Positivity and prevalence of human papillomavirus among a large population of Hakka women in southern China

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Research article

Keywords: Human papillomavirus (HPV), cervical cancer, prevalence, genotypes, Hakka

Posted Date: May 7th, 2019

DOI: <https://doi.org/10.21203/rs.2.9332/v1>

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Abstract

Background Human papillomavirus (HPV) infections are known to be the main cause of cervical cancer. Thus, detecting HPV genotypes is of great significance for cervical cancer screening. To formulate strategies for prevention of cervical cancer, we studied the HPV infection prevalence and age-specific distribution of female in Hakka area of southern China. **Method** From January, 2016 to July, 2018, 36,871 women from gynecological or reproductive center of Meizhou People's Hospital (Huangtang Hospital), Meizhou Hospital Affiliated to Sun Yat-sen University were recruited in this study. HPV genotypes was detected by flow cytometry method. **Results** HPV infection was observed in 18.34% of the participants, and approximately 79.98% of them were infected with high-risk HPV. The 5 most prevalent genotypes were HPV 52 (18.18%), HPV 16 (16.06%), HPV 58 (11.37%), HPV 53 (8.82%) and HPV 39 (6.42%). The most intensive HPV infections were observed in women aged 40-49 and women aged 30-59 added up to 79.62% of positive patients. **Conclusion** The high HPV infection rate in this Hakka area highlighted the necessity of screening program for HPV infection and cervical cancer among women. The findings of HPV genotypes and the age specific distribution of HPV infection in this area will contribute to the future vaccine development.

Background

Human papillomavirus (HPV), a spherical DNA virus with a genome of 7.9k bp, infects the skin or epithelial mucosa tissue [1]. It is now widely accepted that HPV infection is highly correlated with cervical cancers and other genital diseases [2]. In addition, a recent study has suggested that HPV infection caused at least 25% of head and neck cancers, of which up to 60% were oropharyngeal cancers [3].

Cervical cancer is acknowledged as the third most common type of cancer in females, leading to 530,000 new cases and 275,000 deaths each year [4]. It is worth noting that the prevalence of cervical cancer are high in developing countries. While most cases are detected at a later stage, the outcomes are quite unsatisfactory [5]. The prevalence of cervical cancer of women in China was estimated to be 8.7-11.3/100,000 annually and nearly half of these women died of cervical cancer [6-8]. It is certain that most patients with cervical cancer have infected with HPV [9]. Women under the age of 25 had highest prevalence, and most were infected shortly after sexual activity [10]. In different countries or regions, the distribution of HPV infections and genotypes are various [11, 12]. Guangdong is one of the most important provinces in southern China, with a high-risk HPV infection rates (20.02%) above the average [6].

Since cervical cytology testing for HPV was subjective and comparatively insensitive, molecular testing methods such as HPV genotyping, has been recommended for HPV detection [9]. About a total of 200 HPV subtypes have been identified, which characterized with the HPV acronym and assigned number. Meanwhile, these subtypes were classified as high-risk or low-risk based on the oncogenic risk. There are 12 HPVs, including HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58 and HPV59, which are considered as high-risk and HPV68 is considered as less evident high-risk [9]. These 13 HPVs cause more than 96% of cervical cancers [13]. In addition, another 11 HPVs including HPV26, HPV30, HPV34, HPV53, HPV66, HPV69, HPV70, HPV73, HPV82 and HPV97 are classified as high-risk because of the close association with cervical cancer [14]. Low-risk genotypes, including HPV6, HPV11, HPV40, HPV42, HPV43, HPV44, HPV55, HPV61, HPV81, and HPV83, typically result in benign conditions [15]. The progression from HPV infection to cervical cancer usually takes several years, and women with persistent HPV infection are more likely to suffer cervical cancer [16]. It can be seen that HPV genotyping is of great importance in early diagnosis, treatment and prognosis of cervical cancer.

It has been proved that HPV vaccine can prevent persistent infection and cervical disease [17]. So far, three virus-like particle vaccines are available on the market, including HPV16/18 (Cervarix), HPV6/11/16/18 (Gardasil) and HPV6/11/16/18/31/33/45/52/58 (Gardasil) [18]. The detection and genotyping of HPV are significantly. meaningful, which providing guidance for development of HPV vaccine.

Meizhou, located in the northeastern of Guangdong Province, has an area of 15,876 square kilometers and a population of 5.15 million. It is one of the most concentrated settlements of Hakka people and known for its unique traditions and culture [19]. The physical characteristics of Hakka are differ from the populations of northern and southern China [20]. However, few reports on the prevalence and genotype distribution of HPV infection among Hakka people in Meizhou.

In the present study, we tested and genotyped HPV infection among women attending gynecological or reproductive center of Meizhou People's Hospital from January 1, 2016 and July 7, 2018. The aim of this study was to assess HPV prevalence and genotype distribution among Hakka women and to provide epidemiological evidence for the clinical application of HPV vaccines in these regions.

Methods

Study subjects

A total of 36,871 study subjects enrolled in this research were Hakka women who attending gynecological or reproductive center of Meizhou People's Hospital (Huangtang Hospital), Meizhou Hospital Affiliated to Sun Yat-sen University during January 1, 2016 and July 7, 2018. The eligible participants should meet three criterions: (i) age 15 to 89; (ii) never infected HPV before; (iii) provided sufficient DNA for HPV screen. This study was approved by Ethics Committee of Meizhou People's Hospital. Informed consent was obtained from all participants. If the participants were children (under 16 years of age), informed consents were collected from the parents. To protect privacy, the data were de-identified before analysis.

Sample collection

Cervical specimens were collected by gynecologists using plastic cervical brushes. Briefly, cell brush was inserted into cervix, rotated five full turns clockwise, put the head of brush at transport medium tube (Tellgen Life Science Co., Shanghai, China) and stored at 4 °C.

DNA extraction

DNA was isolated from cervical specimen using DNA extraction kit (Tellgen Life Science Co., Shanghai, China) following manufacturer's protocol. Briefly, samples were divided into four groups according to the turbidity: (i) clarified, (ii) mild turbid, (iii) moderately turbid and (iv) severely turbid. Vibrated samples for 2 minutes, transferred various volumes of samples to 1.5 ml vessels based on sample quality, i.e. 800ul, 500ul, 300ul and 100ul for i, ii, iii and iv, respectively. Then added transport medium to 1 ml, mixed, centrifuged at 12,000 rpm for 3 minutes. Remove supernatant and suspend pellet with 200ul extraction reagent and place in metal bath for 15 minutes at 100 °C. Centrifuge at 12000 rpm for 5 minutes and supernatant are ready for use.

Genotyping of HPV

HPV DNA genotyping was performed using Tellgenplex® HPV27 genotyping assay (Tellgen Life Science Co., Shanghai, China) and following manufacturer's protocol. This method detected 27 HPV genotypes, including 17 high-risk (HR) genotypes, i.e. HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 82, and 10 low-risk (LR) genotypes i.e. HPV 6, 11, 40, 42, 43, 44, 55, 61, 81, 83. Briefly, each color-coded bead was coated with a pre-designed HPV type-specific probe. The HPV DNA sample was amplified using biotin-labeled consensus PCR primers. The products were hybridized to beads bearing type-specific probes, then incubating with phycoerythrin (PE)-conjugated streptavidin (SA-PE), and signal were analyzed on a Luminex 200 system (Luminex Corporation, Texas) [21].

Data analysis

The total accounts and percentage of females in different age groups per year was shown. One, two or more types were identified as single, double and multiple HPV infection. The incidence of specific HPV and corresponding 95% confidence interval (CI) were provided. Data statistical analysis was performed using SPSS (Statistical Package for the Social Sciences, Chicago, IL). Pearson 2 or Fisher exact tests were used appropriately. $P < 0.05$ was considered statistically significant.

Results

Prevalence of HPV infection

A total of 38,851 women attending gynecological or reproductive center of Meizhou People's Hospital that received HPV DNA test during January 1, 2016 and July 7, 2018. 1980 women were excluded in our study as they didn't meet the inclusion criterions (Fig.1). In the end, 36,871 participants were included in this study for analysis. The average age of the participants was 42 years.

The number of women infected with HPV was 6,761 (18.34%) in this period. Among these women, 5,408 (14.34%), 1,353 (3.67%) and 760 (2.06%) had HR infection, LR infection, both HR and LR infection, respectively. In the meanwhile, 3.06% women were infected with double HPV and 1.17% women were infected with multiple HPV (Table 1).

Genotype distribution of HPV infection

Twenty-seven HPV types were detected in our study. It was observed that HPV 52 was the most predominant genotype, with a proportion of 18.18%, other subtypes in top ten were followed by HPV 16, 58, 53, 39, 18, 81, 51, 61 and 44, with corresponding proportions of 16.06%, 11.37%, 8.82%, 6.42%, 5.81%, 5.80%, 5.50%, 4.90% and 4.82% (Table 2). Most of these infections were HR HPVs, while HPV 81, 61 and 44 were LR HPVs. Multiple HPV infections were general, HR subtypes like HPV 52, 16 and 58 were observed with high infection rates in women.

Annual HPV type-specific positivity from 2016 to 2018 was shown in Table 3. Obviously, most HPV positivity, HR and LR infections tend to stabilize during this period, but HPV53 and HPV68 shown an increasing trend.

Age-specific prevalence of HPV infection

Age-specific prevalence of HPV infection was determined in Hakka area of Southern China in our study. As shown in Table 4, most HPV infections were occurred in women with age of 30-59, followed by 20-29. The 30-59 age women account for over 79% of the HPV positivity.

Furthermore, positive rates of HPV subtypes in different age groups were diverse. As illustrated in Fig. 2, the age-specific prevalence of total HPV infections appeared as a U-shaped curve. Positive rates of HPV combined infection (4v HPV, 9v HPV, HR HPV) peaked among participants aged 15-19 years, stabilized among participants aged 20-59 years, then increased again among participants older than 60 years old. Combined HPV 6/11 infection declined with age, while combined HPV 16/18 infection increased with age.

Discussion

In the present study, we investigated the prevalence of HPV infection in Hakka region during 2016 to 2018 with a large scale of study participants. We identified the top 10 HPV genotypes that infected Hakka women, and found that HPV 52 was the most prevalent single HPV genotype. Combined HPV infections were common. We have revealed the age-specific distribution of HPV infection. To our knowledge, this is the first large-scale study to investigate the genotypes (27 subtypes) of HPV infection among women in Hakka area of southern China.

It is doubtless that persistent HPV infection is a major risk factor for cervical cancer and most common carcinogenic subtypes are HPV16 and 18 [22, 23]. In China, cervical cancer was the second leading cause of cancer-related death among women aged 15 to 44 years [24]. Our study found that over 90 percent of HPV infections occurred in participants between 20 and 59 years, which emphasizes the protection of these women against HPV infection. In developed countries where cervical cancer screening systems have been established, the prevalence of cervical cancer and the resulting mortality of patients have shown a significant downward trend after screening [25, 26]. Molecular detection of HPV for cervical cancer screening and early diagnosis has been widely recognized with its high sensitivity and specificity [27, 28].

In addition to HPV screening, it has been proven that HPV vaccination can fundamentally block HPV transmission. At the end of 2014, World Health Organization (WHO) issued a position paper on HPV vaccine, and recommended that 9 to 13-year-old girls should receive HPV vaccine to prevent cervical cancer [18]. Until now, three HPV vaccines were applied to protect HPV infection, including bivalent vaccine (HPV16/18), tetravalent vaccine (HPV6/11/16/18) and nonavalent vaccine (HPV6/11/16/18/31/33/45/52/58) [29-31]. Clinical trials have confirmed that the bivalent vaccine and the tetravalent vaccine can prevent 90% to 98% of cervical intraepithelial neoplasia, however, these two vaccines have no preventive effect on anal and genital precancerous lesions in women with HPV subtypes infected [32].

Our study found that the infection rate of the overall HPV was 18.34% among participants who received HPV screening in Hakka area of southern China. Forman et al. found that the infection rate of HPV among women worldwide is 11% to 12% [5]. As the reports showed, the overall HPV infection rate of women was 11.4-20.3% in China, and total prevalence of HR HPV was 21.07% in Guangdong Province [6, 11]. However, HPV infection rates varied widely in different cities in Guangdong Province. Studies showed that the

infection rate of HPV was 10% in Guangzhou, 12.3% in Heyuan, 12.6% in Chaozhou and 18.4% in Shenzhen [33-36]. Definite evidence suggested that several factors were conducive to the prevalence of HPV, including genetic susceptibility, sexual behavior (earlier age of sexual activity, too many sexual partners), long-term use of contraceptives and life-style [37, 38]. HPV infection rate in this study were lower than our previous report (19.81%) [39], which due to large samples were collected from women attending both gynecological and reproductive center in present study. Furthermore, of the twenty-seven HPV genotypes we screened, HPV52, 16, 58 and 53 were observed to be the top four common types among women in Hakka area, which totally up to 54.43% of the HPV infection. This is similar to those results of the studies on specific population in China [6, 40]. This highlights that HPV vaccines for HPV16, 18, 58 and 52 would be valuable for people in Hakka area in southern China and vaccines for HPV53 might be study in future.

A previous meta analysis reported that HPV infection rate was higher in the younger and older age females all over the world [11]. The previous studies showed that in the cities of China, such as Kunming and HongKong, HPV prevalence also presented the similar pattern [33, 41]. Our present study observed a similar pattern, with peaks in participants aged 15-19 years or 80-89 years. The precise mechanism between HPV infection and age is unclear. The likely reason is that women in these ages have lower level of hormones and weaker immune functions, so the ability to resist HPV infection is weak. In addition, a large number of younger women fail to protect themselves during sexual activity, which makes them susceptible to HPV infection [9]. Also, our data presented the prevalence rate of age-specific HPV infection in women, which found that most HPV infection occurred in women between 40 to 49 years old. It might cause the largest number of participants in these age groups. In addition, women of these ages were gravely affected by HPV infection. As the large-scale study reporting year- and age-specific HPV positivity in Hakka area of China, the information provided in present study would be beneficial for protecting women from HPV infection.

Conclusion

Our study urges the need of a screening program for HPV infection and cervical cancer for both younger and older women. Additionally, vaccination about HPV infection and sexual education campaigns should begin in youth. The findings provide useful information for vaccine development and vaccination strategies against HPV infection in Hakka area of southern China.

Abbreviations

HPV: Human papillomavirus; HR: High-risk; LR: Low-risk; PE: phycoerythrin; SA-PE: phycoerythrin-conjugated streptavidin

Declarations

Ethics approval and consent to participate

This study was approved by the Human Ethics Committees of Meizhou People's Hospital (Huangtang Hospital), Meizhou Hospital Affiliated to Sun Yat-sen University, Guangdong Province, China. Informed consent was obtained from all participants. If the participants were children (under 16 years of age), informed consents were collected from the parents.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated during the current study are not publicly available yet, due to privacy concerns and ongoing additional research. Data can be made available for peer review on reasonable request through contacting the corresponding author.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by the grants from Science and Technology Program of Meizhou (Grant No.:2018B027), Key Scientific and Technological Project of Meizhou People's Hospital, (Grant No.: MPHKSTP-20170101).

Authors' contributions

SL conceived and designed the experiments; XG contributed to the data collection and the manuscript draft. RW, JL and ZZ helped to collect clinical data, conducted the clinical performances and researches; SL and XG analyzed the data; SL and XG wrote the paper.

Acknowledgements

The authors would like to thank other colleagues whom were not listed in the authorship of Clinical Core Laboratory, Meizhou People's Hospital (Huangtang Hospital), Meizhou Hospital Affiliated to Sun Yat-sen University for their helpful comments on the manuscript.

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Tables

Table 1 Prevalence of HPV infection among Hakka women in Southern China

	Single, n (%)	Double, n (%)	Multiple, n (%)	Overall, n (%)
Negative	-	-	-	30110(81.66%)
Positive	5201(14.11%)	1130(3.06%)	430(1.17%)	6761(18.34%)
HR	3951(10.72%)	987(2.68%)	470(1.27%)	5408(14.34%)
LR	1250(3.39%)	99(0.27%)	4(0.01%)	1353(3.67%)
HR+LR	-	461(1.25%)	229(0.81%)	760(2.06%)

HR: high-risk; LR: low-risk

Table 2 Genotypes of HPV infection among Hakka women in Southern China

HPV Type	Single, n (%)	Double, n (%)	Multiple, n (%)	Overall, n (%)
6	142(2.10%)	81(1.20%)	32(0.47%)	255(3.77%)
11	84(1.24%)	53(0.78%)	31(0.46%)	168(2.48%)
16	776(11.48%)	204(3.02%)	106(1.57%)	1086(16.06%)
18	241(3.56%)	85(1.26%)	67(0.99%)	393(5.81%)
26	9(0.13%)	3(0.04%)	4(0.06%)	16(0.24%)
31	80(1.18%)	43(0.64%)	14(0.21%)	137(2.03%)
33	155(2.29%)	63(0.93%)	60(0.89%)	278(4.11%)
35	69(1.02%)	38(0.56%)	26(0.38%)	133(1.97%)
39	243(3.59%)	111(1.64%)	80(1.18%)	434(6.42%)
40	34(0.50%)	21(0.31%)	22(0.33%)	77(1.14%)
42	83(1.23%)	56(0.83%)	26(0.38%)	165(2.44%)
43	161(2.38%)	85(1.26%)	55(0.81%)	301(4.45%)
44	194(2.87%)	83(1.23%)	54(0.80%)	331(4.90%)
45	52(0.77%)	27(0.40%)	14(0.21%)	93(1.38%)
51	210(3.11%)	113(1.67%)	49(0.72%)	372(5.50%)
52	807(11.94%)	329(4.87%)	93(1.38%)	1229(18.18%)
53	324(4.79%)	171(2.53%)	101(1.49%)	596(8.82%)
56	164(2.43%)	116(1.72%)	46(0.68%)	326(4.82%)
58	478(7.07%)	182(2.69%)	109(1.61%)	769(11.37%)
59	117(1.73%)	84(1.24%)	59(0.87%)	260(3.85%)
61	188(2.78%)	97(1.43%)	58(0.86%)	343(5.07%)
66	91(1.35%)	85(1.26%)	32(0.47%)	208(3.08%)
68	76(0.67%)	45(0.67%)	14(0.21%)	135(2.00%)
81	220(3.25%)	108(1.60%)	64(0.95%)	392(5.80%)
82	59(0.87%)	28(0.41%)	22(0.33%)	109(1.61%)
83	35(0.52%)	30(0.44%)	21(0.31%)	86(1.27%)

Table 3 Positivity of HPV infection among Hakka women in Southern China from 2016 to 2018

HPV type	2016(N=13632)		2017(N=15286)		2018(N=7953)		P
	n	%(95% CI)	n	%(95% CI)	n	%(95% CI)	
6	99	0.73(0.58-0.87)	105	0.69(0.56-0.82)	53	0.67(0.61-0.72)	0.861
11	66	0.48(0.37-0.60)	75	0.49(0.38-0.60)	28	0.35(0.31-0.39)	0.284
16	424	3.11(2.82-3.40)	419	2.74(2.48-3.00)	243	3.06(2.94-3.18)	0.145
18	136	1.00(0.83-1.16)	179	1.17(1.00-1.34)	78	0.98(0.91-1.05)	0.253
26	4	0.03(0.00-0.06)	6	0.04(0.01-0.07)	6	0.08(0.06-0.09)	0.136
31	53	0.39(0.28-0.49)	56	0.37(0.27-0.46)	28	0.35(0.31-0.39)	0.904
33	101	0.82(0.68-0.97)	110	0.72(0.59-0.85)	67	0.84(0.78-0.91)	0.576
35	64	0.47(0.35-0.58)	46	0.30(0.21-0.39)	23	0.29(0.25-0.33)	< 0.05
39	186	1.36(1.17-1.56)	169	1.11(0.94-1.27)	79	0.99(0.92-1.06)	< 0.05
40	25	0.18(0.11-0.26)	39	0.26(0.18-0.34)	13	0.16(0.14-0.19)	0.249
42	57	0.42(0.31-0.53)	63	0.41(0.31-0.51)	45	0.57(0.51-0.62)	0.203
43	102	0.75(0.60-0.89)	120	0.79(0.65-0.92)	53	1.28(1.04-1.52)	0.608
44	135	1.18(1.00-1.36)	126	0.82(0.68-0.97)	70	0.88(0.82-0.95)	0.322
45	34	0.25(0.17-0.33)	43	0.28(0.20-0.37)	16	0.20(0.11-0.30)	0.511
51	124	0.98(0.82-1.14)	180	1.18(1.01-1.35)	68	0.86(0.79-0.92)	< 0.05
52	431	3.16(2.87-3.46)	530	3.47(3.18-3.76)	268	3.37(3.24-3.50)	0.345
53	224	1.64(1.43-1.86)	219	1.43(1.24-1.62)	153	1.92(1.83-2.02)	< 0.05
56	135	0.99(0.82-1.16)	124	0.81(0.67-0.95)	67	0.84(0.78-0.91)	0.242
58	279	2.05(1.81-2.28)	315	2.06(1.84-2.29)	175	2.20(2.10-2.30)	0.719
59	93	0.68(0.54-0.82)	109	0.71(0.58-0.85)	58	0.73(0.67-0.79)	0.913
61	112	0.82(0.67-0.97)	140	0.92(0.76-1.07)	91	1.46(1.20-1.71)	0.057
66	82	0.60(0.47-0.73)	80	0.52(0.41-0.64)	46	0.58(0.53-0.63)	0.663
68	44	0.32(0.23-0.42)	49	0.32(0.23-0.41)	42	0.53(0.48-0.58)	< 0.05

81	146	1.07(0.90-1.24)	167	1.09(0.93-1.26)	79	0.99(0.92-1.06)	0.778
82	48	0.35(0.25-0.45)	42	0.27(0.19-0.36)	19	0.24(0.20-0.27)	0.277
83	30	0.22(0.14-0.30)	36	0.24(0.16-0.31)	8	0.10(0.08-0.12)	0.076
6/11	165	1.21(1.03-1.39)	175	1.14(0.98-1.31)	82	1.03(0.96-1.10)	0.490
16/18	547	4.01(3.68-4.34)	581	3.80(3.50-4.10)	318	4.00(3.86-4.13)	0.602
4v HPV	698	5.12(4.75-5.49)	735	4.81(4.47-5.15)	395	4.97(4.82-5.12)	0.475
9v HPV	1472	10.80(10.28-11.32)	1605	10.50(10.01-10.99)	845	10.62(10.41-10.84)	0.713
HR HPV	2052	15.05(14.45-15.65)	2204	14.42(13.86-14.98)	1152	14.49(14.24-14.73)	0.274
LR HPV	513	3.76(3.44-4.08)	549	3.59(3.30-3.89)	291	3.66(3.53-3.79)	0.739
ANY HPV	2565	18.82(18.16-19.47)	2753	18.01(17.40-18.62)	1443	18.14(17.88-18.41)	0.185

4vHPV: 4-valent HPV (HPV6, 11, 16 and 18); 9vHPV: 9-valent HPV (HPV6, 11, 16, 18, 31, 33, 45, 52 and 58); HR HPV: high risk HPV (HPV16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68 and 82); LR HPV: low risk HPV (HPV6, 11, 40, 42, 43, 44, 55, 61, 81 and 83). Any HPV: any of the 27 HPV types mentioned above.

Table 4 Age-specific distribution of HPV among Hakka women in Southern China

Age(year)	2016(N=13632)		2017(N=15286)		2018(N=7953)		HPV positive(N=6761)	
	n	%	n	%	n	%	n	%(95%CI)
15-19	153	1.12	81	0.50	29	0.37	51	0.75(0.55-0.96)
20-29	1484	10.89	1719	10.55	769	9.66	738	10.92(10.17-11.66)
30-39	3051	22.38	3552	21.80	1768	22.23	1567	23.18(22.17-24.18)
40-49	5194	38.10	5553	34.07	2749	34.56	2461	36.40(35.25-37.55)
50-59	2687	19.71	3019	18.53	1810	22.76	1355	20.04(19.09-21.00)
60-69	809	5.93	1069	6.56	615	7.73	435	6.43(5.85-7.02)
70-79	223	1.64	239	1.47	162	2.04	126	1.86(1.54-2.19)
80-89	31	0.23	53	0.32	51	0.64	28	0.41(0.26-0.57)

Figures

Figure 1

Flow chart of participant inclusion and exclusion

Figure 2

Age-specific prevalence of HPV infection among Hakka women in Southern China by age group. 4v HPV: 4-valent HPV (HPV6, 11, 16 and 18); 9v HPV: 9-valent HPV (HPV6, 11, 16, 18, 31, 33, 45, 52 and 58); HR HPV: high risk HPV (HPV16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68 and 82); LR HPV: low risk HPV (HPV6, 11, 40, 42, 43, 44, 55, 61, 81 and 83). Any HPV: any of the 27 HPV types mentioned above.