High-risk Human Papillomavirus Infection in Putian, China: A Cross-sectional Analysis of 98085 Women

Rujing Zhang[1,2], Guihua Hong[1,2], Changxi Liao[1], Zhonghui Chen[1,2], Hua Lin[1,2] and Liumin Yu[1,2]

[1]*The Affiliated Hospital of Putian University, Fujian, China*
[2]*Key Laboratory of Medical Microecology (Putian University), Fujian Province University, Putian University, Fujian, China*

ABSTRACT

Objective: To assess high-risk human papillomavirus (hrHPV) infection among women undergoing cervical cancer screening in Putian for establishing an optimal cervical cancer screening mode and preventive vaccination strategy for HPV.

Study Design: Cross-sectional study.

Place and Duration of the Study: The Affiliated Hospital of Putian University for cervical cancer screening period, from August 2020 to December 2022.

Methodology: Cervical cell specimens were obtained using 'two cancer screening platforms'. qRT–PCR and flow-FISH were used for hrHPV typing. The pathological diagnostic test was performed for the hrHPV-positive samples. The results concerning the relationships between hrHPV infection at different age groups and pathological diagnosis were analysed retrospectively.

Results: A total of 98085 hrHPV preliminary screening results in the Putian region and 9036 hrHPV-positive samples were included. The infection rate of hrHPV for the three infection modes increased with age. The 41–50 age group is the highest incidence which the phase from cervical intraepithelial neoplasia to cervical cancer. The top three hrHPV subtypes were HPV52, HPV58, and HPV16. The positive rate of HPV16 was positively correlated with the progression of cervical intraepithelial neoplasia.

Conclusion: Effective screening, vaccination, and education must be provided because HPV infections are district-specific and age-specific. HPV16 is correlated with cervical cancer progression. Pathological diagnosis and prevention of cervical cancer infected with HPV16 must be conducted.

Key Words: *hrHPV, Cervical cancer, Pathological diagnosis.*

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INTRODUCTION

Cervical cancer is a common malignant tumour among women. There were approximately 110,000 new cases of cervical cancer in China, accounting for 18.2% of the new cases of cervical cancer worldwide in 2020.[1] The incidence of cervical cancer in the country has increased in recent years, and it has mostly affected young women between the ages of 15 and 44.[2] Human papillomavirus (HPV) infection is considered the main cause of cervical cancer. HPV can be divided into low-risk and high-risk categories. The high-risk subtypes include HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, and HPV59.[3]

Correspondence to: Dr. Changxi Liao, The Affiliated Hospital of Putian University, Fujian, China
E-mail: changxiliao@163.com

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Persistent high-risk human papillomavirus (hrHPV) promotes cervical cancer progression.[2] The current cervical cancer vaccine cannot meet the needs of people because of regional differences and their distribution with respect to the many HPV subtypes.[4] Therefore, obtaining baseline data on early diagnosis and treatment of high-risk populations in specific regions can optimise cervical cancer screening programs and evaluate the effectiveness of vaccination. However, only a few studies on the distribution of hrHPV cases in the Putian region have been published. The aim of this study was to assess hrHPV infection among women undergoing cervical cancer screening in Putian for establishing an optimal cervical cancer screening mode and preventive vaccination strategy for HPV.

METHODOLOGY

All data were collected from the free cervical cancer screening project conducted in Putian City from August 2020 to December 2022 in accordance with China's Direct Reporting System for Information on Major Public Health Services for Women and Children. The exclusion criteria of the current study were age <35 years or >64 years; no medical insurance for urban and rural residents in Putian city; tumour and other

systemic diseases; vaginal medication three days before sampling; menstrual period, pregnancy, or lactation; and a history of uterine and cervical surgery. All subjects voluntarily received cervical cancer screening and signed informed consent forms. This study was reviewed by the Ethics Committee of the Affiliated Hospital of Putian University (Approval No. 202037).

The project was conducted in strict accordance with the cervical cancer screening program under the Chinese government's public health plan. The hrHPV-DNA typing of the specimens was completed via multiplex PCR amplification and flow fluorescence hybridization. The ThinPrep cytological test (TCT) was performed on hrHPV samples except for HPV16 and HPV18 and then classified according to TBS (Bethesda System). Colposcopy and subsequent histopathological examination were performed in patients with HPV16 and HPV18 infection or on atypical squamous cells of undetermined significance. The pathological results were characterised as (a) a normal cervix; (b) inflammation and other benign lesions; (c) a low-grade squamous intraepithelial lesion, which describes those samples with cervical intraepithelial neoplasia grade (CIN) I; (d) a high grade squamous intraepithelial lesion (HSIL), which describes those samples with CIN II or CIN III; and (e) adenocarcinoma in situ, micro-invasive carcinoma (squamous cell carcinoma/adenocarcinoma), and invasive carcinoma (squamous cell carcinoma/adenocarcinoma), which describes those samples with cervical cancer.

SPSS 22.0 was used for data analysis. Origin 2021 was used for image output. As the subtypes of HPV, multiple infections were counted by each subtype, the total frequency of HPV subtype infection was greater than the number of positive infections. The association between age and infection categories was determined by initially classifying the ages into four groups, namely, 35–40, 41–50, 51–60, and 61–64 year age groups. Then, hrHPV infections were classified into three types, namely, single, double, triple or more infections. The χ^2 test was used to compare the difference in the positive rate of hrHPV infection at all levels in different age groups and the difference in the positive rate of hrHPV subtypes in different pathological outcomes. The difference was statistically significant when $p < 0.05$.

RESULTS

A total of 98085 participants were examined, with an average age of 49.7±7.8 year. Among them, 9036 (9.2%) tested positive for hrHPV (Supplement Table I). The results indicate a significant increase in hrHPV infection rate as age progressed in all infection categories ($p < 0.001$) and a significant decrease in having more infection subtypes in the same age group ($p < 0.001$, Figure1).

The normal group showed the highest incidence in the 61-64 year age group (27.3%), whereas the lowest prevalence was in the 51-60 year age group (23.7%) (Figure 2, Supplement Table II). The incidences of CIN1 and CIN2–CIN3 were highest in the 41-50 year age group (6.6% and 4.7%), while that of cervical cancer development was highest in the 51-60 year age group (0.5%, Figure 2, Supplement Table II). Therefore, hrHPV patients older than 40 years old have a higher risk of precancerous lesions. If not treated in a timely manner, then the incidence of cervical cancer may increase in the later stage.

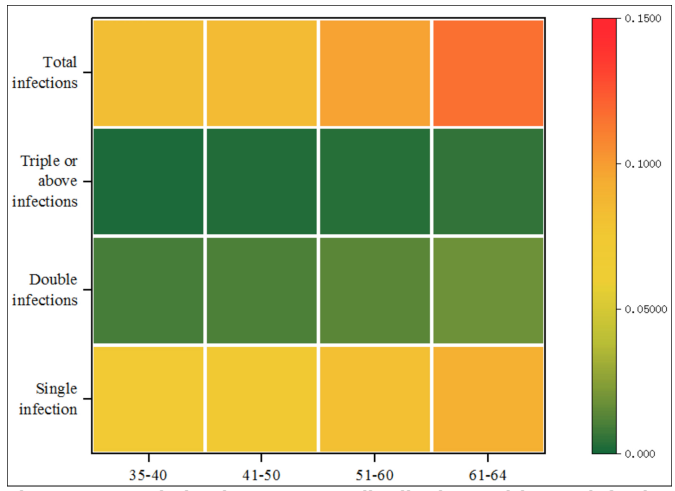

Figure 1: Association between age distribution and hrHPV infection patterns. The colour indicates the prevalence of the corresponding group.

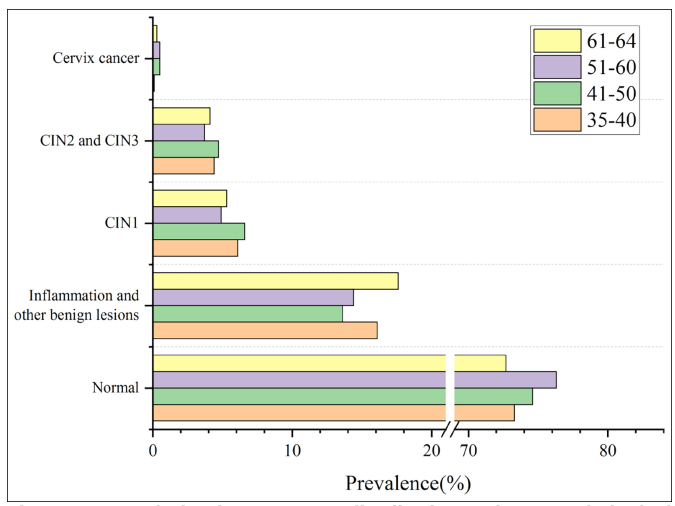

Figure 2: Association between age distribution and HPV pathological diagnoses.

The positive rates of 13 hrHPV subtypes in the screened population ranged from 0.1% (HPV45) to 3.4% (HPV52). The top five hrHPV subtypes were HPV52 (3.4%), HPV58 (1.6%), HPV16 (1.2%), HPV39 (0.8%), and HPV18 (0.7%). These trends depict the distribution characteristics of hrHPV in Putian. The analyses of different pathological outcomes of hrHPV subtypes also showed increasing infection rates of HPV16, HPV18, and HPV33 with the severity of cervical lesions ($\chi^2_{trend} = 1576.38$, $p < 0.001$; $\chi^2_{trend} = 414.10$, $p < 0.001$; $\chi^2_{trend} = 5.664$, $p = 0.017$, Table I. The distribution of different HPV types in pathology was clearly observable in the chord graph. Infections of HPV16 and HPV18 are highly correlated with HPV lesions. Furthermore, although the number of people infected with HPV52 is large, the pathogenic rate is relatively low, as shown in Figure 3A. Figure 3B shows the role HPV16 plays in the occurrence and development of cervical cancer.

Table I: Distribution of 13 hrHPV infection subtypes in the pathological results.

hrHPV subtype	Total [n(%)]	The proportion of hrHPV infection subtypes in pathological diagnosis [n(%)]						
		Normal (N=6769)	inflammation and other benign lesions (N=1332)	CIN1 (N=515)	CIN2 and CIN3 (N=380)	Cervix cancer (N=40)	χ^2 (p)	χ^2_{trend} (p)
HPV16	1128(1.2)	271(4.0)	475(35.6)	174(33.8)	184(48.4)	24(60.0)	**1846.29(0.000)**	**1576.38(<0.001)**
HPV18	665(0.7)	187(2.8)	332(24.9)	98(19.0)	40(10.5)	8(20.0)	**930.44(0.000)**	**414.10 (<0.001)**
HPV31	280(0.3)	210(3.1)	33(2.5)	15(2.9)	22(5.8)	0(0.0)	12.21(0.016)	1.345(0.246)
HPV33	582(0.6)	442(6.5)	49(3.7)	45(8.7)	42(11.1)	4(10.0)	**35.72(0.000)**	**5.664 (0.017)**
HPV35	164(0.2)	121(1.8)	21(1.6)	15(2.9)	7(1.8)	0(0.0)	4.676(0.322)	0.288(0.591)
HPV39	747(0.8)	695(10.3)	19(1.4)	21(4.1)	12(3.2)	0(0.0)	**146.51(0.000)**	**93.51 (<0.001)**
HPV45	108(0.1)	88(1.3)	13(1.0)	4(0.8)	2(0.5)	1(2.5)	3.952(0.413)	2.313(0.128)
HPV51	641(0.7)	532(7.9)	70(5.3)	31(6.0)	7(1.8)	1(2.5)	**30.94(0.000)**	**26.85 (<0.001)**
HPV52	3304(3.4)	2757(40.7)	308(23.1)	148(28.7)	88(23.2)	3(7.5)	**212.00(0.000)**	**152.19 (<0.001)**
HPV56	444(0.5)	374(5.5)	46(3.5)	18(3.5)	5(1.3)	1(2.5)	**24.74(0.000)**	**22.36 (<0.001)**
HPV58	1553(1.6)	1233(18.2)	177(13.3)	69(13.4)	73(19.2)	1(2.5)	**31.61(0.000)**	**9.830 (0.002)**
HPV59	330(0.3)	275(4.1)	37(2.8)	12(2.3)	5(1.3)	1(2.5)	**14.74(0.005)**	**13.751 (<0.001)**
HPV68	449(0.5)	375(5.5)	51(3.8)	15(2.9)	7(1.8)	1(2.5)	**21.34(0.000)**	**20.48 (<0.001)**

Note: Non-precancerous lesion group includes normal cervix, inflammation and other benign lesions. The numbers in bold are significant differences.

Table II: Distribution of the number of hrHPV infections among hrHPV-infected populations.

hrHPV infection mode	Total [n(%)]	The proportion of hrHPV infection patterns in pathological diagnosis [n(%)]				
		Normal (N=6769)	Inflammation and other benign lesions (N=1332)	CIN1 (N=515)	CIN2 and CIN3 (N=380)	Cervix cancer (N=40)
Single infection	7654(7.8)	5924(87.5)	1030(77.3)	386(75.0)	280(73.7)	34(85.0)
Double infections	1152(1.2)	719(10.6)	240(18.0)	102(19.8)	85(22.4)	6(15.0)
Triple or above infections	230(0.2)	126(1.9)	62(4.7)	27(5.2)	15(3.9)	0(0.0)

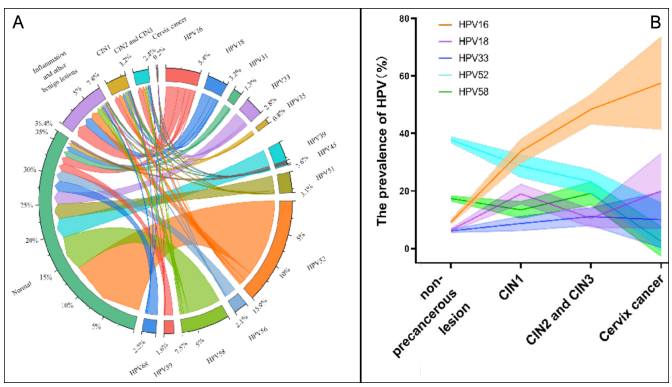

Figure 3: Relation of hrHPV subtypes in different pathological diagnoses. (A) Chord diagram of correlations of HPV type with pathological diagnoses. (B) Prevalence of HPV subtypes in the development of cervical cancer. Shaded areas represent 95% CIs. Non-precancerous lesion group includes normal cervix, inflammation and other benign lesions.

Certain HPV subtypes play a positive role in the development of cervical cancer. However, whether multiple infections also promote the development of cervical cancer is largely unknown. The results of this study showed that single hrHPV infection dominates the pathological results at all levels. The single-infection rate was the highest in the normal cervix group (87.5%), followed by the cervical cancer group (85.0%). In the pathological diagnosis of dual infection, the CIN2–CIN3 group (22.4%) and the CIN1 group (19.8%) accounted for relatively high proportions. However, the probabilities of CIN1 (5.2%) and inflammation and other benign lesions (4.7%) were higher in patients with triple infection or more subtypes (Table II). These data did not show any association between the frequency of hrHPV infection and the severity of cervical lesions.

DISCUSSION

Cervical cancer is a common malignant tumour. Previous HPV infection data on 1.7 million women showed that the overall HPV infection rate in Chinese women was 15.5%, and the hrHPV infection rate was 11.9%.[5] The data analysed in this study showed that the total infection rate of female hrHPV was 9.2%, a value higher than that of Hunan province (8.5%), Xinjiang province (7.4%), and Italia (6.8%), but lower than that of Ningxia province (9.9%), Yunnan province (12.7%) and Malaysia (10.7%).[6-10] This scenario signifies obvious differences in hrHPV infection in terms of regional distribution.

The significant differences in the age profile of global HPV infection rates are attributable to a number of factors, including regional sexual habits, vaccination, and cervical cancer screening programs.[11] In this study, both single and multiple hrHPV infections increased with age in screened women aged 35-64 years. Combined with pathological diagnosis, this study found that the 61-64 year age group had the highest inflammatory and other benign outcomes compared with other age groups. The results are likely related to lower hormone levels in older women, which affect their local immune resistance in the reproductive tract, increase their susceptibility to HPV, and weaken their self--clearing ability. Interestingly, the CIN1–CIN3 incidences were the highest in the 41-50 years age group, while cervical cancer incidences were the highest in the 51-60 year age group, which comprised elderly individuals. The finding approximates the time difference of approximately 10 years from the natural development of precancerous lesions to cervical cancer; furthermore, the same result is consistent

with the findings of Liu *et al*.[12] Therefore, more attention should be given to cervical cancer screening in local elderly women, and age should be included in the risk-based screening shunt strategy.[13]

Currently, the cervical cancer screening program of China adopts the protocol of immediate referral colposcopy for HPV16 and HPV18 and cytological shunt for other high-risk subtypes, which applies to the 2019 guidelines of the American Society for Endoscopic and Cervical Pathology.[14] The evidence-based medical data of this program mainly originate from European and American populations. However, the latest data from ICO/IARC have shown a significantly higher cervical cancer detection rate for HPV58, HPV52, and HPV33 subtypes in China and even the rest of Asia compared with the global level.[15] This study also found that the HPV52 and HPV58 subtypes in Putian, which ranked as the top two hrHPV infections, exceeded the HPV16 and HPV18 subtypes. Nonetheless, while the incidence of HPV52 and HPV58 subtypes decreased with the progression of cervical lesions, their proportions in the local area were extremely high, and they were the most common genotypes found in CIN1–CIN3 following HPV16 and HPV18, with HPV33 ranking fifth. From the seventh hrHPV subtype in the local ranking, HPV33 ranked third in the incidence of cervical cancer, following the HPV16 and HPV18 subtypes. The trends signify the obvious regional differences in HPV infection and potential risk differences. The results of this study can be used for clinical grading and shunt management of local patients.

On the basis of the absolute advantage of using the single hrHPV infection model in the hrHPV-positive population in the region, each pathological result group also seemed dominated by a single hrHPV infection. Some scholars believe that only one HPV is preferentially expressed in invasive cervical cancer infected with multiple HPV genotypes.[16] Meanwhile, other scholars have found that multiple infections with HPV16 can reduce the pathogenicity of HPV16 patients,[17,18] which may be related to much higher clearance rates.[19] Accordingly, the pathological results of different infection frequencies were analysed in this study. Without considering the subtype of infection, multiple hrHPV infections appeared more likely to lead to the development of precancerous lesions; the incidence of cervical cancer in single-infection patients was listed second, following the normal group. This scenario indicates the noncorrelation of cervical lesion occurrence and progression with the number of hrHPV subtype infections; rather, it is more likely related to the type of hrHPV infection, the persistence of infection ability, and subtype interaction.

In this study, certain limitations were encountered. First, local cervical cancer screening programs did not require low-risk HPV detection. Thus, the local HPV infection data were not fully considered in this study. Second, the age limit for the mass screening of women was set to 35–64 years old, which is not conducive to the comprehensive epidemiological study of HPV in the local female population. Third, the study data originated from cross-sectional screening results in the past three years, but dynamic monitoring of the hrHPV-infected population was not implemented. Thus, relevant information about persistent infection and the clearance ability of specific subtypes could not be obtained. A follow-up study can help to extend the time of case monitoring while expanding the scope of screening. Understanding the local HPV infection status is of great significance for the accurate and efficient prevention and control of cervical cancer. The research results recommend by a focus on the education of cervical cancer screening for elderly women, strengthening the health management of other hrHPV subtypes (HPV33, HPV52, and HPV58 in addition to the HPV16 and HPV18 subtypes), and adopting necessary monitoring or diversion strategies to focus on quantification and refinement based on the occurrence of HSIL and their risk value. In addition, health departments need to increase the publicity of local hrHPV infection characteristics and recommend that Chinese women use nine-valent HPV vaccines with HPV33, HPV52, and HPV58 subtypes as soon as possible to reduce the risk of cervical cancer.

CONCLUSION

Regional hrHPV screening can provide data support for exploring China's preferred cervical cancer screening model and vaccination strategy. Age gradients and regional differences in hrHPV infection were evident in past results, and these differences may affect future risks of cervical cancer and precancerous lesions.

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ETHICAL APPROVAL:
This study was approved by the Affiliated Hospital of Putian University ethics committee. (Approval Number: 202037).

PATIENTS' CONSENT:
Written informed consent were obtained from all patients.

COMPETING INTEREST:
The authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTION
RZ, CL: Research design and manuscript writing.
ZC, HL: Data collection.
GH, LY: Statistical analysis and manuscript writing.
All the authors have approved the final version of the manuscript to be published.

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