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Age-related distribution of human papillomavirus genotypes in women with cervical squamous cell carcinoma from Linyi, China, 2015–2023

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Abstract

Background Understanding the regional HPV genotype profile is critical for informing targeted vaccination strategies and optimizing cervical cancer screening programs to enhance their effectiveness. This study investigated the prevalence and distribution of human papillomavirus (HPV) genotypes among women with cervical squamous cell carcinoma (CSCC) in Linyi city, China, from 2015 to 2023.

Methods Data were obtained from 606 women histologically diagnosed with CSCC at Linyi Cancer Hospital between January 2015 and December 2023. DNA was extracted from paraffin-embedded tissue samples. HPV genotyping was performed via gene chip-based polymerase chain reaction (PCR) technology. Temporal trends and age-specific variations in HPV genotype distribution were analyzed to provide a comprehensive epidemiological assessment.

Results The overall prevalence of HPV infection was 94.7% among 606 women with CSCC. HPV 16 was the most prevalent genotype (80.5%), followed by HPV 18 (5.2%), HPV 33 (2.8%), HPV 31 (1.8%), and HPV 58 (1.8%). Single infections were predominant (95.5%), while coinfections were observed in 4.5% of the cases. Age-specific analysis revealed that non-HPV 16 infections were more prevalent in women aged > 45 years, with greater genotype diversity in older age groups. Temporal trends indicated a decline in the prevalence of younger CSCC patients (26–45 years), whereas the prevalence of older women significantly increased.

Conclusion Our study revealed that HPV genotype diversity in CSCC patients varies with age, highlighting the need for age-stratified and personalized cervical cancer prevention strategies. Enhanced screening efforts for older women are essential because of the greater genotypes diversity in this group. Additionally, the observed trends in HPV prevalence over time suggest that HPV vaccination has effectively reduced the incidence of CSCC in women under 45 years of age. These findings emphasize the importance of expanding vaccination coverage and optimizing screening programs to further reduce the cervical cancer burden across different age groups.

Keywords Human papillomavirus, HPV genotypes, Cervical squamous cell carcinoma, Prevalence, Age, China

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Background

Cervical cancer ranks as the fourth most common malignancy among women worldwide [1, 2]. The disease is primarily categorized into two major histological subtypes: cervical adenocarcinoma and cervical squamous cell carcinoma (CSCC), with CSCC accounting for approximately 60–70% of all cases [3]. Persistent infection with human papillomavirus (HPV), the most prevalent sexually transmitted virus globally, is recognized as the principal factor for cervical cancer development [4, 5]. HPV is a non-enveloped, circular double-stranded DNA virus belonging to the *Papillomaviridae* family [6]. The oncogenic potential of specific HPV genotypes has been well established, leading to their classification into high-risk and low-risk groups on the basis of carcinogenicity. Low-risk HPV (LR-HPV) types, including HPV types 6, 11, 42, 43, and 44, are typically associated with benign lesions, whereas high-risk HPV (HR-HPV) types, such as HPV types 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70, are strongly associated with cervical cancer and precancerous lesions [7].

HPV infection is a well-established etiological factor in cervical cancer, and HPV vaccination is globally recognized as the most effective preventive measure against HPV infection [8, 9]. In developed countries, the widespread implementation of government-funded HPV vaccination programs and cervical cancer screening has led to a steady decline in the incidence and mortality rates of cervical cancer [10]. However, in China — the world's largest and most populous low- and middle-income country — the burden of cervical cancer remains substantial. According to 2020 statistics, the proportion of women aged 9–45 years who have completed HPV vaccination in China is only 3% [11], and in the 2020 Global Burden of Cancer Study, China has the second highest cervical cancer incidence and mortality rate in the world, with approximately 110,000 new cases and 59,000 deaths [12]. As of 2022, the cumulative HPV vaccination coverage rate in Shandong Province was approximately 7.4%, markedly lower than in cities, such as Beijing and Shanghai, highlighting a significant regional disparity that may influence HPV-related disease burden. Although the Chinese government has recently launched free HPV vaccination programs for adolescent girls, overall vaccination coverage remains low and highly heterogeneous across different regions. This disparity is attributed to various factors, including economic constraints, limited healthcare resources, and differences in public awareness and vaccine acceptance. Consequently, the incidence and mortality rates of cervical cancer in China continue to rise, particularly among young women [3, 14].

Numerous studies, including global and regional surveillance efforts, have demonstrated that the distribution patterns of HPV genotypes vary significantly across countries and regions. For instance, studies, including those conducted by Luo et al. [15] and Guan et al. [16] have provided comprehensive global data showing regional variations in the prevalence of high-risk HPV genotypes. In China, recent multicenter surveillance studies, such as those conducted by Zheng et al. [17] and Chen et al. [18], have begun to map HPV genotype distribution in southern and central regions. However, data on the distribution of HPV genotypes across different regions of China remain relatively limited, particularly in northern provinces. Surveillance studies investigating the distribution patterns of HPV genotypes and cervical lesions in specific regions and age groups are essential not only for providing comprehensive insights into the regional epidemiological characteristics of HPV infection but also for informing the development of region-specific cervical cancer screening programs, optimizing vaccination strategies, and improving public health interventions [15–18].

Linyi, the most populous city in Shandong Province, had a resident population of 10.99 million by the end of 2022. Shandong, located in northern China, is the second most populous province and ranks third in national gross domestic product (GDP). Given its large population size and geographic location within a key economic region, Linyi can serve as a meaningful site for studying regional patterns of HPV genotype distribution. Findings from studies conducted in Linyi may offer important insights for improving HPV infection screening and cervical cancer prevention strategies in northern China.

In this retrospective clinical study, we analyzed the distribution of HPV genotypes among women with CSCC in Linyi, China, over a nine-year period. By assessing genotype-specific and age-specific prevalence patterns, we aimed to establish a regional baseline for HPV-related disease burden. Our findings provide valuable epidemiological insights that may guide cervical cancer prevention efforts, optimize HPV vaccination strategies, and improve region-specific screening programs. Additionally, this study contributes to the currently limited body of evidence on HPV genotype distribution in northern China, helping to address a critical gap in cervical cancer epidemiology. By providing region-specific data, the findings support more informed public health decision-making and the development of targeted cervical cancer screening and vaccination strategies in underrepresented areas.

Methods

General materials

All data in this study were collected from clinical records between January 2015 and December 2023 in the Department of Pathology, Linyi Cancer Hospital, Linyi, Shandong Province, China. Although this study is based

on a single center, all eligible cases were consecutively enrolled during the study period. Linyi Cancer Hospital is the leading oncology-specialized institution in the region and serves as the primary referral center for cervical cancer patients, including those diagnosed at other medical facilities throughout Linyi. This broad referral base ensures comprehensive and representative case collection.

The inclusion criteria were: (1) female patients aged 18 years or older; (2) histopathologically confirmed diagnosis of CSCC based on the WHO 2020 classification; (3) availability of sufficient formalin-fixed paraffin-embedded (FFPE) tissue for HPV DNA extraction and genotyping; and (4) complete clinical records and follow-up data.

The exclusion criteria included: (1) patients diagnosed with cervical adenocarcinoma or other histological subtypes of cervical cancer; (2) prior history of cervical cancer treatment, including chemotherapy, radiotherapy, or surgery, before tissue sampling; (3) inadequate or poor-quality tissue samples unsuitable for DNA extraction; and (4) coexisting malignancies or systemic diseases that may influence HPV infection or immune response.

Histopathological slides from all 606 CSCC patients were independently reviewed and confirmed by at least two attending pathologists. For cases diagnosed prior to the publication of the fifth edition of the World Health Organization (WHO) classification of female genital tract tumors in 2020, the original slides were retrospectively re-evaluated to ensure consistent application of the updated diagnostic criteria.

As this was a retrospective observational study, all eligible patients with histologically confirmed CSCC diagnosed at Linyi Cancer Hospital between January 2015 and December 2023 were consecutively included. Therefore, no formal sample size calculation was applicable.

DNA extraction from paraffin-embedded tissues

The FFPE tissue samples used in this study were obtained from cervical cancer resection specimens rather than biopsy materials. Each FFPE block represented a single tumor lesion that had been histopathologically confirmed as CSCC. Sections were taken from tumor-rich areas as identified on corresponding hematoxylin and eosin (H&E) slides to ensure representative sampling. Given that a single HPV genotype is typically associated with one lesion, care was taken to avoid sampling areas that might contain more than one discrete lesion within a block. No FFPE blocks included in the study were known to contain multifocal lesions prior to DNA extraction.

Paraffin-embedded tissues were sectioned into 5 μm thick slices, with 4–6 sections collected per sample. The tissue sections were carefully transferred into sterile centrifuge tubes using dedicated tweezers. To prevent cross-contamination between samples, instruments (blades and tweezers) were first wiped with 75% ethanol to remove paraffin and organic debris, followed by thorough decontamination with DNA AWAY™ (Thermo Fisher Scientific) to degrade any residual nucleic acids. Instruments were then allowed to air dry before reuse. Deparaffinization was performed using xylene and ethanol washes, following standard protocols for formalin-fixed paraffin-embedded (FFPE) tissues. Specifically, tissue sections were incubated in xylene for dewaxing, followed by rehydration through a graded ethanol series and brief drying. A total of 150 μL of lysis buffer from the FFPE DNA Extraction Kit (Xiamen Aide Biomedical Technology Co., Ltd.) was added to each sample, followed by thorough mixing. The mixture was centrifuged at 13,000 rpm for 10 min, and the middle layer of the DNA mixture was carefully collected. Genomic DNA was then extracted according to the manufacturer's instructions, including enzymatic digestion, lysis, and removal of contaminants. The purity and concentration of the extracted DNA were measured using spectrophotometry, and DNA samples were stored at − 20 °C until further analysis.

HPV genotyping test

HPV genotyping was performed using the HPV Genotyping Detection Kit (23 Types), catalog number YN-PDH-023, manufactured by Shenzhen Yaneng Biotechnology Co., Ltd. This assay employs reverse dot blot hybridization technology, which is particularly well-suited for the analysis of fragmented DNA commonly obtained from FFPE samples. The assay detects 23 HPV genotypes, including high-risk types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68) and low-risk types (HPV 6, 11, 42, 43, 73, 81, 82, and 83). Target DNA fragments were amplified via PCR using type-specific primers, with amplicon sizes ranging from 120 to 220 base pairs to ensure efficient amplification from degraded FFPE-derived DNA templates. Each reaction included an internal amplification control targeting the human β-globin gene to verify DNA integrity and detect potential PCR inhibition.

All procedures were carried out strictly according to the manufacturer's instructions. Positive and negative controls were included in each batch to ensure assay validity. Genotyping was conducted in triplicate to enhance reliability and minimize the risk of missing low-abundance HPV types. According to the manufacturer's validation report and previous studies utilizing this platform, the assay demonstrated high diagnostic performance, with an analytical sensitivity of 95.3% and specificity of 98.1%, a positive predictive value (PPV) of 96.7%, and a negative predictive value (NPV) of 97.5% for high-risk HPV genotypes in FFPE cervical cancer tissues. These characteristics make the assay a robust and clinically reliable method for HPV genotyping in CSCC

samples. In cases where discordant results were observed among the triplicate PCR tests, the majority result (i.e., at least two consistent outcomes) was used for genotype determination. If all three replicates yielded inconsistent results, the test was repeated using a newly extracted DNA aliquot from the same FFPE block to ensure result reliability.

Statistical analysis

Statistical analyses were performed via SAS 9.4, R 4.4.3 and Microsoft Excel 2019. The datasets were visualized via MATLAB 2016b. Categorical variables were presented as absolute frequencies with corresponding proportions. Differences in proportions across distinct HPV genotypes, age-stratified cohorts, and temporal intervals were assessed using the Chi-square (χ^2) test. HPV genotype diversity was quantified using the Simpson index, with statistical significance of differences assessed via Bootstrap confidence intervals and permutation tests. Temporal and age-related trends in HPV positive rate were evaluated using correlation/regression analysis and the Mann-Kendall trend test. A two-sided $p < 0.05$ was considered statistically significant throughout all analyses.

Ethics statement

This study was approved by the Ethics Committee of Nanjing Chinese Medicine Hospital (NJCMH), China, on February 10, 2012 (Institutional Review Board Reference No. 2012NJL008). Although the data were collected at Linyi Cancer Hospital, the ethical review was conducted at NJCMH because this study was part of a larger, multicenter project organized by the China HPV Infection Disease Committee, for which NJCMH served as the coordinating institution. Centralized ethics approval applied to all participating sites, including Linyi Cancer Hospital, as consistent with previously published multicenter studies under the same framework. Informed consent was obtained from all patients to use their clinical data and personal information for research purposes.

Results

Overall prevalence of HPV infection

A total of 606 patients met the inclusion criteria of this study and were included in the final statistical analysis. The median age of the women was 50.0 (42.0, 57.0) years, with a range of 19–81 years. The analysis revealed HPV infection in 574 women (94.7%, 574/606), with HR-HPV and LR-HPV genotypes constituting 94.6% (573/606) and 0.2% (1/606) of the total cases, respectively. HPV infection was not detected in 32/606 (5.3%) women.

HPV genotypes frequency distribution

A total of 18 HPV genotypes were identified among the 574 HPV-positive CSCC patients. As shown in Table 1, the five most prevalent genotypes were HPV 16 (80.5%), HPV 18 (5.2%), HPV 33 (2.8%), HPV 31 (1.8%), and HPV 58 (1.8%). HPV 16 remains the predominant genotype associated with CSCC, accounting for the vast majority of cases. Notably, some patients harbored multiple HPV

Table 1 The frequency of 18 HPV genotypes among 574 HPV-positive CSCC patients

HPV Type	No. of Cases (*n*)	Rate on Positive Cases($n=574$)[1]	Rate on Total Genotypes($n=601$)[2]
16	484	84.3%	80.5%
18	31	5.4%	5.2%
33	17	3.0%	2.8%
31	11	1.9%	1.8%
58	11	1.9%	1.8%
59	10	1.7%	1.7%
56	7	1.2%	1.2%
45	5	0.9%	0.8%
52	4	0.7%	0.7%
35	3	0.5%	0.5%
39	3	0.5%	0.5%
53	3	0.5%	0.5%
68	3	0.5%	0.5%
73	3	0.5%	0.5%
66	2	0.3%	0.3%
82	2	0.3%	0.3%
42	1	0.2%	0.2%
51	1	0.2%	0.2%
Total	601		

[1]Rate on Positive Cases ($n=574$): (number of patients with specific genotype / 574 total HPV-positive patients) × 100

[2]Rate on Total Genotypes ($n=601$): (number of detections of a genotype / 601 total genotype detections) × 100

Note: Multiple genotypes may be present in a single individual

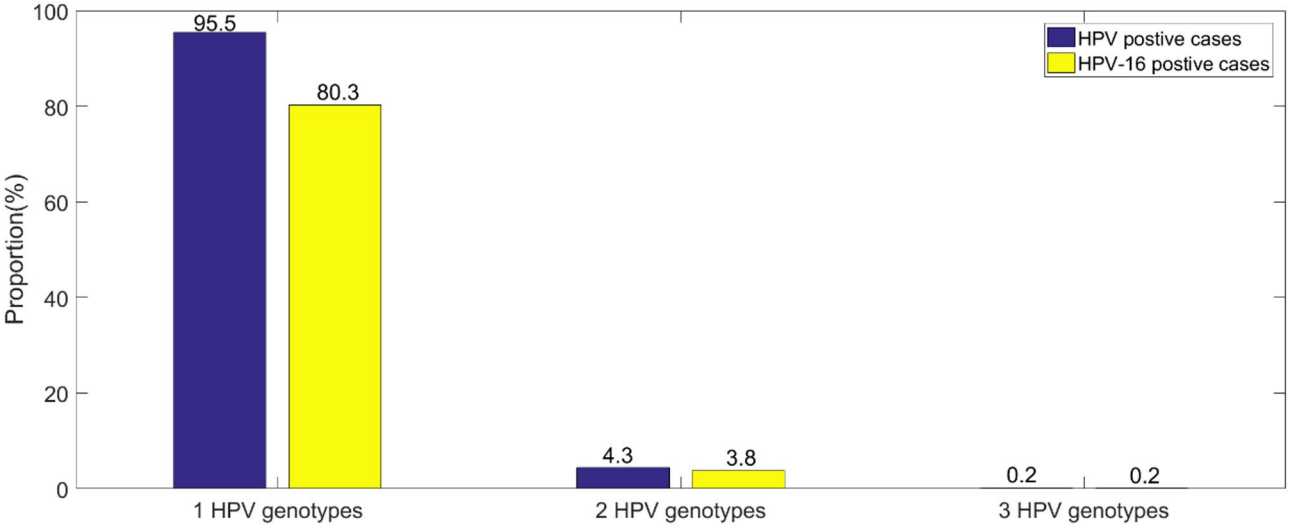

Fig. 1 The proportions of single-type HPV infections, two-type HPV mixed infections, three-type HPV mixed infections and the proportions of HPV-16 infections in different HPV infection form

Table 2 Frequency distribution of HPV genotypes in CSCC showing numbers and percentages of multiple-type HPV infections

HPV Genotype	Frequency (*n*)	Percent (%)
16&33	5	19.2
16&59	4	15.4
16&18	2	7.7
16&53	2	7.7
16&56	2	7.7
16&58	2	7.7
16&31	1	3.8
18&31	1	3.8
16&73	1	3.8
16&42	1	3.8
16&52	1	3.8
16&68	1	3.8
18&56	1	3.8
39&66	1	3.8
16&18&58	1	3.8
Total	26	100.0

genotypes, yielding a total of 601 genotype detections among 574 positive cases.

Analysis of HPV single infection and coinfection

Among the 574 HPV-positive cases, single infections were detected in 548 cases (95.5%), while 26 patients (4.5%) presented coinfections with multiple HPV genotypes. Specifically, 25/26 patients (96.2%) were coinfected with two HPV types, and only 1/26 patients (3.8%) carried three types of HPV infections. Notably, HPV 16 demonstrated epidemiological dominance across infection types, 84.3% of total HPV-positive cases were infected with HPV 16 (Fig. 1, Table S1). In the details, HPV 16 account for 461/548 (84.1%) single infections and 23/26 (88.5%) coinfections. The distribution of coinfections is detailed in Table 2, with the most frequently observed combination being HPV 16 and HPV 33.

Age-specific distribution features of HPV genotypes

The classification into six age groups (19–25, 26–35, 36–45, 46–55, 56–65, >65 years) was based on developmental and epidemiological stages relevant to cervical cancer risk: HPV vaccination coverage and early sexual debut (19–25), peak reproductive and screening age (26–35), rising incidence of cervical dysplasia and early malignancy (36–45), perimenopausal transition (46–55), immunosenescence and increased cancer risk (56–65), and geriatric oncology relevance (>65). Relevant epidemiological and clinical staging frameworks have been

previously described in studies on age-related HPV persistence and cancer risk [19–21].

While overall HPV positive rates did not vary significantly among age groups (Table S2), the age-stratified distribution of HPV genotypes showed important features (Table S3). HPV 16 consistently remained the dominant genotype across all ages (Fig. 2A). In contrast, the prevalence of non-HPV 16 infections appeared to rise with age, reaching its peak in the >65 years cohort, constituting 25.3% of infections within that group. Comparative analysis confirmed a significantly higher non-HPV 16 prevalence in postmenopausal women (>45 years) versus younger counterparts ($\chi 2$ trend test, $p = 0.0072$, Table S4).

Further stratification of non-HPV 16 infections (Fig. 2B) revealed that HPV 18 and HPV 33 were consistently among the most prevalent non-HPV 16 genotypes across all age groups, rather than being confined to younger women (≤45 years). However, a key age-related distinction was the increasing diversity of HPV genotypes with advancing age. A significant difference in HPV genotype diversity was found between the two age groups (≤45 years and >45 years) following quantification and statistical analysis (Simpson's biodiversity index ($1 - D_k$) of ≤45 years group = 0.2475, ($1 - D_k$) of ≤45 years group = 0.3949, $p < 0.01$). In women over 45 years, a broader range of genotypes, such as HPV 52, HPV 58, and HPV 59, was more frequently detected, in contrast to the narrower distribution observed in younger women. This pattern indicates an age-related shift in genotype diversity, with older CSCC patients exhibiting a more heterogeneous HPV infection profile. In this study, women who aged >45 years were considered postmenopausal based on the average age of natural menopause among Chinese women (approximately 49–51 years) and established clinical practice guidelines that recommend using age >45 as a proxy cutoff for menopausal transition in large-scale epidemiological studies where menstrual history is unavailable.

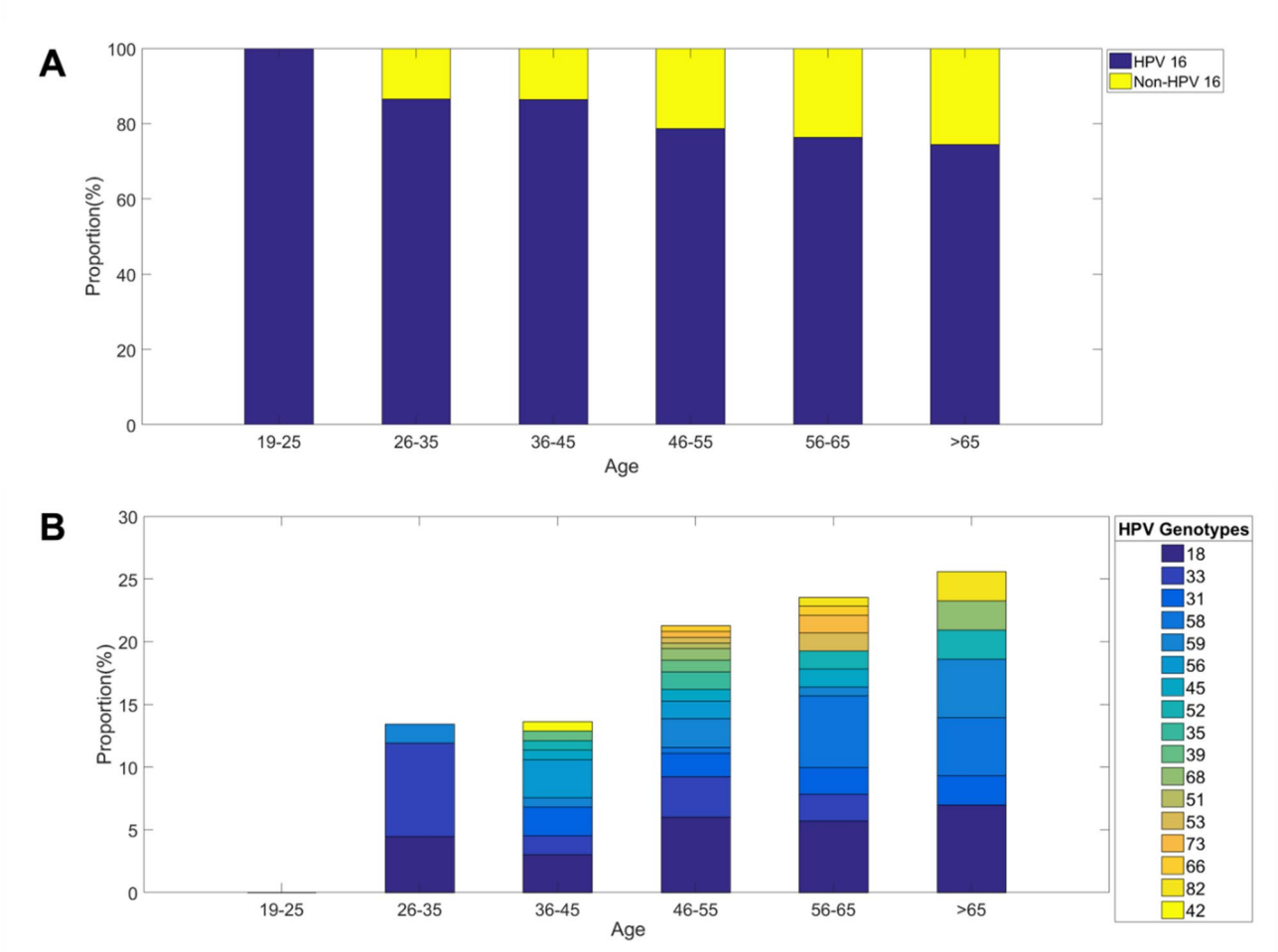

Fig. 2 Age-specific distribution of HPV genotypes in cervical squamous cell carcinoma (CSCC) patients from Linyi, China (2015–2023). (**A**) Proportions of HPV 16 and non-HPV 16 genotypes across different age groups. (**B**) Distribution of non-HPV 16 high-risk genotypes stratified by age

Temporal trends in the proportions of HPV genotypes

The detected HPV positive rate among patients with CSCC did not differ significantly across the study years (Table S5). Therefore, we proceeded to analyze the HPV-positive subgroup by stratifying patients according to age ($n_{2015}=39$, $n_{2016}=36$, $n_{2017}=59$, $n_{2018}=79$, $n_{2019}=74$, $n_{2020}=75$, $n_{2021}=84$, $n_{2022}=58$, $n_{2023}=70$). The temporal trends in the proportions of different age groups among cervical cancer patients from 2015 to 2023 are presented in Fig. 3. The 46–55 years age group consistently represented the highest proportion throughout the study period, remaining relatively stable overall despite minor fluctuations. The 56–65 years group demonstrated an overall increasing trend (Mann-Kendall trend test: $Z=3.232$, $p=0.0012$), surpassing the 36–45 years group after 2017. For the 36–45 years age group, a non-significant decline before 2018 was followed by a period of stabilization. Nevertheless, the group exhibited an overall downward trend across the study duration (Mann-Kendall trend test: Z = -2.3062, $p=0.0211$). The 26–35 years age group fluctuated prior to 2017 before entering a period of slow decline (Mann-Kendall trend test: Z = -2.1026, $p=0.0355$). The 0–25 years group consistently had the lowest proportion, showing negligible variation over time. Additionally, while the rate for the >65 years age group remained at a comparatively low level, it also showed an upward trend throughout the period (Mann-Kendall trend test: $Z=2.40$, $p=0.0165$) (Table S6).

Changes in the distribution of HPV vaccine-targeted genotypes over time

To evaluate the changing prevalence patterns of HPV vaccine-covered genotypes among CSCC patients and assess the effectiveness of the three commercially available HPV vaccines for preventing cervical cancer, we conducted a comprehensive analysis of the infection rates of vaccine-covered genotypes across different age groups and study years.

The distribution of HPV genotypes among CSCC patients in Linyi, China, from 2015 to 2023, revealed several key trends (Fig. 4). HR-HPV consistently presented the highest proportion across all age groups and study years, confirming its critical role in cervical carcinogenesis. The proportions of 2v/4v/ 9v-HPV showed a slight but steady decline with increasing age (2v/4v-HPV:β=-0.3873, (95%CI: -0.5497, -0.2250), $p<0.0001$; 9v-HPV:β=-0.1859, (95%CI: -0.3415, -0.0303), $p=0.0294$), particularly after 45 years of age (Fig. 4A, Table S3).

Over time, HR-HPV and vaccine-targeted genotypes exhibited relatively stable but high proportions, with minor interannual variations. Due to the absence of HPV 6 and 11 in the dataset, the trends for 2v-HPV and 4v-HPV overlapped. The 9v-HPV group showed a peak in 2020, with fluctuations in 2017, 2019, and 2021 (Fig. 4B). Chi-squared analysis indicated a significant difference in the 2v/4v-HPV positive rate between 2018 and 2020 ($p<0.05$), while no significant differences were observed among the rates for other years.LR-HPV types were detected at consistently low levels across all age groups and years, and were only observed in co-infections with HR-HPV types, with no cases attributed solely to LR-HPV (Table S7).

Discussion

Distribution of HPV genotypes: similarities and differences with other regions in China

Our findings indicate that HPV 16 is the predominant genotype among CSCC patients in Linyi, accounting

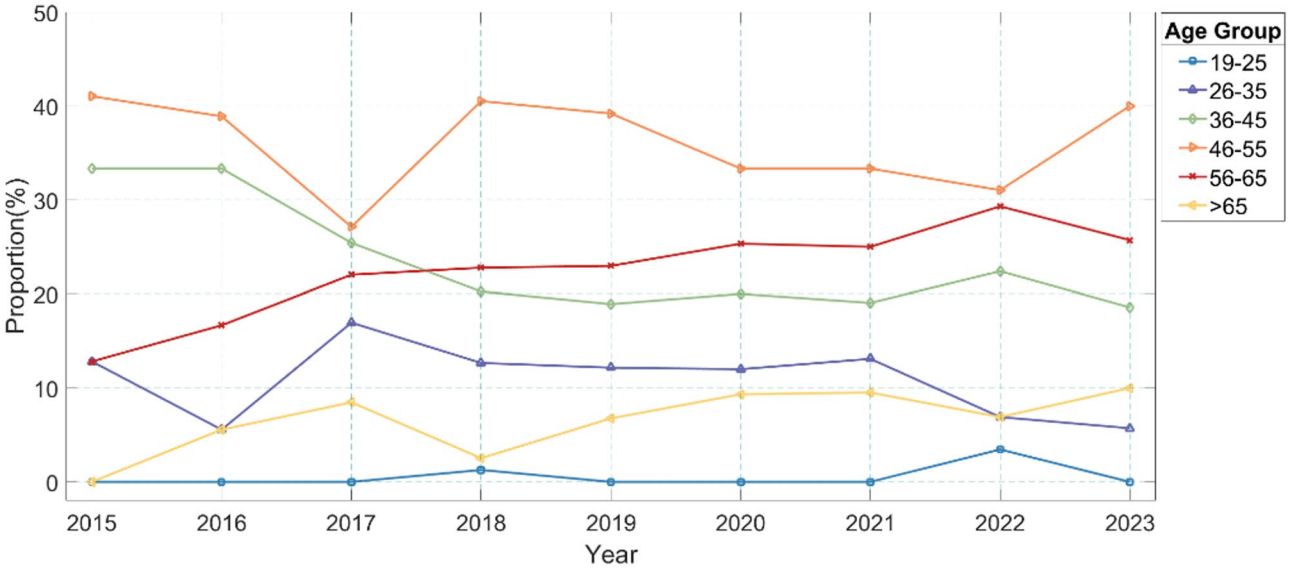

Fig. 3 The temporal trends in the proportions of different age groups among *HPV-positive* cervical cancer patients from 2015 to 2023

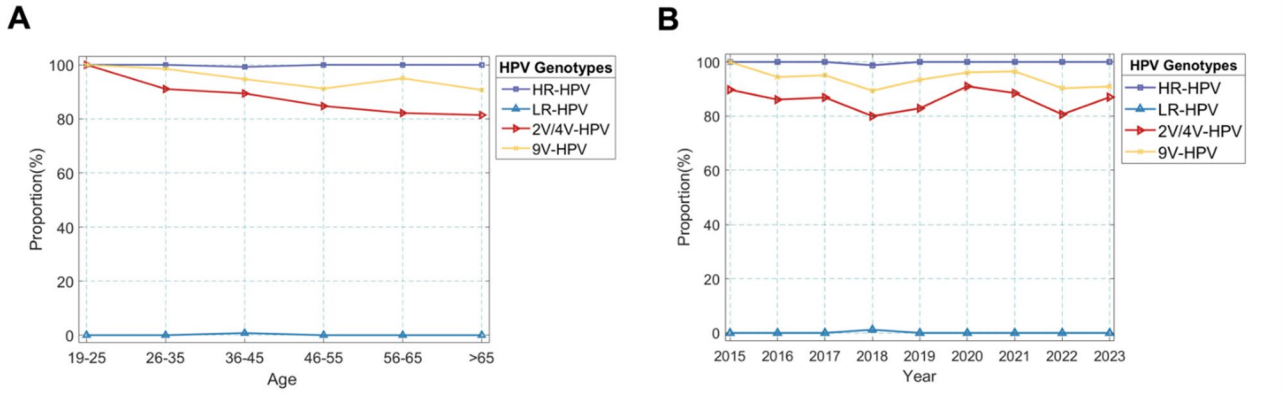

Fig. 4 Changes in the proportions of 2vHPV,4vHPV,9vHPV, LR-HPV and HR-HPV among different age groups and different years, 2015–2023. (**A**) Age-specific proportions of different HPV genotypes. (**B**) Time-specific proportions of different HPV genotypes. HR HPV includes: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82. LR HPV includes: HPV 42. 2vHPV includes: HPV 16 and 18. 4vHPV includes: HPV 6, 11, 16 and 18. 9vHPV includes: HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58

for 84.3% of positive cases. This result is consistent with global trends and studies from other regions of China, as HPV 16 is widely recognized as the most oncogenic genotype associated with cervical cancer [22–24]. However, the distribution of other HR-HPV genotypes in Linyi exhibits notable regional variations compared to other areas of China. For instance, in our study, the prevalence of HPV 58 was relatively low. In contrast, studies conducted in the Yangtze River Delta, Central China, and North China have reported that HPV 58 is the third most common genotype following HPV 16 and HPV 18 among women with CSCC [25, 26]. These regional variations in HPV genotype distribution of CSCC women may be attributed to differences in population genetics, sexual behavior, environmental factors, and healthcare access across different areas of China. Additionally, variations in HPV vaccination coverage, screening programs, and public health policies could influence the prevalence of specific genotypes, underscoring the need for tailoring HPV vaccination and screening strategies to specific geographic populations.

Our study is among the first to provide detailed data on the distribution of HPV genotypes in CSCC patients from northern China, particularly Shandong Province. Previous research has predominantly focused on southern regions, such as Guangzhou and Shenzhen, leading to a significant knowledge gap regarding the epidemiology of HPV in cervical cancer patients from northern China. By addressing this gap, our study contributes to a more comprehensive understanding of HPV genotype distribution across different regions of China, which is crucial for developing effective public health interventions tailored to regional epidemiological patterns.

Single infections play a key role in the pathogenesis of CSCC

Another key finding of our study is the high prevalence of single HPV infections (95.5%) compared to coinfections (4.5%) in Linyi. Among the coinfections, the most common combination was HPV 16 and HPV 33. This finding is consistent with previous studies that have reported HPV 16 as the most frequently detected genotype in both single and coinfections [27]. The relatively low prevalence of coinfections in our research suggests that single HPV infections may play a more significant role in the pathogenesis of CSCC in the population of this region.

The predominance of single HPV infections has important implications for cervical cancer prevention and treatment. This finding suggests that targeting the most prevalent genotypes, particularly HPV 16, through vaccination could significantly reduce the burden of cervical cancer in this region. Additionally, the low prevalence of coinfections may indicate that the immune response to one HPV genotype could potentially provide cross-protection against other genotypes, although further research is needed to confirm this hypothesis.

Regarding the low frequency of co- or multiple HPV infections in this study, several factors may contribute to this observation. First, CSCC is primarily associated with persistent infection by a single high-risk HPV genotype, particularly HPV 16, which remains the predominant genotype in our cohort. This aligns with existing literature, which suggests that single genotype infections, especially with HPV 16, are strongly implicated in the pathogenesis of cervical cancer, while multiple HPV infections are more commonly observed in low-grade lesions or in populations undergoing screening, rather than in those with malignant disease. Quint et al. [28] provided valuable insights into the patterns of single and multiple HPV infections and their association with

cervical disease. In their study, they observed that multiple HPV infections occurred more frequently than would be expected by chance, indicating a non-random pattern of coinfection. However, they found no evidence that specific HPV types have a tendency to be found more or less often than others in coinfections, suggesting that the occurrence of multiple infections is not due to specific interactions between HPV types.

Additionally, the methodology employed in this study, including the use of paraffin-embedded tissue samples and PCR-based genotyping, may have influenced the detection of co-infections. Although triplicate PCR testing was used to ensure result accuracy, the quality of DNA extracted from formalin-fixed paraffin-embedded tissues could limit the sensitivity for detecting low-abundance co-infections. Furthermore, the predominance of HPV 16 in this study may reflect a viral dominance effect, wherein HPV 16 suppresses or outcompetes other HPV types in the context of CSCC. This phenomenon has been observed in other studies, emphasizing the oncogenic potential of HPV 16 and its role in driving the progression to cervical cancer.

Age-specific stratified screening and preventive management of HPV infection should be implemented

Our age-specific analysis revealed that HPV 16 remains the dominant genotype across all age groups, but the proportion of non-HPV 16 infections increases with age, particularly in women over 46 years. Older women (> 45 years) also showed a greater diversity of HPV genotypes infections, including HPV 52, 58, and 59, than younger women (≤ 45 years).

The observed age-related shift in the HPV genotype distribution may be attributed to several factors. First, the increasing proportion of non-HPV 16 infections in older women may reflect a cohort effect, where younger generations have been more exposed to HPV 16 because of changes in sexual behavior patterns over time [29]. Additionally, differences in immune system efficiency across age groups could play a role, as older women may have a reduced ability to clear persistent non-HPV 16 infections, leading to a higher prevalence of diverse HPV genotypes in this population. Furthermore, the greater diversity of HPV genotypes in women aged > 45 years suggests cumulative lifetime exposure to multiple HPV strains, possibly due to longer infection persistence and lower immune clearance rates [30].

These findings emphasize the need for age-specific cervical cancer screening and prevention strategies. These programs should consider the changing distribution of HPV genotypes with age. Especially for older women, who may carry more HR-HPV genotypes and have an increasing proportion of non-HPV 16 infections, they require more comprehensive screening strategies for non-HPV 16 infections. This could improve the effectiveness of cervical cancer screening programs and better protect high-risk populations.

Despite the availability of HPV vaccines targeting HPV 16, this genotype remains overwhelmingly prevalent among CSCC patients in Linyi. Several factors may account for this phenomenon. First, widespread HPV vaccination was not introduced in China until 2016, and vaccine coverage remains relatively low, especially among older women who constitute the majority of CSCC cases in this study. Second, the catch-up vaccination strategy for women aged ≥ 26 years has only recently gained attention, leaving a large cohort unprotected during their peak years of HPV exposure. Third, public awareness, accessibility, and socioeconomic barriers may further limit vaccine uptake in some areas. Finally, the high oncogenic potential and biological fitness of HPV 16 may contribute to its persistence and dominance, even in partially vaccinated populations. Together, these factors may explain the continued high burden of HPV 16-associated cervical cancer despite the availability of effective vaccines.

HPV infection and cervical cancer screening need to be strengthened for women over 45 years of age in China

The consistently high number of cases in the 46–55 age group among HPV-positive patients throughout the study period reflects the expected burden of disease in this cohort, likely due to prolonged HPV persistence and cumulative cellular damage with age. Notably, the proportion of cases of the 56–65 age group increased over time, surpassing that of the 36–45 age group after 2017. This shift may indicate a gradual transition of disease burden toward older women, potentially related to improved survival among younger patients, changing sexual behavior patterns, or the reactivation of latent HPV infections in postmenopausal women due to immune senescence [21, 31].

Conversely, the declining proportions of CSCC cases in the 26–35 and 36–45 age groups are more likely attributable to enhanced cervical cancer screening programs targeting women in these age groups, as HPV vaccination coverage was still limited among women over 26 during the study period.

According to information released by the National Health Commission of China, in July 2016, GlaxoSmithKline's bivalent HPV vaccine, Cervarix, received approval from the China Food and Drug Administration, making it the first HPV vaccine authorized in China for females aged 9–25 years. In April 2018, Merck's nonavalent HPV vaccine, Gardasil 9, was also approved for use in China, expanding the range of preventable HPV types and extending the vaccination age to 9–45 years. Since 2018, China has continuously adjusted its public health policies to promote nationwide HPV vaccination. As of

October 2024, free HPV vaccination services have been provided to 106,000 eligible girls, achieving a coverage rate of 91.5% [32]. The introduction and wider adoption of HPV vaccination in China, particularly among younger cohorts, may have contributed to a reduction in the number of HPV-related cervical cancer cases in these age groups [33]. Additionally, improved awareness and access to early screening programs may have facilitated earlier detection and treatment of precancerous lesions, preventing progression to invasive carcinoma. The consistently low proportion of CSCC cases in the 0–25 years age group aligns with the natural history of HPV-related carcinogenesis, which typically requires several years or decades to progress from infection to malignancy. The fluctuating yet relatively low proportion of cases in the >65 years may reflect a combination of factors, including under-screening in older populations, reduced healthcare-seeking behavior, and competing mortality risks that reduce the overall case burden in this age cohort.

Our nine-year study (2015–2023) represents one of the most prolonged observational analyses of HPV infection rates among cervical cancer patients in China. These temporal trends highlight the effectiveness of vaccination and the urgent need to increase coverage, especially in low- and middle-income regions [34]. These findings significantly affect HPV research in CSCC patients. The persistent predominance of the 46–55 years age group and the increasing burden among women aged 56–65 years highlight the need for targeted screening strategies for middle-aged and older populations, as prolonged HPV persistence and immune senescence may contribute to increased cancer risk. The declining incidence in younger women after 2018 suggests that HPV vaccination and improved screening programs effectively reduce CSCC cases in this population. These results underscore the importance of expanding vaccination coverage and optimizing prevention efforts in women over 45 years of age to further reduce the burden of HPV-related CSCC.

HPV vaccination has good prospects

The prevalence of vaccine-covered genotypes (2v-HPV, 4v-HPV, and 9v-HPV) remained consistently high throughout the study period, with minor interannual fluctuations. Notably, the 9-valent vaccine, which covers HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58, exhibited a similarly high prevalence over the years. These findings suggest that the 9-valent vaccine could be particularly effective in reducing the burden of cervical cancer in this region, and that further expansion of the proportion of vaccination with the 9-valent vaccine in China may be able to reduce the prevalence of CSCC in the future. However, consistent with previous reports, including large-scale epidemiological studies, no cases of HPV 6 or 11 were detected in cervical cancer samples in our dataset. This absence reflects the well-established low oncogenic potential of these genotypes and supports the predominance of high-risk HPV types in cervical carcinogenesis.

Research strengths and limitations

From a methodological standpoint, this study comprehensively analyzed both single and multiple HPV infections, HR-HPV and LR-HPV genotypes, and age- and time-related patterns—offering a multidimensional understanding of HPV epidemiology in CSCC. Unlike most previous studies that focused primarily on general screening populations, our work specifically targets histologically confirmed CSCC cases, thus providing more clinically relevant insights into the link between genotype distribution and disease progression. This distinction improves the applicability of our study in clinical and preventive oncology. Additionally, our study used a long-term observational design, which provides a comprehensive overview of the HPV genotype distribution over a nine-year period. This longitudinal approach allows us to track temporal trends and assess the impact of vaccination efforts on HPV prevalence, as well as providing some guidance on current cervical cancer screening programmes and HPV vaccination strategies in China. Additionally, our study is one of the first to focus specifically on CSCC patients in northern China, providing valuable baseline data for future research and public health interventions.

However, our study also has several limitations. First, the data were collected from a single center, which may limit the generalizability of our findings to other regions. Second, the study population consisted exclusively of women diagnosed with CSCC, which may not fully represent the broader population of women with cervical lesions. Future studies should include a more diverse population to validate our findings and provide a more comprehensive understanding of the HPV genotype distribution in northern China.

Abbreviations

HPV	Human papillomavirus
CSCC	Cervical squamous cell carcinoma
PCR	Polymerase chain reaction
HR-HPV	High-risk human papillomavirus
LR-HPV	Low-risk human papillomavirus

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

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Author contributions
Y.W. wrote the manuscript and data extraction; H.H. completed the work of follow-up and revised the manuscript; G.D. and conducted the statistical analysis; H.Z., X.Z. and M.X. completed the analysis of pathology; Y.Z., Z.W. and J.G. collected the data and were responsible for project administration; L.L. conceived and designed the study.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study followed the ethical guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of Nanjing Chinese Medicine Hospital, China.

Consent for publication
All of the patients signed an informed consent form.

Competing interests
The authors declare no competing interests.

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