

Distribution and Prevalence of Human Papillomavirus (HPV) Genotypes in 2473 Cytological Samples Of Xinjiang Uyghur Women

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ABSTRACT Objective: To investigate the types distribution of human papillomavirus (HPV) in women in Xinjiang Uyghur women.

Methods: Women between 30 and 59 were chosen in cluster stratified sampling from XiaPuTaoLe Town, Xinjiang and received a population-based cervical cancer screening. After consent, every woman was detected and genotyped HPV DNA by PCR and Gene Chip. **Results:** 2473 women were chosen. The total infection rate of HPV was 9.1%. The positive rate of HPV-16 infection was 6.9% among the High-Risk HPV indicating it was the most common infection. Other High-Risk HPV infection rates were ranked from high to low: HPV-59, HPV-56, HPV-18, HPV-33, HPV-58, HPV-51, HPV-31, HPV-45, HPV-52, HPV-35, HPV-39, HPV-68. HPV-11 was the most common infection among the Low-Risk HPV types. Other Low-Risk HPV infection rates were ranked from high to low: HPV-42, HPV-43, HPV-6, HPV-53, HPV-66, HPV-73. HPV-44, -83, -MM4 were not detected. The multiple infection rate was 34.2%. **Conclusion:** HPV-16 was the most common types in the study population. The next most common types of HPV were HPV59, 56, 18, 33. Xinjiang Uyghur women had the relatively higher risk of suffering HPV59 infection indicating the specificity of HPV infection in Xinjiang Uyghur women.

Key words: Xinjiang; Human papillomavirus (HPV); Genotyping; Cervical cancer

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Introduction

Human papillomomaviruses (HPVs) constitute a group of more than 100 different genotypes associated with benign and malignant neoplasms of skin and mucous membranes. Approximately 40 different HPV genotypes have been detected in the anogenital mucosa. On the basis of their epidemiological association with the development of cervical cancer, a group of so-called high-risk HPV genotypes has been defined. These include HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -68, Other genotypes, such as HPV-6, -11, -42, -43, and -44, which associated benign lesions, are classified as low-risk types^[1]. Some research demonstrates that Human papillomavirus is a necessary cause of invasive cervical cancer worldwide^[2]. The study of HPV genotyping in invasive cervical cancers shows that the distribution and prevalence of HPV vary by geographic region and race^[3-5]. The prevalence rate of cervical cancer in Xinjiang Uyghur women approximately is 459~590/100thousand. The mortality was about 15.78/100thousand^[6]. Both of them were higher than average national level. However, limited data on HPV type distribution among Xinjiang Uyghur women are available to date. This study was to investigate distributions of Human Papillomavirus types in Xinjiang Uyghur women, in order to provide important molecular epidemic basis for the development of HPV vaccine.

1 Materials and method

1.1 Source of specimens

Women were recruited to participate in a cervical cancer-screening program in the town of Xiaputule, west Xinjiang province. The case group consisted of 2473 women which covered 75% women of the town (aged 30-59 years, without any operation on uterus). Women who agreed to participate then signed informed consent forms approved by Shihezi University School of Medicine. Cervical cells were obtained from all women of normal and abnormal cytology. The cervical cell scrapings were collected with a cytobrush from the ecto- and endocervix of the uterus of each woman by gynaecologists. The cell samples on the cytobrush were suspended in PreservCCT solution (CCT) and stored at 4℃ until DNA extraction.

1.2 Method

1.2.1 DNA extraction The cervical cells were suspended in 50 mM Tris-HCl (pH 8.0) with 10 mM EDTA containing 200 mg/ml proteinase K and incubated overnight at 37℃ for cell lysis. DNA was extracted from this lysis solution by the phenol-chloroform-isoamylalcohol method^[7]. To avoid contamination, we used disposable utensils and discarded them immediately after a single use. (Reagents, purchased from Sangon Biotech (Shanghai) Co. Ltd)

1.2.2 PCR General consensus primers GP5+/GP6+ (GP5+ 5'-TTTGT TACTG TGGTA GATAC TAC-3', GP6+ 5'-GAAAA ATAAA CTGTA AATCA TATTC3')^[8] were used to amplify the corresponding part of the HPV L1 gene. Presence of human ge-

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nomie DNA was verified by amplification of a 268 bp fragment of theβ-globin gene by using primers Glob-F (5'-CAACTTCATC-CACGTTCCACC-3') and Glob-R (5'-GAAGAGCCAAG-GACAGGTAC-3'). A reaction mixture without template DNA was included in every set of PCR runs as a negative control. Reaction system and conditions refer the reference [9]. Positive PCR products were used for HPV Genotyping. (Reagents, purchased from Tiangen Biotech (Beijing) Co. Ltd)

1.2.3 HPV Genotyping HPV genotypes were analyzed by commercial HPV DNA Chip (Yaneng Bioscience(Shenzhen) Co. Ltd) for detection and genotyping of 23 HPV types (HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -68, -73, -82, -6, -11, -42, -43, -44, -53, -66,-MM4), including all the 13 high-risk HPV genotypes. All the operations were conducted in strict accordance with the manufacturer's protocol. The results were also identified according to the manufacturer's instructions(Fig.1).

2 Results

2.1 Distribution of HPV subtypes

Of the 2473 samples analyzed, 9.7% (240) were HPV-positive by Gene Chip. 20 of the 23 HPV genotypes,which the Gene

Chip contains, were detected. HPV 16 was identified totally 152 among the 240 positive cases, was the most frequent viral type, with sequence as HPV-11 (52), HPV-59 (20),HPV-42 (20), HPV-56 (19),HPV-18 (15), HPV-43 (12), HPV-33 (11), HPV-58 (9), HPV-6 (7), HPV-31 (5), HPV-51 (5), HPV-45 (4), HPV-53 (4), HPV-66 (4), HPV-73 (4), HPV52 (3) , HPV-35 (2), HPV-39 (2), HPV-68 (2). All of the 13 high risk genotypes were detected. 7of the 10 low risk genotypes were detected. HPV 11 was the most frequent viral type, with sequence as HPV-42, 43, 6, 54, 66, 73. HPV-44、83、MM4 were not detected.(Table 1).

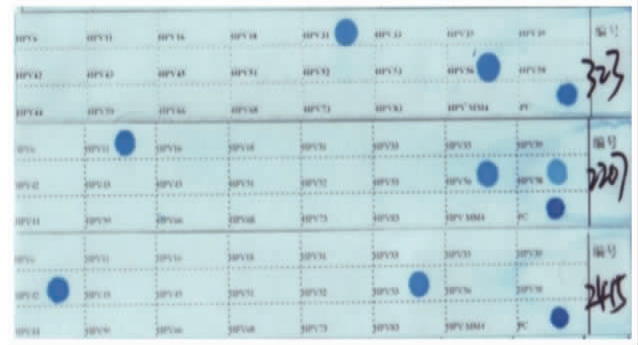


Fig.1 The results of the Gene Chip

Table 1 Distribution of HPV subtypes in 2473 samples

HPV Subtypes High-Risk	Total(%) Infection	HPV Subtypes Low-Risk	Total(%) Infection
HPV-16	152(63.3%)	HPV-11	52(21.7%)
HPV-59	20(8.3%)	HPV-42	20(8.3%)
HPV-56	19(7.9%)	HPV-43	12(5.0%)
HPV-18	15(6.3%)	HPV-6	7(2.9%)
HPV-33	11(4.6%)	HPV-54	4(1.7%)
HPV-58	9(3.8%)	HPV-66	4(1.7%)
HPV-31	5(2.1%)	HPV-73	4(1.7%)
HPV-51	5(2.1%)	HPV-44	0(0%)
HPV-45	4(1.7%)	HPV-83	0(0%)
HPV-52	3(1.3%)	HPV-MM4	0(0%)
HPV-35	2(0.8%)		
HPV-39	2(0.8%)		
HPV-68	2(0.8%)		

2.2 Multiple Infection

Multiple infections were identified 82 cases, accounts for 65.8%. Doule infection was predominated among the Multiple infections, accounts for 70.7%(58/82). The more types the samples infected, the lower cases were detected.(Table 2). Among the double infection, HPV16 combined with other types was dominant.

HPV16 combined HPV33 was the most frequent forms, (10/58). In the second place is HPV11, (6/58). Among the 20 cases triple infection, HPV16 combined with other types was also the most common forms. HPV16 combined HPV-43,59 was the most frequent forms, (5/20). 1 sample was detected infecting 7 HPV genotypes (HPV-11, 16, 42, 66, 53, 59, 68) simultaneously.

Table 2 The overall infection ratio between Single Infection and Multiple Infection

Infection type	Positive	Positive ratio
Single Infection	158	65.8%
Double Infection	58	24.2%
Triple Infection	20	8.3%
Four Infection	3	1.3%
Five Infection	0	0%
Six Infection	0	0%
Seven Infection	1	0.04%
Total	240	100%

3 Discussion

The research on molecular epidemiology has been indicated that persistent infection with high risk HPV is a necessary cause for invasive cervical cancer [10]. Over 100 HPV genotypes have been isolated so far. 13 of them (genotypes HPV-16,-18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -68) have been found to be associated with cervical cancer or high-grade cervical intraepithelial neoplasia and therefore classified as high-risk types. Studies on HPV type distribution among ICC cases worldwide have shown some geographical variation[3]. Because the immunity conferred by vaccines is type-specific, the need for HPV genotyping in routine screening population is increasingly recognized. Limited data on HPV type distribution among Xinjiang Uyghur women are available to date.

We made a cross-sectional survey to the women residing in the town of Xiaputule, west Xinjiang province, China. Totally 2473 women were enrolled to detect and genotype HPV DNA by PCR and Gene Chip. This study shows that HPV-16, 59,56 were the most frequent viral type. The percentage in high-risk cases was 63.3% , 8.3% , 7.9% respectively, with sequence as HPV-18 (6.3%), HPV-33 (4.6%), and HPV-58 (3.8%). A global study shows HPV-16, -31, -18 were the main types among the women with normal TCT (Thinprep cytologic test) diagnose in Europe, and HPV-16, -18, -33 in Asia, HPV-16, -58, -18 in South America [11]. The survey of HPV genotyping in cervical cancer in 14 province of China, conducted by Li Jie et al [12], has revealed that HPV-16, 58 were the most frequent viral type in cervical cancer. A cross-sectional survey led by Shi Jufang et al [13] in Shenzhen Nanshan District indicated that the prevalence of HPV 58 infection was similar to HPV 18, beside the highest infection rates of HPV 16. Those data suggested that HPV 58 may be one of the most important HPV type in China. This study shows that HPV -16, -59, -56 were the most important HPV type in the town of Xiaputule, west Xinjiang province, China. However, HPV18, HPV58 ranked the fourth place and sixth place respectively. The result of this study indicated that the women in the town of Xiaputule were vul-

nerable to infect HPV59. That embodied the special characteristics of HPV infection among the women in west Xinjiang province. However, Guzhalinuer ABLZ genotyped the HPV in 100 cervical cancer cases in west Xinjiang province [14]. The result shows that HPV -16, -18, -68 were the most important HPV type. There exist large differences with this study. A great disparity in sample size may attributed to the difference. A global research in 85 medical centers indicated that the average HPV16 infection rate was 51.0% (45.9%-62.6%)[15]. The data shows that HPV distribute with nationalistic differences [4,5]. The results of this study conform to this viewpoint.

Lee SH considered that multiple HPV infection has higher risk than single HPV infection in the oncogenesis of cervical cancer [16]. Tao PPlled a study shows that multiple HPV infection rate was 44.6% in cervical benign lesion, however 48.1% in cervical cancer cases, indicated that the higher multiple HPV infection rates, the higher malignant degree[17]. The result of this study shows that the multiple HPV infection rate is 34.2% in normal population, a seven HPV types infection involved. The data indicated that the risk of occurring and developing is higher among the women dwelled in this region than others. A data shows that the prevalence of cervical cancer in Xinjiang Uyghur women is 459~590 /100thousand, obviously higher than the women reside in the same region [6]. The result of this study was in accordance with the data. As for multiple infection, this study shows that double infection takes the lead. HPV16 combining other HPV types was the commonest form among most of the double infection. The result was also in accordance with other report.

In conclusion, this study considered that HPV -16, -59, -56 were the most important HPV type in Xinjiang Uyghur women, embodied the special characteristics of HPV infection among the women reside in this region. The data of the this study could provide theoretical basis for researching HPV vaccine suitable for the women. Meanwhile, multiple infection rate up to 34.2% also indicated that the Uyghur women reside in this region have higher risk than others. The prevention and therapeucy of cervical cancer should be enhanced.

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新疆维族妇女感染的 HPV 型别分布的研究 *

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摘要 目的:探讨人乳头瘤状病毒(Human papillomavirus, HPV)在新疆南部维族妇女人群中的型别分布情况。方法:以年龄在30-59岁的新疆伽师县夏普吐勒乡维吾尔族妇女人群为基础进行筛查,签署知情同意后,采集受试者宫颈脱落细胞,利用PCR和基因芯片技术检测 HPV DNA 并分型。结果:共2473名妇女入选。HPV总的感染率为9.1%,高危型中 HPV-16 的感染率最高为6.9%,其他高危型的感染率从高到低依次为:HPV-59、HPV-56、HPV-18、HPV-33、HPV-58、HPV-51、HPV-31、HPV-45、HPV-52、HPV-68、HPV-35、HPV-39。低危型中 HPV11 感染率最高,其他低危型的感染率从高到低依次为 HPV-42、HPV-43、HPV-6、HPV-53、HPV-66、HPV-73。HPV-44、-83、-MM4 没检测到。多重感染率为34.2%。结论:新疆维吾尔族妇女人群中以 HPV16 感染为主,其次为 HPV59、56、18、33 等。HPV59 可能是新疆维吾尔族妇女较易感染的类型。体现了新疆维吾尔族妇女感染 HPV 的特殊性。

关键词 新疆;人乳头瘤状病毒(HPV)基因分型;子宫颈癌

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