Retrospective Analysis of the Association Between Cervical Squamous Intraepithelial Lesions and Human Papillomavirus Genotypes

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Research

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Abstract

Background: Human papillomavirus (HPV) has been confirmed as a major causative factor for malignant transformation of cervical epithelial cells and for the development of cervical intraepithelial neoplasia (CIN) and invasive cervical cancer.

Methods: We collected the cervical cell samples of women who visited the gynecological clinic of Peking Union Medical College Hospital between October 2017 and May 2020 and submitted them to the HPV genotyping test. We analyzed the distribution of single-type HPV genotypes in CIN of different severities and the age-dependent prevalence for single-type HPV infection.

Results: In both CIN2 and CIN3 group, HPV 16, 58, 52, 33 and 31/18 were detected as top 5 HPV types, which accounts for 89.25% and 88.54% of single HPV infection incidence respectively. HPV 16 was the dominant genotype in both CIN2 and CIN3, accounted for 46.24% and 55.21%, respectively. The prevalence of HPV 16 was the most frequent in all the age groups, except >64 years group in CIN3. The prevalence of HPV 16 and 33 increased obviously with increasing grade of CIN (chi-squared test for trend, $P < 0.001$), while HPV 18, 31, 52 and 58 showed the opposite trends. The peak of the incidence of CIN3 was observed at 25~34 years (33.68%), followed by 35-44 years (31.58%).

Conclusion: High grade CIN peak at 25-44 years, women of this age are recommended for normative screening if conditions permit. HPV 16 was particularly aggressive in the development of cervical premalignant lesions and malignant lesions in almost all age groups, except >64 years group in CIN3. For women >64 years old, patients infected with other HPV types should be also taken seriously. In general, HPV 16, 58, 52, 33, 31 and 18 were the most common genotypes in high grade CIN, and vaccine including these predominant genotypes might be of great significance for cervical cancer prevention in China.

1. Background

Cervical cancer is the second most frequent cancer among women globally, especially in developing countries. It has been estimated that about 529000 women are diagnosed with cervical cancer annually, and causing approximately 275000 deaths every year [1]. In 2015, there were 98900 new cases of cervical cancer and 30500 cervical cancer-related deaths in China [2]. Human papillomavirus (HPV), the most common sexually transmitted virus, has been confirmed as a major causative factor for malignant transformation of cervical epithelial cells and for the development of cervical intraepithelial neoplasia (CIN) and invasive cervical cancer [3]. To date, 14 HPV types have been classified as “high-risk” for their strong carcinogenic potentials, which contribute to 96.6% of invasive cervical cancers diagnosed worldwide [4]. Among the high-risk HPV types, HPV16 and HPV18 are well-known carcinogenic genotypes, additionally, HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59 are also classified as “carcinogenic to humans” [5]. Besides, the low-risk types, such as HPV6, and 11, are classified as “non-oncogenic to humans”, which are associated with hyperplastic lesions [5].

As there are differences in pathogenicity between different HPV genotypes, it is important to understand the information on HPV prevalence and type distribution in cervical lesions, particularly precancerous lesions. In January 2019, aglobal strategy towards the elimination of cervical cancer as a public health problem was approved by World Health Organization, which outlines key goals and agreed targets to be reached by 2030 and set the world on track to elimination [6]. In the era of widespread HPV-based primary screening and HPV-based triage of screen-detected cervical abnormalities, HPV genotyping will provide evidence for the future selection of vaccines targeting HPV types common to a specific region and further aid the development of public health policy programs of eliminating cervical cancer by 2030.

Therefore, updated information on type-specific HPV prevalence and distribution is of guiding significance for cervical cancer prevention. In this study, HPV prevalence and genotype distribution in Negative tissue and CIN patients were described, and the age-dependent prevalence of single-type HPV infection were also investigated. These data will provide information for estimating the potential impact of HPV prophylactic vaccines in Chinese women.

2. Method

2.1 The aim

To investigate the correlation between cervical squamous intraepithelial lesions and HPV genotypes and to understand the importance of HPV genotypes distribution.

2.2 The design

We collected the cervical cell samples of women who visited the gynecological clinic of Peking Union Medical College Hospital between October 2017 and May 2020 and submitted them to the HPV genotyping test. A total of 24199 cases were submitted to individual HPV genotyping test, among which, 1661 cases underwent colposcopy biopsy and were included in the current study. Among the cases, 456 cases were diagnosed as Negative tissue, 899 cases were diagnosed as CIN1, 156 cases were diagnosed as CIN2, 144 cases were diagnosed with CIN3, 6 cases were diagnosed with SCC. We analyzed the distribution of single-type HPV genotypes in CIN of different severities and the age-dependent prevalence for single-type HPV infection. As this study was retrospective, which was approved by the Ethics Committee of our hospital (approval number S-K1604).

2.3 HPV DNA test

Cervical samples were collected by gynecologists and stored in standard preservative media provided by manufacturers along with their kits. The testing was based on the polymerase chain reaction (PCR) and the TaqMan technique using a commercially available HPV Genotyping Real Time PCR kit (ZJ Bio-Tech Co., Ltd, Shanghai, China), which could detect HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 82 and 6/11 simultaneous individually.

2.4 Pathological examination

Pathological diagnosis of cervical lesions was used as the gold standard. The results of pathology were classified into negative (normal or inflammation), CIN1, CIN2, CIN3 and SCC. All the histologic specimens were reviewed by 2 independent expert pathologists.

2.5 Statistical analysis

SPSS v25.0 was used for statistical analysis. The count data are expressed as a percentage or n (%). The prevalence of HPV across cervical lesions was compared by Chi-square test. The *P* value was used to indicate the significance, the test level was $\alpha = 0.05$, and $P < 0.05$ was considered statistically significant.

3. Results

A total of 1661 cases with histopathologic diagnoses and HPV genotyping test results were included in the study. 456 women were diagnosed as Negative tissue, 1199 women were diagnosed with CIN and 6 women were diagnosed as cervical cancer. Among women diagnosed with CIN, 899 (75.29%) had CIN1, 156 (13.06%) had CIN2 and 144 (11.65%) had CIN3. HPV-positive results reported in Negative, CIN1, CIN2, CIN3 and SCC were 91.45%, 90.99%, 98.08%, 96.53% and 100%, respectively ($x^2 = 14.577$, $P = 0.006$). The proportion of single HPV infection were 64.75%, 58.19%, 60.78%, 69.06% and 100% in Negative, CIN1, CIN2, CIN3 and SCC, respectively, which increased with increasing grade of CIN ($x^2 = 5.906$, $P = 0.052$) (Fig. 1). And infections with multiple types were identified in 35.25%, 41.81%, 39.22%, 30.94% and 0% of individuals in Negative, CIN1, CIN2, CIN3 and SCC, respectively, which decreased with increasing grade of CIN ($x^2 = 5.906$, $P = 0.052$) (Fig. 1).

3.1 Distribution of single HPV genotypes

In the present study, when only the cases with single type HPV were evaluated, HPV 16 accounts for 24.07%, 22.06%, 46.24% and 55.21% of infections in Negative, CIN1, CIN2 and CIN3, respectively, which increased significantly with increasing grade of CIN (chi-squared test for trend, $P < 0.001$). The prevalence of HPV 33 was 2.22%, 3.15%, 5.38% and 8.33% in Negative, CIN1, CIN2 and CIN3, respectively, which also increased with increasing grade of CIN (chi-squared test for trend, $P = 0.033$). Besides, the incidence of HPV 16/18 was 33.33%, 29.41%, 50.54% and 59.38% in Negative, CIN1, CIN2 and CIN3, respectively, which increased significantly with the increasing grade of CIN (chi-squared test for trend, $P < 0.001$). However, the prevalence of HPV 51, 56 and 66 were lower than 8.00% in all the CIN grades and decreased obviously with the increasing grade of CIN (chi-squared test for trend, $P < 0.05$). Then, the incidence of other HPV types, namely genotypes excluding 16, 18, 6 and 11, was 66.67%, 70.59%, 49.46% and 40.63% in Negative, CIN1, CIN2 and CIN3, respectively, which also exhibited the same trend, which decreased with increasing grade of CIN (chi-squared test for trend, $P < 0.001$).

In the Negative group, the 5 most frequent genotypes were, in descending order of frequency, HPV types 16, 52, 58, 18 and 51/56 (ranged from 24.07–5.19%), the total incidence of these 5 genotypes was 67.41%. In CIN1 patients, the 5 most common HPV types were 16 (22.06%), 52 (17.02%), 58 (14.92%), 18 (7.35%) and 66 (7.14%), with a total incidence of 68.49%. The 5 predominant genotypes were 16

(46.24%), 52 (15.05%), 58 (15.05%), 31 (7.53%) and 33 (5.38%), with a total incidence of 89.25% in CIN2 patients. In the CIN3 group, in order of prevalence, HPV 16 (55.21%), 58 (11.46%), 52 (9.38%), 33 (8.33%), 31/18 (4.17%) were detected as top 5 HPV genotypes, which accounts for 88.54% of infection incidence. Moreover, single type infection of 45 and 6/11 were not observed in both CIN2 and CIN3. Additionally, all of the six SCC cases were infected with single-type HPV 16. (Table 1)

Table 1
Distribution of HPV genotypes in CIN

HPV types	Negative (n = 270)	CIN1 (n = 476)	CIN2 (n = 93)	CIN3 (n = 96)	*P*-value
16	24.07%	22.06%	46.24%	55.21%	< 0.001
18	9.26%	7.35%	4.30%	4.17%	0.243
31	3.70%	2.10%	7.53%	4.17%	0.053
33	2.22%	3.15%	5.38%	8.33%	0.033
35	1.48%	2.10%	1.08%	2.08%	0.871
39	4.81%	2.94%	1.08%	0.00%	0.065
45	1.48%	0.63%	0.00%	0.00%	0.325
51	5.19%	5.67%	1.08%	0.00%	0.031
52	14.81%	17.02%	15.05%	9.38%	0.295
56	5.19%	5.04%	0.00%	1.04%	0.046
58	14.07%	14.92%	15.05%	11.46%	0.842
59	2.96%	3.36%	2.15%	1.04%	0.630
66	4.81%	7.14%	1.08%	0.00%	0.006
68	4.81%	4.62%	0.00%	1.04%	0.065
82	0.37%	0.84%	0.00%	2.08%	0.311
6 + 11	0.74%	1.05%	0.00%	0.00%	0.571
16/18	33.33%	29.41%	50.54%	59.38%	< 0.001
Others*	66.67%	70.59%	49.46%	40.63%	< 0.001
*Others: HPV includes 6, 11, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 82					

3.2 Distribution patten of predominant HPV genotypes

As Fig. 2 showed, in single-type infection, HPV 16 was the most frequent genotype in Negative, CIN1, CIN2 and CIN3, whose prevalence increased significantly with the aggravation of the CIN (chi-squared test for

trend, $P < 0.001$). HPV 33 also increased with increasing grade of CIN (chi-squared test for trend, $P = 0.033$), with prevalence lower than 10% in all the CIN grades. However, HPV18 (chi-squared test for trend, $P = 0.243$), 31 (chi-squared test for trend, $P = 0.053$), 52 (chi-squared test for trend, $P = 0.295$), 58 (chi-squared test for trend, $P = 0.842$) showed the opposite trends, which decreased with increasing grade of CIN. Besides, other HPV types also decreased with increasing grade of CIN (chi-squared test for trend, $P < 0.001$). Regarding single infection, the cumulative positive rate of HPV 16, 18, 31, 33, 52 and 58 was 68.15%, 66.60%, 93.55% and 92.71% in Negative group, CIN1, CIN2 and CIN3, respectively.

3.3 Coloscopy referral number for detecting 1 CIN2+/CIN3

To detect one CIN2 + cases, 2.8, 8.5, 3.2 and 7.1 women should receive colposcopy referrals if using HPV16, HPV 18, HPV 16/18 and HPV others as screening methods for detecting cervical lesions, respectively. Meanwhile, to detect one CIN3 case, the referral numbers for HPV16, HPV 18, HPV 16/18 and HPV others were 5.0, 17.0, 5.9 and 15.4 women, respectively. The colposcopy referral rate for HPV others is 2.5 times and 3 times higher than HPV 16 for detecting one CIN2 + case and one CIN3 case, respectively (Table 2).

Table 2
Colposcopy referral number for detecting one CIN2 + and CIN3 by using different HPV genotypes

HPV type	Negative % (n/N)	CIN1 % (n/N)	CIN2 % (n/N)	CIN3 % (n/N)	Coloscopy referral number for detecting 1 CIN2+	Coloscopy referral number for detecting 1 CIN3
HPV 16 (n = 266)	24.07% (65/270)	22.06% (105/476)	46.24% (43/93)	55.21% (53/96)	2.8	5.0
HPV 18 (n = 68)	9.26% (25/270)	7.35% (35/476)	4.30% (4/93)	4.17% (4/96)	8.5	17.0
HPV 16/18 (n = 334)	33.33% (90/270)	29.41% (140/476)	49.46% (46/93)	59.37% (57/96)	3.2	5.9
HPV others (n = 601)	66.67% (180/270)	70.59% (336/476)	49.46% (46/93)	40.62% (39/96)	7.1	15.4

3.4 Age-dependent prevalence for single-type HPV infection

In the present study, the average age of CIN2 and CIN3 were (41.51 ± 10.53) years and (40.75 ± 10.51) years, respectively. In CIN2, the predominant morbidity age were 35–44 years (36.46%) and 25–34 years (33.33%), and the prevalence of CIN2 decreased obviously with increasing age in women over 45 years old. While the peak of the incidence of CIN3 was observed at 25 ~ 34 years (33.68%), followed by 35–44 years (31.58%), and decreased obviously with increasing age. Moreover, no single type infections were identified under 25 years old in both CIN2 and CIN3. (Fig. 3)

When age-dependent prevalence for single-type HPV infection in Negative, CIN1, CIN2 and CIN3 were evaluated, the top 5 most frequent HPV types in different age groups were analyzed (Fig. 4). In general, the prevalence of HPV 16 was the most frequent in all the age groups, except > 64 years group in CIN3. Among CIN1 patients, HPV 16, 52 and 58 were the common predominant HPV types in all the age groups except > 64 years. In CIN2 patients, the most prevalent HPV types in different age groups were included in 16, 52, 58, 33, 31 and 18, with relative proportion differed somewhat by age. In CIN3 patients, HPV16, 52, 58 and 33 were the common frequent types in the age groups of 25 ~ 34 years, 35 ~ 44 years and 45 ~ 54 years. Moreover, the prevalence of HPV 16 in younger groups (25 ~ 34 years, 35 ~ 44 years and 45 ~ 54 years) was significantly higher than older groups (55 ~ 64 years and > 64 years), while HPV58 showed the opposite trends.

4. Discussion

In the present study, HPV-positive rate reported in Negative, CIN1, CIN2 and CIN3 were 91.45%, 90.99%, 98.08% and 96.53%, respectively, which were higher than the positive rate in cervical cancer screening [7, 8]. The main reason for the difference is due to the fact that this study was a retrospective analysis based on opportunistic screening, the patients included were detected with abnormal cytology/HPV results and underwent a coloscopy test and a potential cervical biopsy according to the 2012 American Society of Colposcopy and Cervical Pathology (ASCCP) guideline [9]. Therefore, the HPV positive rate of Negative group and CIN1 group were relatively much higher in the current study. In addition, the proportions of multiple HPV infection were 35.25%, 41.81%, 39.22%, 30.94% and 0% in Negative, CIN1, CIN2, CIN3 and SCC, respectively, which decreased with increasing grade of CIN. Thus, a negative relationship between multiple infection and the progression of CIN was found, which revealed that multiple infection was not the leading factor for the progression of CIN2+, the finding was consistent with previous study [10–12].

When only the cases with single type HPV infection were evaluated, HPV 16 and 33 increased significantly with increasing CIN grades, while HPV18, 31, 52, 58 showed the opposite trends. Besides, all of the 6 SCC cases in this study were infected with single-type HPV 16. These results indicated that HPV 16 is the most aggressive Hr-HPV in the development of cervical premalignant lesions and malignant lesions and is less likely to regress compared with other HPV genotypes, the finding is consistent with previous studies [13, 14]. Moreover, the frequency of HPV 33 also increased with the severity of the cervical lesion grade, which deserves further attention due to the limited cases in this analysis. However, the present study demonstrated that the prevalence of HPV 18 was less than 10% and decreased with the severity of cervical lesions, which was not the most common HPV genotype in high grade CIN, the finding

was similar with other Chinese studies [11, 12, 15], but was different with international data [14, 16]. Furthermore, only 7 cases (0.94%) with single-type HPV 45 was found in ≤ CIN1 patients, while no cases of single-type HPV 45 was identified in both CIN2 and CIN3 in this study. Although HPV 18 and 45 showed a relative lower prevalence in the current analysis, they were reported to be associated with glandular lesions in the endocervical canal [9, 17], which are still of great importance. In consequence, patients with HPV 18/45 infection should pay close attention on endocervical canal lesions when referring coloscopy. In addition, the referrals and colposcopies number were as low as 2.7 and 5.0 if using HPV16 as screening method for detecting one CIN2 + and one CIN3, respectively, which is much lower than that of HPV others. Meanwhile, the data of HPV others showed in Table 2 was actually not using HPV others alone as screening method, but in combination with the results of abnormal cytology. Therefore, the actual referrals and colposcopies number using HPV others alone as screening method would be higher.

HPV genotype distribution in high-grade cervical lesions has been reported to vary significantly in different geographic population [17, 18]. According to a meta-analysis, the prevalent HPV types in high-grade cervical lesions were 16 (57.90%), 31 (15.80%), 33 (4.40%), 18 (4.00%) and 52 (2.90%) in Europe, while the top five HPV types in CIN3 were 16, 31, 18, 52 and 59 in Canada [19]. In this study, the 5 predominant genotypes were 16 (46.24%), 52 (15.05%), 58 (15.05%), 31 (7.53%) and 33 (5.38%) in CIN2 patients and the most prevalent HPV genotypes among CIN3 patients were HPV 16 (36.81%), 58 (7.64%), 52 (6.25%), 33 (5.56%) and 31/18 (2.78%). The prevalence of these predominant genotypes comprised 93.55% and 92.71% of total single HPV infection in CIN2 and CIN3, respectively. It is worth mentioning that most of the patients in our gynecological clinic came from all over China with confirmed HPV infection or suspected cervical lesions, which does not belong to the category of regional cervical cancer screening, but opportunistic screening. Our finding was consistent with a previous analysis [20], which reported the predominant types of CIN2/3 in Asia were HPV 16, 58, 52, 18, 33 and 31. Regarding the distribution of HPV genotypes in China, little regional differences among high-grade cervical lesions were observed. In northern China, the most prevalent HPV genotypes were found to be HPV16, 58, 33, 52 and 18 [15]. In a large cohort study based on western Chinese women, the most commonly detected HPV genotypes in CIN2/CIN3 cases were HPV 16 (48.1%), 58 (19.3%), 52 (10.0%), 33 (9.6%) and 18 (4.6%) [11]. In eastern China, the top 5 predominant genotypes in CIN2 were 16, 58, 52, 33 and 31, while in CIN3 were HPV 16, 58, 33, 52 and 31 [12]. Thus, based on the prior studies and the current study, HPV 16, 58, 52, 33, 31 and 18 were the predominant genotypes in the majority of Chinese women. Therefore, vaccine including HPV 16, 18, 31, 33, 52 and 58 is potentially very effective for Chinese women, which might reduce the morbidity of cervical cancer in China. Of cause, large multicenter studies and long-term follow up are needed to further confirm this hypothesis.

In the present study, the incidence of high-grade cervical lesions was significantly higher among women age 25 ~ 34 years and 35 ~ 44 years than among the other age groups, the results were similar with previous studies. For example, in a previous population-based study in Beijing, a peak of 2.2% at age 30 ~ 34 years was observed in CIN2+ [21]. Besides, a cross-sectional study of Yangtze River Delta area (China) showed that the prevalence of CIN2 and CIN3 peaked at 40 ~ 44 years and 35 ~ 39 years,

respectively, and followed by 35 ~ 39 years and 30 ~ 34 years, respectively [12]. According to the evolution of cervical lesions, it takes several years for the occurrence of high-grade CIN [22]. Consequently, women over 25 years old were recommended for a standardized screening when conditions permit. When age-dependent prevalence for single-type infection was examined in CIN3 groups, we found that the prevalence of HPV 16 was significantly lower in the patients older than 55 years. In contrast, the prevalence of HPV 58 was obviously higher in the patients older than 55 years. This data suggested that HPV 16 is the most malignant HPV type, which has a strong potential for CIN trends, and whose progression from benign to premalignant (or malignant lesions) is earlier than that of other types. However, other HPV types, such as HPV 58, may need longer time to progress into premalignant lesions. In this study, 3 cases of CIN3 were found in > 64 years group, with 2 cases infected with HPV 58 and one case infected with HPV 16. As a country with a vast territory, China still faces many difficulties in cervical cancer screening, many women older than 64 years old have not yet received routine cervical cancer screening before. Therefore, women older than 64 years old with other HPV types infection should be paid special attention as well.

5. Conclusions

In summary, age-dependent distribution suggested that high grade CIN peak at 25-44 years of age, women aged 25 years and older in China are recommended for a routine screening if conditions permit. For women >64 years old, patients infected with other HPV types should be also taken seriously. For women 25 to 64 years old, HPV 16 was particularly aggressive in the development of cervical premalignant lesions and malignant lesions. In general, HPV 16, 58, 52, 33, 31 and 18 were the most common genotypes in high grade CIN, vaccine including these main genotypes might be of great value for cervical cancer prevention in China. The current study was a single-center and retrospective analysis with a relatively small sample size, further confirmation and validation are needed in future multicenter prospective study with large samples.

6. Abbreviations

Number	Abbreviations	Full name
1	CIN	Cervical intraepithelial neoplasia
2	Hr-HPV	High-risk human papillomavirus
3	SCC	Squamous cervical carcinoma
4	PCR	Polymerase chain reaction
5	ASCCP	American Society of Colposcopy and Cervical Pathology

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Peking Union Medical College Hospital (approval number S-K1604) and the study was retrospective, with only data been used and analyzed, no consent was need to participate, which was in compliance with the institutional and national policies concerning research approvals.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LK and YX conceived of the original idea for the study, interpreted results, obtained ethical approval, edited the paper and was overall guarantor. TX and XX carried out the statistical analysis and contributed to the preparation of the data set. LK, XX and RW interpreted results and contributed to the writing of the paper. All authors read and approved the final manuscript.

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Figures

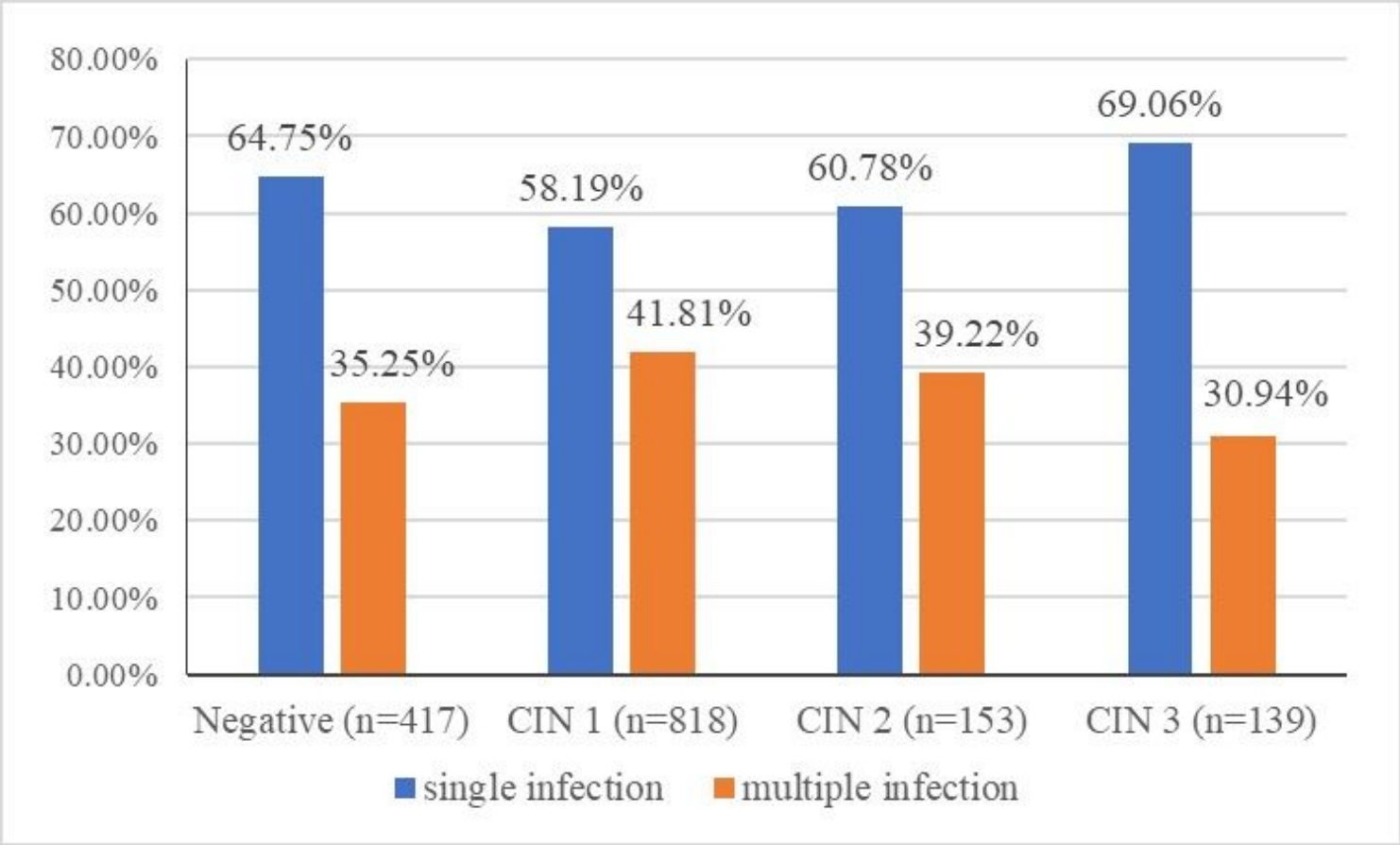

Figure 1

The single infection and multiple infection of HPV among different groups

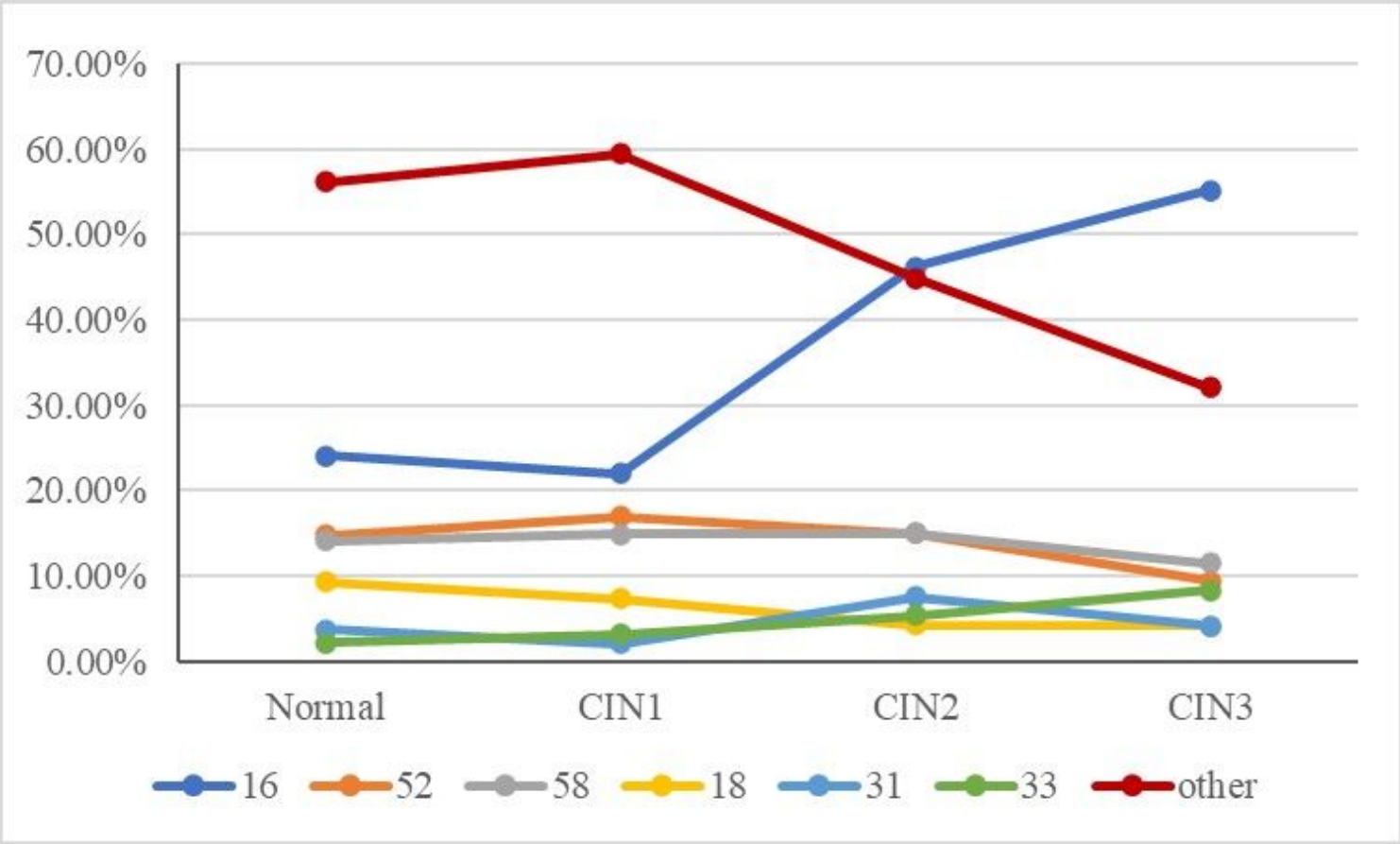

Figure 2

The distribution pattern of predominant HPV genotypes with single-type infection

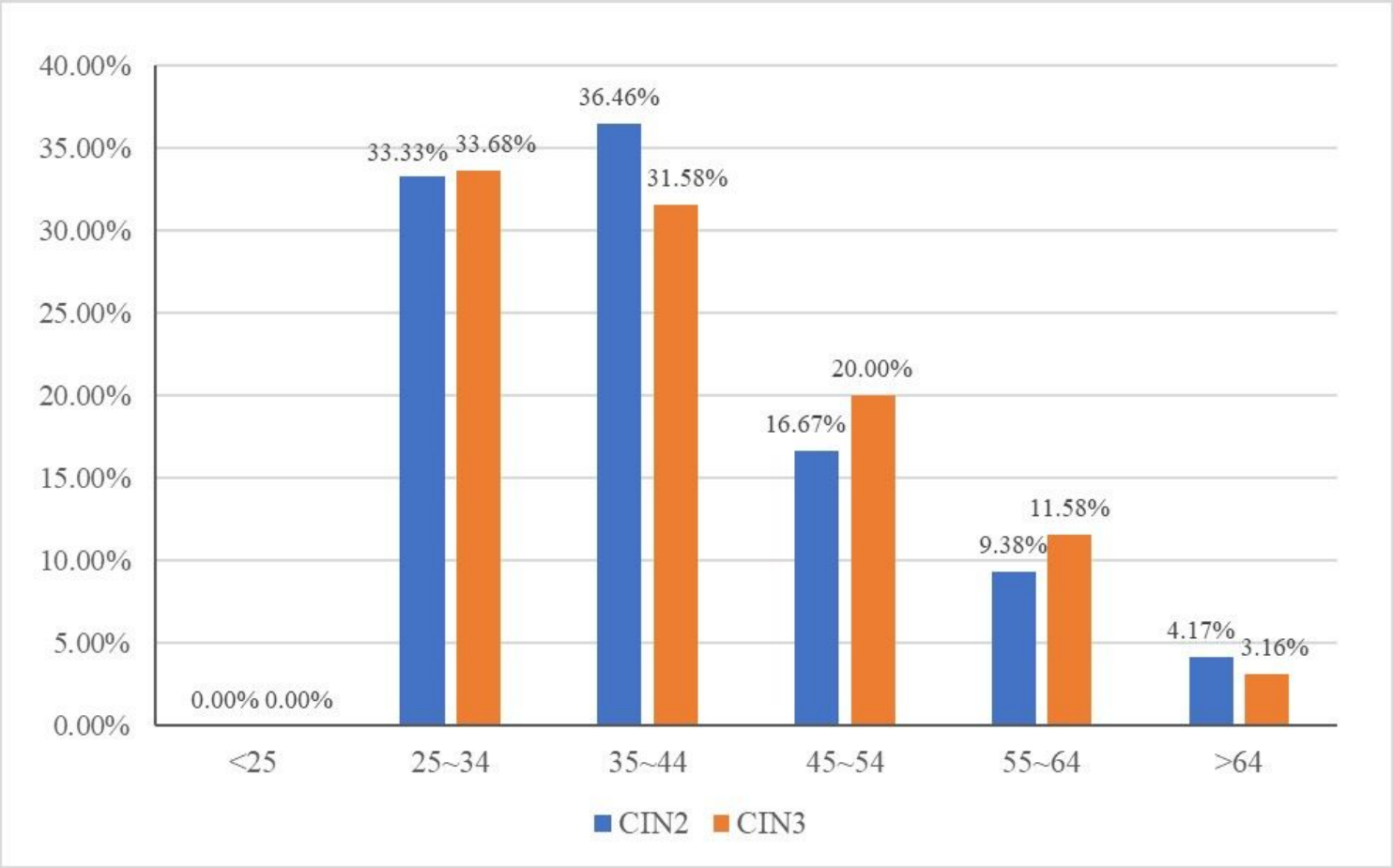

Figure 3

The age distribution of the incidence of CIN2 and CIN3

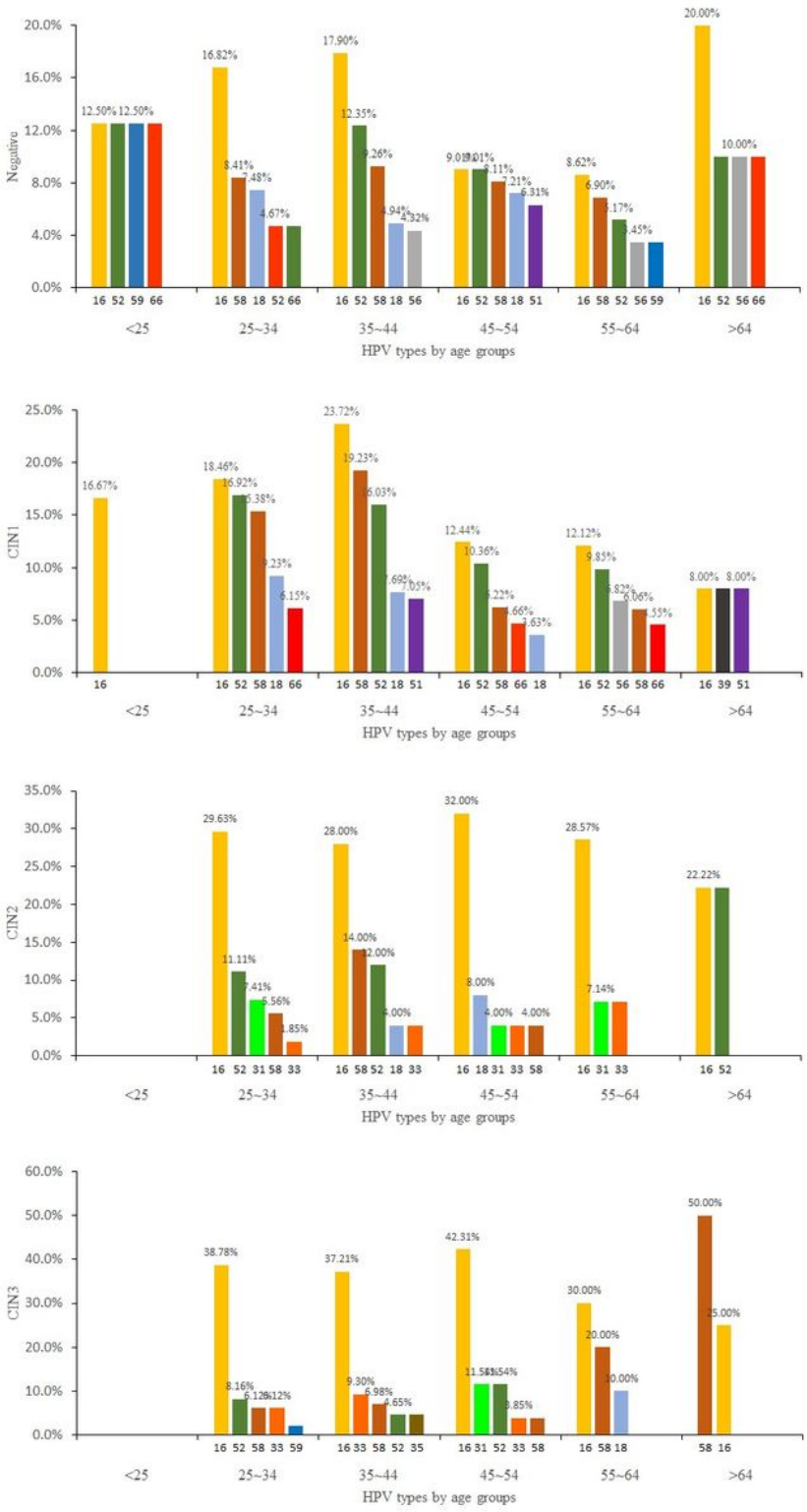

Figure 4

HPV genotypes by age groups in CIN