96)	5 (19)	3 (11)
CSCC	80	77 (96)	14 (17)	2 (2.5)

Table 3. — *Association of HPV infection with differentiation states of cervical cancer [cases (%)].*

Clinical stage	Cases	Positive cases of infection (%)			
		Total HPV	HPV 16	HPV 18	HPV 58
All SCC	91	80 (88)	77 (85)	14 (15)	2 (2)
Grade 1	15	13 (87)	12 (80)	1 (7)	0 (0)
Grade 2	49	43 (88)	42 (86)	5 (10)	1 (2)
Grade 3	27	24 (89)	23 (85)	8 (30)	1 (4)

Discussion

Cervical cancer is a potentially preventable disease; it is a common cancer and a serious threat to women's lives in developing countries. Squamous cell carcinoma of the cervix remains the most predominant phenotype. Persistent infection with high-risk HPV is the main etiological factor in the development of cervical cancer and may depend on HPV genotypes and variants. HPV 16 and HPV 18 are the most prevalent genotypes associated with cervical carcinomas globally [14, 15].

To evaluate the health impact of HPV infections and in order to design HPV vaccines, it is necessary to know the distribution of oncogenic HPV in cervical cancer and precancerous disease among the different geographical regions and different populations. In the present study, among 91 cervical cancer patients the overall HPV positive rate detected by MY09/11 PCR was 87.9% in SCC (80/91), which is close to the value obtained in a meta-analysis on HPV prevalence in 5,954 ICC cases in Asia (85.9%) [16], and higher than the rate reported in a meta-analysis for China only (83.7%) [17]. In this study it was shown that HPV 16 is the most common high-risk HPV detected in Uighur cancer patients in the Xinjiang region. HPV 16 was found in 84.6% (77/91) of all cervical cancer cases and 96.3% (77/80) of all HPV-positive SCCs. This is higher than the results of a meta-analysis comprising all China (58.7%) [17]. Detection rate of HPV 18 (15.4%) was similar to the results reported for Asia (14.9%) [16]. HPV 58 was found in 2.2% (2/91) of all cervical cancer cases.

The overall rate of HPV positivity in CIN cases was 51.1 % (48/94), whereby the rates for HPV 16, 18 and 58 were 43.6 % (41/94), 8.5 % (8/94), or 5.3 % (5/94), respectively. Results obtained from HPV high-risk typing

in SCC and CIN have shown that HPV 16 was the most frequent type also in the Uighur patients, which is inconsistent with the results of other authors who have described HPV16 as the main oncogenic type of HPV associated with cervical cancer in Japanese, Latvian and Chinese women [18-20]. In this study we identified HPV 18 followed by HPV 58 as the second and third prevalent types after HPV 16, HPV 18, and HPV 58 may be more important in glandular lesions and adenocarcinoma, but this will be analyzed in more detail in further studies. Other HPV types could not be detected in the present study.

HPV 16 infection may also play a central role in the pathogenesis and development of cervical squamous cancer and precancerous lesions in Uighur women and contribute to the high mortality from cervical cancer. Many reports have demonstrated that persistent HPV 16 infection could induce oncogenic potency and proposed to use it as a marker, in addition to morphology, for progression of cervical precancerous lesions [21-24]. However, the high-risk HPV types did not show any significant association with histological grading of squamous cervical cancer. Preliminary clinical trials in humans demonstrated that HPV vaccine can prevent HPV infection and precancerous lesions [25]. Recently, vaccines designed to protect against the worldwide most common HPV high-risk types HPV 16 and HPV 18 have become available [26]. As these two types of HPV are the most prominent in the Uighur population we assume that this vaccination could be of great benefit. Therefore, in screening programs HPV DNA testing and cervical cytology are very important to identify women at risk for cervical cancer. HPV DNA testing is a promising alternative or complementary test to improve the efficacy of cervical cytology, to reduce cervical cancer incidence and mortality. Prospective studies have shown that HPV DNA-positive women are significantly more likely to develop high-grade squamous intraepithelial lesions within ten years than women with a negative HPV DNA test [27, 28]. Fortunately, the transition to cancer usually takes years or decades, thus allowing the opportunity for detection by a cervical screening test.

Our study showed that women between the ages of 35 and 44 years had the highest number of HPV infections and CIN. Similar results have been reported in other studies [29, 30]. Education of women about HPV genital infections and the performance of Pap smear screening and HPV testing for all women 35 years of age and older is critical.

Conclusions

HPV 16 is the most common HPV type detected in Uighur patients while the prevalence of HPV 18 and 58 was relatively low, and all other types were absent. These results could explain the high cervical cancer incidence and mortality. They also could be the basis for the development of prevention measures against cervical cancer e.g., HPV vaccination. This study also shows the necessity of epidemical studies and the performance of further research on the molecular variants of HPV 16 in Uighur population.

Acknowledgment

The authors gratefully acknowledge the support of the "Xinjiang High Education Research Fund", XJEDU2007I25, and "Natural Science Foundation of China (NSFC)", 30860279 and 30801065.

References

[1] Franco E.L., Schlecht N.F., Saslow D.: "The epidemiology of cervical cancer". *Cancer J.,* 2003, *9,* 348.
[2] Parkin D.M., Bray F., Ferlay J., Pisani P.: "Estimating the world cancer burden: Globocan 2000". *Int. J. Cancer,* 2001, *94,* 153.
[3] Parkin D.M.: "Global cancer statistics in the year 2000". *Lancet Oncol.,* 2001, *2,* 533.
[4] Ferlay J.B.F.P.P., Parkin D.M.: "GLOBOCAN 2002: Cancer Incidence, Mortality and Prevalence Worldwide". IARC Cancer Base No. 5, version 2.0. IARC Press, 2004.
[5] Suzuke L., Peng Y., Zhou K.: "The analysis of pathogenetic tendency of cervical cancer in various ethnic groups in Xinjiang". *J. Xinjiang Med. University*, 2006, *29,* 569.
[6] Guzalinuer A., Peng Z., Guo Y.: "HPV and its subtypes in the Han nationality in Sichuan and the northwestern region of Xinjiang Uygur southern region of cervical tissue of women differentially expressed". *Chinese Journal of Microbiology and Immunology,* 2004, *24*, 402.
[7] Villa LL.: "Human papillomaviruses and cervical cancer". *Adv. Cancer Res.,* 1997, *71*, 321.
[8] Ho G.Y., Burk R.D., Klein S., Kadish A.S., Chang C.J., Palan P. *et al*.: "Persistent genital human papillomavirus infection as a risk factor for persistent cervical dysplasia". *J. Nat. Cancer Inst.,* 1995, *87,* 1365.
[9] Villiers E.M., Fauquet C., Broker T.R., Bernard H.U., zur Hausen H.: "Classification of papillomaviruses". *Virology,* 2004, *324,* 17.
[10] Munoz N., Bosch F.X., de Sanjose S., Herrero R.,Castellsague X., Shah K.V. *et al*.: "Epidemiologic classification of human papillomavirus types associated with cervical cancer". *N. Engl. J. Med.,* 2003, *348,* 518.
[11] Asato T., Maehama T., Nagai Y., Kanazawa K., Uezato H., Kariya K.: "A large case-control study of cervical cancer risk associated with human papillomavirus infection in Japan, by nucleotide sequencing-based genotyping". *J. Infect. Disease,* 2004, *189,* 1829.
[12] Andersson S., Rylander E., Larson B., Sigurdardottir S., Backlund I., Sallstrom J. *et al*.: "Types of human papillomavirus revealed in cervical adenocarcinomas after DNA sequencing". *Oncol. Rep.,* 2003, *10,* 175.
[13] Clifford G.M., Gallus S., Herrero R., Muñoz N., Snijders P.J., Vaccarella S. *et al*.: "Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis". *Lancet*, 2005, *366*, 991.
[14] Bosch F.X., Manos M.M., Muñoz N., Sherman M., Jansen A.M., Peto J. *et al*.: " Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group". *J. Natl. Cancer Inst.,* 1995, *87,* 796.
[15] Muñoz N., Bosch F.X., de Sanjose S. *et al*.: "Epidemiologic classification of human papillomavirus types associated with cervical cancer". *N. Engl. J. Med.,* 2003, *348,* 518.
[16] Bao Y.P., Li N., Smith JS., Qiao YL.: "Human papillomavirus type distribution in women from Asia: a meta-analysis". *Int. J. Gynecol. Cancer,* 2008, *18*, 71.
[17] Bao Y.P., Li N., Smith J.S., Qiao Y.L.: "Human papillomavirus type-distribution in the cervix of Chinese women: a meta-analysis". *Int. J. STD AIDS,* 2008, *19*, 106.
[18] Ma C.L., Li Y.J., Zhang F.C., Wang G.Q., Zheng Y.J., Kai L.M. *et al*.: "Detection of HPV16 E6 gene in cervical tissues by quantitative polymerase chain reaction". *Zhonghua Yi Xue Za Zhi.,* 2004, *84*, 469.

[19] Masumoto N., Fujii T., Ishikawa M., Mukai M., Ono A., Iwata T. *et al*.: "Dominant human papillomavirus 16 infection in cervical neoplasia in young Japanese women; study of 881 outpatients". *Gynecol. Oncol.,* 2004, *94*, 509.
[20] Silins I., Wang X., Tadesse A., Jansen KU., Schiller JT., Avall-Lundqvist E. *et al*.: "A population based study of cervical carcinoma and HPV infection in Latvia". *Gynecol. Oncol.,* 2004, *93,* 484.
[21] Kjaer S.K., van den Brule A.J., Paull G. *et al*.: "Type specific persistence of high risk human papillomavirus (HPV) as indicator of high grade cervical squamous intraepithelial lesions in young women: Population based prospective follow up study". *BMJ,* 2002, *325*, 572.
[22] Schiffman M., Herrero R., Desalle R., Hildesheim A., Wacholder S., Rodriguez A.C. *et al*.: "The carcinogenicity of human papillomavirus types reflects viral evolution". *Virology,* 2005, *337*, 76.
[23] Dalstein V., Riethmuller D., Pretet J.L., Le Bail Carval K., Sautiere J.L., Carbillet J.P. *et al*.: "Persistence and load of high-risk HPV are predictors for development of high-grade cervical lesions: A longitudinal French cohort study". *Int. J. Cancer*, 2003, *106,* 396.
[24] van Duin M., Snijders P.J., Schrijnemakers H.F., Voorhorst F.J., Rozendaal L., Nobbenhuis M.A. *et al*.: "Human papillomavirus 16 load in normal and abnormal cervical scrapes: An indicator of CIN II/III and viral clearance". *Int. J. Cancer,* 2002, *98*, 590.
[25] Koutsky L.A., Ault K.A., Wheeler C.M., Brown D.R., Barr E., Alvarez F.B. *et al*.: "A controlled trial of a human papillomavirus type 16 vaccine". *N. Engl. J. Med.,* 2002, *347*, 1645.
[26] Paavonen J., Jenkins D., Bosch F.X., Naud P., Salmerón J., Wheeler C.M. *et al*.: "Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine against infection with human papillomavirus types 16 and 18 in young women: An interim analysis of a phase III double-blind, randomised controlled trial". *Lancet,* 2007, *369,* 2161.
[27] Bulkmans N.W., Rozendaal L., Voorhorst F.J., Snijders P.J., Meijer C.J.: "Long-term protective effect of high risk human papillomavirus testing in population-based cervical screening". *Br. J. Cancer,* 2005, *92*, 1800.
[28] Khan M.J., Castle P.E., Lorincz A.T., Wacholder S., Sherman M., Scott D.R. *et al*.: "The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of typespecific HPV testing in clinical practice". *J. Natl. Cancer Inst.,* 2005, *97*, 10729.
[29] Liu J., Rose B., Huang X., Liao G.., Carter J., Wu X. Thompson C: "Comparative analysis of characteristics of women with cervical cancer in high-versus low-incidence regions". *Gynecol. Oncol.,* 2004, *94,* 803.
[30] Wall S.R., Scherf C.F., Morison L., Hart K.W., West B., Ekpo G. *et al*.: "Cervical human papillomavirus infection and squamous intraepithelial lesions in rural Gambia, West Africa: viral sequence analysis and epidemiology". *Br. J. Cancer,* 2005, *93,* 1068.

Address reprint requests to:
A. ABUDULA, Ph.D.
Key Laboratory of Molecular Biology
and Endemic Diseases of Xinjiang Uighur
Autonomous Region
and Department of Biochemistry
Medical University of Xinjiang
Xinyi Road 393
830011 Urumqi (P.R. China)
e-mail: abulizi_a@126.com