

## Distribution of human papillomavirus types in local population with cytological abnormalities

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### Abstract

**Background:** Human papillomavirus (HPV) is a non-enveloped, small size, and double stranded DNA virus. HPV are usually classified into Low Risk (LR) and High Risk (HR) types. HPV infection is strongly related to cervical cancer development which is a highly lethal cancer for women. In 2006, the first HPV vaccine was licensed in Hong Kong. During the last decade, the prevalence pattern of HPV subtypes may have been changed considering the progress in vaccine development of bivalent HPV vaccine, quadrivalent HPV vaccine and 9-valent HPV vaccine.

**Objective:** To investigate the prevalence pattern of Human Papillomavirus (HPV) in Hong Kong.

**Methods:** One hundred and nine Thinprep® samples were collected from January 2017 to July 2017 and patients' age, cytological finding and HPV sub-type identification were provided by a private laboratory in Hong Kong. Data analysis was done by Microsoft Excel® and Mintab15 software®.

**Results:** The overall prevalence of HPV was 39.4%. The highest infection rate was in the age group 20-29. The 3 most commonly found HPV high-risk subtypes in HPV positive cases were HR-58 (13.4%), HR-52 (9.0%) and HR-59 (7.5%). The 3 most prevalent high-risk subtypes in HPV infected patients with cytological abnormalities were HR-58 (15.8%), HR-59 (10.5%) and HR-39 (10.5%). The most common low-risk subtype in both populations is LR-54 (7.5% in overall positive cases; 7.9% in cervical abnormality).

**Conclusion:** HR-58 infection is the dominant subtype in this studied population. The most prevailing subtype shifts from HR-16 to HR-58 due to increased efficacy of vaccines. Moreover, young population was vulnerable to HPV infection, which might be attributed to change of hormone during post-menopause age, type-specific immunity and other risk factors.

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

## Introduction

Human papillomavirus (HPV) is a non-enveloped, small size (around 8000 bp) double stranded DNA virus which can infect mucosal or skin cells. Genital HPV infection is commonly transmitted by sex.<sup>1</sup> It is highly related to cervical cancer development which can be mostly attributed by HPV infection. According to the data from Department of Health in 2015, 169 deaths caused by cervical cancer have been reported in Hong Kong. Since HPV vaccines have been licensed in Hong Kong for more than 10 years, the prevalence of HPV could have changed significantly. This study aims to investigate the prevalence of HPV in Hong Kong.

## Materials and Methods

There were 109 samples which were collected from Hong Kong females aged 20-70 during January 2017 to July 2017, from a private laboratory in Hong Kong. They are initially collected by cytobrush and stored in Thinprep® bottles for both cytological study and HPV genotyping. 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. The information was provided by the laboratory including patient's age, cytological finding, and HPV genotyping. The data was analyzed by

Microsoft Excel and Mintab15 software, a P value <0.05 was considered as statistically significant.

## Results

All samples were adequately collected for both cytological study and HPV genotyping. Figure 1 shows that 39.4% (43/109) of all cases were determined as infected by any HPV subtypes.

### HPV Overall Prevalence by Age

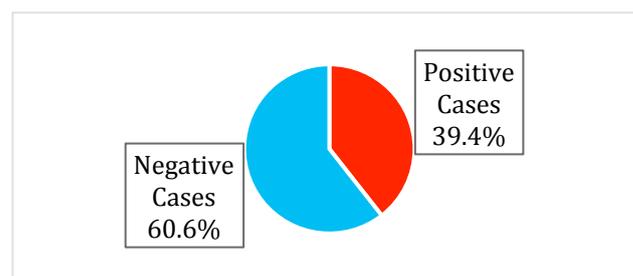


Figure 1: Overall HPV infection rate in 109 specimens (%).

Table 1 showed that the youngest age group 20-29 had the highest infection rate (50%). The age group 50-59 had the second highest infection rate (44%). There was no significant correlation between age and HPV infection rate.

Table 1: The information of HPV infection rate in each age group

Age Group	Cases number	No. of HPV Negative cases	No. of HPV Positive cases	*Distribution of positive cases in aged groups %	^Infection rate in each aged group% (95% CI)
20-29	12	6	6	14.0	50.0 (21.1 - 78.9)
30-39	38	24	14	32.6	36.8 (21.8 - 54.0)
40-49	29	18	11	25.6	37.9 (20.7 - 57.7)
50-59	25	14	11	25.6	44.0 (24.4 - 65.1)
60-69	5	4	1	2.3	20.0 (0.5 - 71.6)
Total	109	66	43	/	/

\*Distribution of positive cases in aged groups

= No. of HPV Positive Cases / 43 (Total positive cases)

^Infection rate in each aged group

= No. of HPV Positive cases/ Cases number in each aged group

(95% Confidence interval was calculated by one proportion calculator with Mintab15)

**Cytological Result vs. HPV Infection**

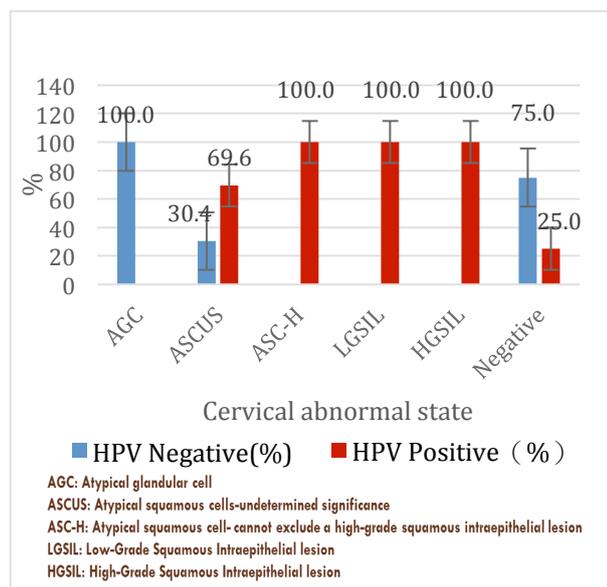


Figure 2 : Infection rate in each cervical abnormal state (%)

There was a significant correlation between cytological state and HPV infection rate

confirmed by Chi Square test (P<0.05). The HPV positive rate in cytologically abnormal state was 72.7% (24/33). Figure 2 shows that the HPV infection rate was more than 50% in most cytological abnormality. Atypical squamous cell-cannot exclude a high-grade squamous intraepithelial lesion (ASC-H), low-grade squamous intraepithelial lesions (LGSIL) and high-grade squamous intraepithelial lesion (HGSIL), which were cervical lesion stage more serious than AGC, showed 100% HPV infection rate (95% CI:  $\geq 5.0\%$ ,  $\geq 60.7\%$  and  $\geq 5.0\%$  respectively).

**Prevalence of HPV in all collected samples vs. cytological samples**

Table 2: The prevalence of HPV subtypes in overall HPV positive and cytological abnormal samples.

HPV Subtypes	Positive Cases (Overall)	*Prevalence rate % (95%CI)	Positive cases (Cervical Abnormality)	^Prevalence rate% (95% CI)
HR-16	2	3.0(0.4 - 10.4)	1	2.6 (0.1 - 13.8)
HR-18	3	4.5(0.9 - 12.5)	1	2.6 (0.1 - 13.8)
HR-33	4	6.0(1.7 - 14.6)	3	7.9 (1.7 - 21.4)
HR-39	4	6.0(1.7 - 14.6)	4	10.5(2.9 - 24.8)
HR-52	6	9.0(3.4 - 18.5)	2	5.3 (0.6 - 17.7)
HR-58	9	13.4(6. - 23.9)	6	15.8(0.6 - 31.3)
HR-59	5	7.5(2.5 - 16.6)	4	10.5(2.9 - 24.8)
LR-6	3	4.5(0.9 - 12.5)	2	5.3 (0.6 - 17.7)
LR-54	5	7.5(2.5 - 16.6)	3	7.9 (1.7 - 21.4)

\* Prevalence rate = Positive Cases (Overall) / 67 (The overall frequency of all HPV subtypes)

^Prevalence rate = Positive cases (Cervical Abnormality) / 38 (The total frequency of HPV subtypes in cytological abnormality)

(95% Confidence interval was calculated by one proportion calculator with Mintab15)

PS: The number of frequency of all HPV subtypes is more than total HPV positive cases since multiple infection cases were counted more than once.

Table 2 illustrates that the prevalence of 9 common HPV subtypes in this study. In HPV infected patients, HR-58 (13.4%), HR-52 (9.0%) and HR-59 (7.5%) were the 3 most common high-risk subtypes. Among HPV carriers with cervical cytologically abnormal, the frequency of HR-58 was still the highest. HR-59 and 39 were the second most prevalent (10.5%, 95% CI: 2.9% - 24.8%). LR-54 was the most prevailing low-risk subtype in both conditions.

## Discussions

Three prophylactic vaccines are currently available in Hong Kong. Quadrivalent vaccine Gardasil<sup>®</sup> against LR-6, LR-11, HR-16 and HR-18 was licensed for 9 to 26-year-old women was licensed in Hong Kong in 2006, and was licensed for 9 to 45-year-old aged group in 2010. The bivalent vaccine Cervari<sup>™</sup> was licensed in Hong Kong in 2008 which aims at HR-16 and HR-18 for 10 to 25-year-old women. The anti-HR-16 and anti-HR-18 antibodies generated by vaccination may provide additional cross-protection effect against non-vaccine type of HPV.<sup>1</sup> The 9-valent HPV vaccine aims at against HR-16, 18, 31, 33, 45, 52, 58 and LR-6, 11, which was licensed for women aged 9 or above in the United States in 2014, is now available in Hong Kong. With the implementation of HPV vaccination program in Hong Kong, changes in HPV infection rate and prevalence pattern are expected.

A previous research in 1999 analyzed 332 samples collected from the Queen Elizabeth

Hospital, and the overall HPV positive rate was 44.3%. It showed that the top two prevalent subtypes were HR-16 and HR-58.<sup>2</sup> Another report published in 2002 by the same authors in which 553 cervical samples were investigated showed that the overall infection rate was 30.6%, and HR-16 and HR-58 were the most common high-risk subtypes followed by low-risk subtype LR-11.<sup>3</sup> A study published last year showed that the infection rate in 2344 Chinese women was 19.5% and HR-52, HR-58 and HR-16 were the most frequent subtypes.<sup>4</sup> The prevalence rate in our study (39.4%) is comparable to the reported range within 19.5%-44.3% based on liquid cytological specimen collection. HR-16 is the second lowest subtype in our study in contrast to the previously reported percentage, possibly due to the official implementation of the vaccine after 2006. HR-58 has been covered in 9-valent after 2015; hence whether the prevalence of HR-58 would change in response to the implementation of vaccination warrants further investigation.

In our study, the HPV positive rate in cytological abnormal state was 72.7% (24/33), which was higher than overall HPV infection rate. There is a significant correlation between HPV infection rate and cytological state in this study. A similar analysis was performed by Ng *et al.* to investigate the correlation of HPV incidence rate and ASCUS samples. The HPV positive rate was 92.1% in all ASCUS samples.<sup>5</sup> Another report published in 2011 which investigated 2790 cervical scrap specimens of South China's women illustrated that the

HPV infection rate was higher in patients who were suffering varying degrees of cervical intraepithelial neoplasia (79.2%-86%) and invasive cervical cancer (88.6%-95.9%).<sup>6</sup> These several reports demonstrate that the infection rate in overall population falls within the reported range of 19.5%-44.3% whereas the rate rises to more than 70% in cervical abnormality state. Hence, cervical health status has an impact on HPV prevalence rate.

The most prevailing subtype in this study is HR-58 in both HPV infected patients or who are also suffering cervical abnormality. The geographic pattern of HPV prevalence was investigated in 2008, which showed that the HR-58 was prevailing in Asia, especially in South China area, except south central Asia. HR-58 was also the third most frequent subtype found in invasive cervical cancer, after the HR-16 and 18<sup>7</sup> and highly related with cervical intraepithelial neoplasia (CIN).<sup>8</sup> The bivalent vaccine has poor efficacy against HR-58 and quadrivalent vaccine presented low effectiveness for CIN grade 1 to 3 prevention, if it was associated with non-vaccine types such as HR-58 and 52.<sup>9</sup> Bivalent vaccine can protect against HR-16 and HR-18 genotypes, while providing little or zero protection to prevent other high-risk subtypes such as HPV-33, HPV-52 and HPV-58.<sup>10</sup> The shift of prevalent HPV subtype from HR-16 and 18 to HR-58 could be attributed to the development of specific vaccines to the HPV subtypes.

Previous studies have shown that low risk subtype was related to genital wart

generation. LR-6 and LR-11 accounted for 90% and 10-30% in genital wart cases, and around 20-50% of lesions are associated with co-infections of other HPV high-risk subtypes.<sup>1,11</sup> However, LR-54 was the most common low-risk subtype in this study, though it is less studied compared to LR-6 and LR-11. A previous study in 2008 demonstrated a high prevalence rate (27%) in CIN 1, while the prevalence was much lower in CIN III and invasive carcinoma (0% and 2% respectively).<sup>12</sup> They suggested that LR-54 was related to low potential of invasive cancer causation.<sup>12</sup>

The most HPV susceptible age group was 20-29 years and the second infection peak was observed in 50-59 years, while 60-69 age group was the most HPV resistant group in this study. Although this report shows a statistically insignificant relationship between age and HPV infection, previous studies suggested that HPV was an aged-specific infection virus. A previous local research showed that the subtypes infection rate peaked in the age group of 26-30 and gradually declined by age, except a small increase in age group of 51-55.<sup>13</sup> The lowest prevalence rate in age group >55 was observed.<sup>13</sup> A review in 2008 showed HPV's geographically age-specificity distribution.<sup>14</sup> The HPV infection almost peaked in younger age (aged  $\leq 25$ ) and the second peak was in older age group (aged 30-50).<sup>14</sup> Many factors could affect the infection rate in population such as smoking, sexual habits and hormonal influence. An explanation for the decline in HPV infection rate across increasing age is that the

type-specific immunity in older aged women is stronger.<sup>15</sup> The second peak might be accounted by an undetectable infection in earlier age, thus the onset of infection occurs during the post-menopausal age when a sudden or gradual loss of type-specific immunity due to imbalanced homeostasis of hormones.<sup>16</sup>

Although this study gives some insight into prevalence of HPV in different cytological states, there are still some limitations which dampen the credibility of our findings. Firstly, a larger sample size would increase the representativeness of this study. In the age group of 60-69 years, more recruited cases would be helpful to calculate the infection rate. In addition, patients' clinical histories are important information for the study of alternation of HPV prevalence pattern, such as the vaccination history, which is not included in this study. The susceptibility of HPV infection is affected by many risk factors, and so a qualitative questionnaire would be helpful to understand the demographics.

### Conclusions

HPV vaccine has been licensed for more than 10 years. HR-16 and HR-18 were initially the main target vaccine type HPV. Compared to previous cohort study, the most prevailing subtype was shifted from HR-16 to HR-58 in this study. Moreover, younger population was more vulnerable to HPV infection, which might be due to the hormonal influences, type-specific immunity and other risk factors.

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