Prevalence of human papillomavirus infection and its correlation with age and cervical cytology results in Hong Kong

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Abstract

Background: Human papillomavirus (HPV) is one of the most common sexually transmitted viruses. More than 200 genotypes of HPV have been determined and they were classified into low risk (LR-HPV) and high risk (HR-HPV) types depending on their oncogenic potential. The HPV prevalence is essential for planning diagnostic and preventive strategies of HPV-related diseases, but only a few HPV prevalence studies have been performed in Hong Kong which warrants further update.

Objectives: To determine the prevalence of HPV in Hong Kong as well as their correlation with age and cervical cytology results.

Materials and Methods: A total of one hundred and eight archived ThinPrep specimens with age ranged from 20 to 67 were collected from a private laboratory in Hong Kong from January 2017 to April 2017. Laboratory number, age, cytological findings and HPV subtypes were provided for data analysis by SPSS.

Results: The overall HPV prevalence among 108 women in Hong Kong was found to be 41.7%. The most frequently detected HPV genotypes were in the sequence of HPV-66 (8.3%), HPV-16 (6.5%), HPV-53 (5.6%), HPV-52 (4.6%), HPV-56 (4.6%) and HPV-73 (4.6%) which were not consistent with previous studies. The differences could be attributed to small sample size in this study and recent promotion of HPV vaccination program. There was no significant correlation between HPV prevalence and age. However, the HPV positive cases and HR-HPV cases among different age groups showed a similar pattern with bimodal distribution which is comparable with previous studies. Moreover, there was significant correlation between HPV prevalence and abnormal cervical cytology results. These results indicated that the increased prevalence of HPV infections and increased severity of cervical cancer which was in-line with previous studies.

Conclusion: This study showed that the overall HPV prevalence among 108 women in Hong Kong was found to be 41.7%. HPV-66 was the most prevalent genotype, followed by HPV-16, 53, 52, 56 and 73. HPV prevalence was well correlated with abnormal cervical cytology findings. A larger sample size with more patient demographics could enhance the credibility of the results.

Key words: *Human Papillomavirus (HPV), Age, Cytology*

Introduction

Human papillomavirus (HPV) is one of the most common sexually transmitted viruses,[1] which is a non-enveloped and double-stranded DNA virus.[2] More than 200 genotypes of HPV have been determined and they were classified into two groups which are low risk (LR-HPV) and high risk (HR-HPV) types depending on their oncogenic potential.[3]

The correlation between HR-HPV and cervical cancer has been widely investigated and proven.[2,3] For cytology, cervical cancer could be divided from low to high severity as normal, low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL) and invasive cervical cancer (ICC). Alternatively, the cancer can also be classified as atypical squamous cells of undetermined significance (ASCUS) and atypical glandular cells (AGC).

The HPV prevalence is essential for planning diagnostic and preventive strategies of HPV-related diseases, such as choosing different prophylactic HPV vaccines. However, only a few HPV prevalence studies have been performed in Hong Kong which warrants further update.

The main aims of this study were to determine the prevalence of HPV in Hong Kong as well as their correlation with age and cervical cytology results.

Materials and Methods

A total of one hundred and eight archived ThinPrep specimens with age ranged from 20 to 67 were collected from a private laboratory in Hong Kong from January 2017 to April 2017. The DNA was extracted by high pure viral nucleic acid kit (Roche®, USA). The HPV DNA was detected by SNIPER™ high-throughput DNA microarray HPV Genotyping System. Laboratory number, age, cytological findings and HPV subtypes were provided for data analysis by Chi-Square Test and Fisher's Exact Test. All statistical tests were two-tailed and P-value of <0.05 at 95% confidence level was regarded as statistically significant.

Results

Overall HPV prevalence

The prevalence of HPV infections is shown in Table 1. Among the 108 archived specimens, 45 were found to be HPV positive (41.7%). Out of them, 26 (24.1%) were positive for HPV single genotype infection, while 19 (17.6%) were multiple genotypes infection. The prevalence of HPV genotypes is shown in Figure 1. A total of 24 HPV genotypes were identified. The most frequently detected HR-HPV genotypes in descending order were HPV-66 (8.3%),

HPV-16 (6.5%), HPV-53 (5.6%), HPV-52 (4.6%), HPV-56 (4.6%) and HPV-73 (4.6%). Comparatively, the most common LR-HPV genotypes were HPV-43 (2.8%), HPV-54 (2.8%), HPV-40 (1.9%), HPV-55 (1.9%), HPV-67 (1.9%) and HPV-81 (1.9%). The prevalence of HR-HPV (33.3%) was significantly higher than that of LR-HPV (14.8%), as HR-HPV comprised almost 70.0% of the HPV infections.

Table 1. Prevalence of HPV infections among 108 women in Hong Kong.

HPV infections	**No. of positive (%)**
Any HPV genotypes	45 (41.7)
HR-HPV	36 (33.3)
LR-HPV	16 (14.8)
Single infection	26 (24.1)
Double infection	14 (13.0)
Triple or more infection	5 (4.6)

As multiple infections, women may be counted more than once. HR-HPV includes HPV-66, 16, 53, 52, 56, 73, 59, 31, 58, 18, 39, 68 and 82, while LR-HPV includes HPV-43, 54, 40, 55, 67, 81, 6, 11, 42, 62 and 69. Multiple infections include multiple HR-HPV or LR-HPV infection and coinfection with both HR-HPV and LR-HPV genotypes.

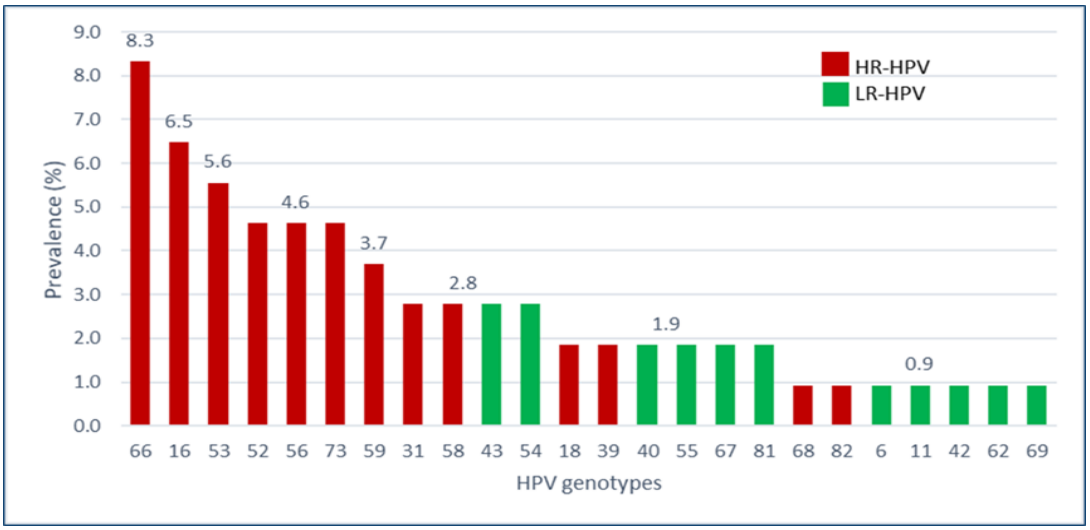

Figure 1. Prevalence of HPV genotypes among 108 women in Hong Kong. As for multiple infections, women may be counted more than once. HR-HPV includes HPV-66, 16, 53, 52, 56, 73, 59, 31, 58, 18, 39, 68 and 82, while LR-HPV includes HPV-43, 54, 40, 55, 67, 81, 6, 11, 42, 62 and 69. The HPV genotypes are arranged according to the prevalence in decreasing order.

Correlation between HPV prevalence with age

The mean age of the 108 recruited women was 39.9 years (range: 20-67, SD: 10.2) with 25 subjects (23.1%) aged ≤30 years, 29 subjects (26.9%) aged 31-40 years, 37 subjects (34.3%) aged 41-50 years, and 17 subjects (15.7%) aged ≥51 years.

The age-stratified prevalence of HPV infections is shown in Figure 2. All the HPV infections among age were in bimodal distribution, as the patterns of HPV positivity and HR-HPV infection were similar, while that of LR-HPV infection was different. The overall HPV prevalence among the age group of ≤30 years was 44.0% and it slightly increased into the first less pronounced peak (44.8%) in the age group of 31-40 years. It declined thereafter with increasing age to 41-50 years (32.2%). The highest overall HPV prevalence was observed in the age group of ≥51 years (52.9%).

For HR-HPV infection among age, the two-peak pattern was similar as HPV positivity. The HR-HPV prevalence among the age groups of ≤30 years was 28.0% and it slightly increased into the first less pronounced peak (34.5%) in the age group of 31-40 years. Then it declined with increasing age to 41-50 years (29.7%). The highest HR-HPV prevalence was observed in the age group of ≥51 years (47.1%).

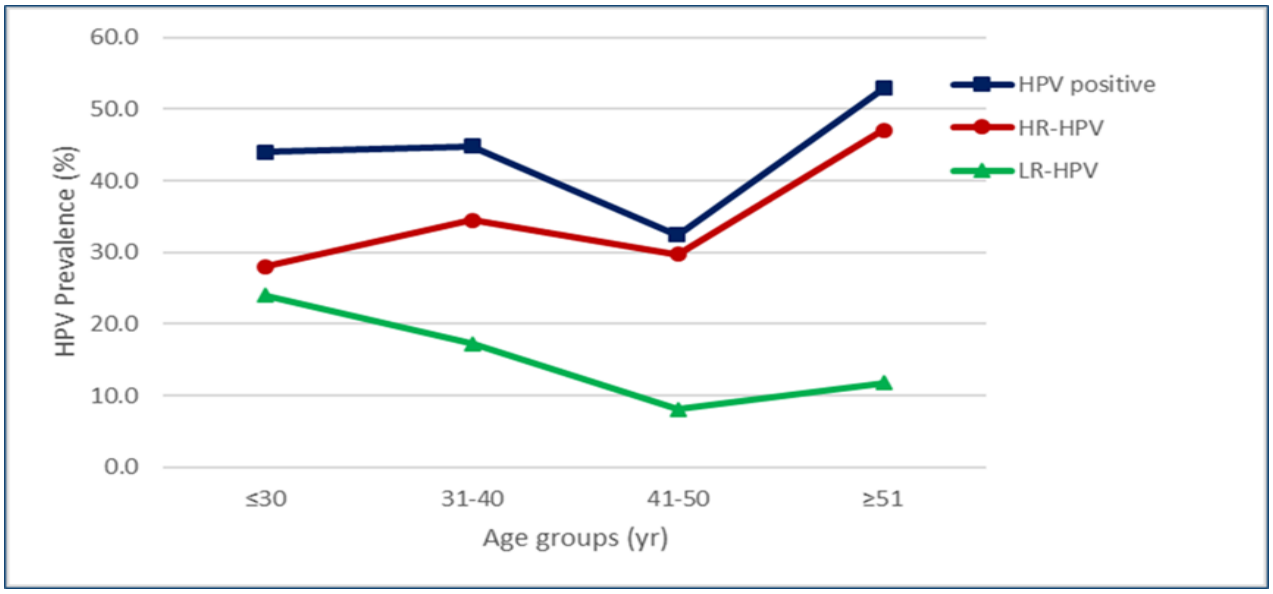

Figure 2. Age-stratified prevalence of HPV infections among 108 women in Hong Kong. HPV positive indicates any HPV genotypes infection, while HR-HPV and LR-HPV indicate the infections of HR-HPV and LR-HPV respectively. All the HPV infections among age were in bimodal distribution.

However, the pattern of LR-HPV infection among age was different with that of HPV positivity and HR-HPV infection. The highest LR-HPV prevalence was observed in the age groups of ≤30 (24.0%). It declined thereafter with increasing age from 31 to 50 years (from 17.2% to 8.1%). The LR-HPV prevalence then increased slightly to the second less pronounced peak in the age group of ≥51 years (11.8%).

Chi-Square Test was applied to determine the correlation between HPV prevalence with age and the correlation is not significant (P=0.501).

Correlation between HPV prevalence with cervical cytology

Among the 108 archived ThinPrep specimens, 72 (66.7%) were found as normal and 36 (33.3%) were found as with abnormal cytology, 16 (14.8%), 1 (0.9%), 18 (16.7%) and 1 (0.9%) specimens were found as with LSIL, HSIL, ASCUS and AGC respectively. Women with abnormal cytology would have higher infection rate of single (41.7%) or multiple (36.1%) HPV genotypes infections.

The prevalence of HPV infections according to cervical cytology results is shown in Figure 3. As expected, women with abnormal cytology results, either LSIL, HSIL or ASCUS, were associated with a higher rate of HPV positivity (77.8%). None of the women with AGC were positively infected with HPV. The distributions of HPV positivity and HR-HPV infection were similar, while distribution of LR-HPV infection was significantly lower in all age groups. For women with normal cytology, there was 23.6% of HPV positive rate. For the abnormal cytology findings, LSIL, HSIL, ASCUS and AGC were found as 87.5%, 100.0%, 72.2% and 0.0% respectively in HPV positive rate.

The distribution of HR-HPV positivity was similar as that of HPV positivity. For women with normal cytology, there was 18.1% with HR-HPV infection. For those with abnormal cytology, 81.3%, 100.0%, 50.0% and 0.0% were found to have LSIL, HSIL, ASCUS and AGC respectively among HR-HPV infection.

However, the distribution of LR-HPV infection was different from HPV positivity and HR-HPV infection. There was 9.7% of LR-HPV infection for women with normal cytology. For abnormal cytology, 25.0%, 0.0%, 27.8% and 0.0% were identified to have LSIL, HSIL, ASCUS and AGC respectively in LR-HPV infection.

Fisher's Exact Test was applied to determine the correlation between HPV prevalence with cervical cytology results and there was a significant correlation between HPV prevalence with cervical cytology ($P < 0.05$).

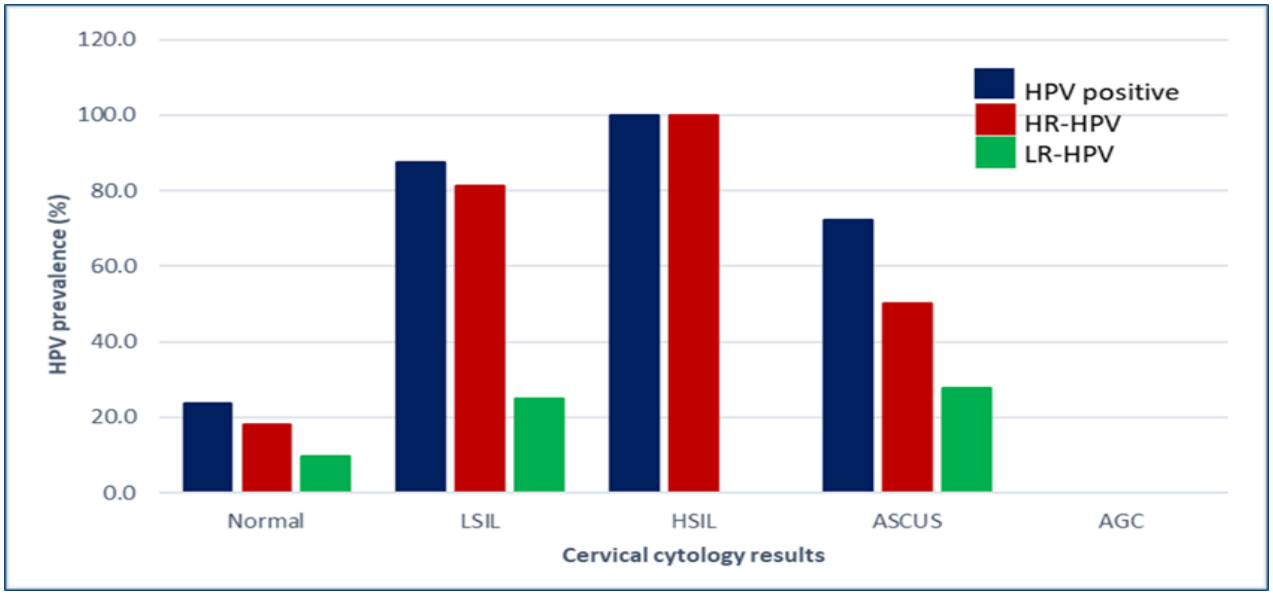

Figure 3. Prevalence of HPV infections according to cervical cytology results among 108 women in Hong Kong. HPV positive indicates any HPV genotypes infection, while HR-HPV and LR-HPV indicate the infections of HR-HPV and LR-HPV respectively. The distributions of HPV positivity and HR-HPV infection were similar, while that of LR-HPV infection was different with others. Women with abnormal cytology results, either LSIL, HSIL or ASCUS, were associated with higher HPV positive rate. None of the women with AGC were HPV positive infection.

Discussion

Overall HPV prevalence

The overall HPV infection rate in this study was higher than that in previous study conducted by Chan *et al*. (41.7% vs 30.6%).[4] The prevalence of HR-HPV (33.3%) and LR-HPV (14.8%) were also higher than that of 14.8% and 10.8% respectively reported in the previous study.[4] The discrepancy observed between studies could be due to different sample selection criteria, in which samples randomly collected in this study were a mixture of normal and abnormal cytology while other studies would select either one.[4,5,6] Since normality of cytology has an impact on prevalence of HPV, it is suggested to compare the results according to cytology subgroups.[4]

From the present study, HPV-66 was found as the most prevalent genotype, followed by HPV-16, 53, 52, 56 and 73, which was different with the previous studies.[5,6] Chan *et al.* showed that the most common HR-HPV correlated with cervical cancer in Hong Kong were HPV-16, 18, 52 and 58, with a prevalence of 50.0%, 21.7%, 12.5% and 8.3% respectively.[5] Lau *et al.* also demonstrated the similar results that the most common HR-HPV correlated with

cervical cancer in Hong Kong were HPV-16, 18, 52 and 58 with a prevalence of 60.2%, 21.6%, 11.9% and 9.3% respectively.[6] The samples recruited in this study came from women with different cytology without cervical cancer cases, while studies of Chan *et al.* and Lau *et al.* selected women with cervical cancer.[5,6] Thus, prevalence of HPV genotype warrants further study and confirmation.

Moreover, the HPV vaccination program may play a role in the prevalence of HPV genotypes in Hong Kong. According to Centre for Health Protection, there are three registered HPV vaccines in Hong Kong which are Cervarix (2-valent vaccine against HPV-16 and 18), Gardasil-4 (4-valent vaccine against HPV-6, 11, 16 and 18) and Gardasil-9 (9-valent vaccine against HPV-6, 11, 16, 18, 31, 33, 45, 52 and 58).[7] Each of them could protect against several HPV genotypes. The prevalence of genotypes covered by the vaccines is lower. For instance, HPV-66 which is not covered with the three registered HPV vaccines was the most prevalent genotype in this study. HPV-18 which is covered with all registered HPV vaccines was relatively less prevalent.[7]

The most common HR-HPV sub-types which are correlated with cervical cancer worldwide were HPV-16, 18, 45, 31 and 33.[7] However, HPV-52 and 58 were found as more common and correlated with cervical cancer in Hong Kong than other regions.[8,9] A recent study conducted by Chan *et al.* showed that the correlation between HPV-52 and 58 with cervical cancer in East Asia including Hong Kong was 3.7 to 4.9 folds higher than other regions.[8] According to another study which recruited 1924 women in Hong Kong, the overall prevalence of HPV-58 was 11.4% and increased with the severity of neoplasia.[9] In the present study, the prevalence of HPV-52 and 58 were 4.6% and 2.8% respectively which were lower than the reported prevalence in other studies. According to Centre for Health Protection, HPV-52 and 58 are covered by Gardasil-9 vaccine and was approved in June 2015 at Hong Kong.[7] Thus, the implementation of vaccine program after 2015 may affect the prevalence of the genotypes.

Correlation between HPV prevalence with age

Age-specific prevalence data on HPV are essential in understanding the trends of HPV prevalence in Hong Kong. In this study, two-peak pattern (bimodal distribution) were found on HPV positivity and HR-HPV prevalence with age. The first peak was found at 31 to 40 years and the second peak at over 51 years. Such distribution is in-line with the worldwide pattern, in which the peaks in HPV prevalence appears among younger women and older women.[10] Besides, the result of this study is also in consonance with the reported meta-analysis study showing that the first peak of HPV prevalence appears in women younger than 35 years of age and a second peak in women aged 45 or older. The two peaks pattern is the predominant prevalence curve reported in women with normal cytology worldwide.[11]

Liu *et al*. found a bimodal distribution of HPV prevalence among age in the Hong Kong.[12] However, the first peak was observed in younger age group of 20 to 29 while the second peak in the group of over 60. Another previous study conducted by Chan *et al.* also found the similar bimodal distribution of HPV prevalence among age in Hong Kong,[4] in which the first peak was observed in younger age group of below 25 years while the second peak in the group of over 46 years. As more young women participated in the vaccination program, the prevalence of HPV in the youngest age group could decrease. In addition, the recruited subjects have aged 5 years older since the Liu *et al.* study, which could account for the shift of HPV prevalence peak as observed in this study.

There are several reasons to account for the bimodal distribution of HPV prevalence with different age groups.[13] The high prevalence of HPV infections among younger women (below 40 years) may be caused by increasing sexual activity or changing high risk sexual behavior. The relatively low prevalence rate among middle age group (41-50 years) may be attributed to development of the immune systems which protect from HPV infections. The second peak among older women (over 51 years) may be resulted from the persistence of HPV infection and decreasing HPV clearance.[13]

Correlation between HPV prevalence with cervical cytology

The correlation between HR-HPV and cervical cancer has been widely investigated and proven.[2,3] In this study, the prevalence of HPV DNA positivity and HR-HPV with more severe lesions were higher than with normal cytology. Such finding resembles the results of previous studies by Muñoz *et al*. and Schiffman *et al*..[2,3] A research conducted by Kjær *et al.* also demonstrated that HR-HPV increased with the severity of neoplasia from 19.2% in women with normal cytology to 100% in women with cervical cancer.[14]

The prevalence of HPV with normal cytology was 15.7% in this study, compared to 25.9% for those with abnormal cytology. A previous study conducted by Bruni *et al.* found that worldwide HPV prevalence in women with normal cytology were 11.7%. The overall HPV prevalence in Southeastern Asia including Hong Kong was 14.0%.[15] Another study conducted by Bao *et al.* showed that prevalence of HPV with ICC, HSIL, LSIL, and normal cytology in Chinese population including China, Hong Kong and Taiwan were 83.7%, 68.3%, 64.7% and 13.0% respectively.[16] The prevalence reported in this study (15.7%) was comparable with the studies of Bruni *et al.* and Bao *et al*..[15,16]

HPV prevalence with other factors

There were some limitations in this study. The number of sample size was not big enough, particularly women aged below 20 or over 60 years and women with HSIL or AGC of cytology findings are lacking, which limits the credibility of the findings.

It is suggested that single and multiple genotype infections may also be a factor that affect HPV prevalence. Thus, further investigation is warranted to establish the correlation between single and multiple genotype infections with HPV prevalence.

Since HPV is mainly sexually transmitted by sex, information on sexual behaviors and number of sexual partners may help to further investigate HPV prevalence. In the study conducted by Baloch *et al.*, it was suggested that sexually transmitted diseases may also be a risk factor for HPV infection.[13] Unfortunately, such information was not available at the present study which deserves further investigation.

The HPV prevalence is essential for planning diagnostic and preventive strategies of HPV-related diseases, such as choosing different prophylactic HPV vaccines. However, only a few local HPV prevalence studies have been performed. The data in present study were collected from private sector for screening purpose which could help formulating better treatment strategies for HPV-related diseases.

Conclusions

This study showed that the overall HPV prevalence among 108 women in Hong Kong was found to be 41.7%. HPV-66 was the most prevalent genotype, followed by HPV-16, 53, 52, 56 and 73. HPV prevalence was well correlated with abnormal cervical cytology findings. A larger sample size with clinical information about sexual behavior of the participants could enhance reliability of the findings in this study.

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