RESEARCH ARTICLE

Genotype Distribution of Human Papillomavirus in Women with Abnormal Cervical Cytology in an Esophageal Carcinoma High Incidence Area of China

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Abstract

Infection with human papillomavirus (HPV) could affect genesis of both cervical and esophageal cancers. The type-specific distribution of HPV in cervical cytology abnormalities of women has remained unclear in Shantou, an esophageal cancer high-incidence area of China. Data from 22,617 women who were subjected to cervical HPV DNA testing with simultaneous cervical cytological examination during 2009-2013 were therefore here retrospectively evaluated in a hospital-based study. Overall, 16.2% (3,584/22,114)of women with normal cytology were HR-HPV positive, with HPV-52 (4.07%) as the most common type followed by -16 (3.63%), and -58 (2.46%). Prevalence of HR-HPV was 50.3% (253/503) in women with cervical cytological abnormalities, of which in ASC-H 71.4%, ASC-US 39.1%, HSIL 80.3% and LSIL 73.7%. HPV-58 (14.12%) was the most common type for all cervical cytological abnormalities, followed by HPV-16 (13.72%), and -52 (12.72%), while the more common HPV-16 type in ASC-H (42.9%) and HSIL (36.1%), HPV-52 and -58 were the most common types for ASC-US (10.3%) and LSIL (25%), respectively. Multiple HPV co-infections were identified in 33.2% (84/253) cytology abnormalities with positive HR-HPV, and the highest prevalence of HPV-58/16 combination in HSIL (28.6%, 6/21) was observed. Our data indicated a relative high prevalence of HPV-58 and -52 in women with cervical cytological abnormalities, which should be considered in the development of next-generation vaccines for Shantou.

Keywords: HPV - cervical cytological abnormalities - prevalence - Shantou, China

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Introduction

Cervical cancer is the third most commonly diagnosed cancer and the fourth leading cause of cancer death in females worldwide, which accounting for 9% of the total new cancer cases and 8% of the total cancer deaths among females with more than 85% of these cases and deaths occur in developing countries (Jemal et al., 2011). The incidence of cervical cancer was estimated about 8.7/100, 000 in females and accounts about 6.3% of all female cancer in China (Kim et al., 2009; Guo et al., 2012; Wang et al., 2012), with obviously geographic variation (Li et al., 2011; Shi et al., 2012). Approximately 9.09% cytological positive rate of cervical cancer and its precancerous lesions had been found in Guangdong, southern China (Zheng et al., 2009). Interestingly, the Shantou coastal area of eastern Guangdong has also a high incidence of esophageal cancer (EC) (Su et al., 2007). Histological type for both cancers is squamous cell carcinoma (SCC) in Shantou, and esophageal SCC (ESCC) and cervical SCC (CSCC) have similar multi-stage histopathological progression pattern: from normal mucosa to dysplasia, carcinoma in situ, and invasive carcinoma. Moreover, the predilection site of both ESCC and CSCC is the junction of the squamous and columnar epithelium (Liu et al., 2014). Those similarities supported that common environment risk factors might have etiologic effect on both ESCC and CSCC.

It is well-established that human papillomavirus (HPV) infection is the central cause in the development of cervical cancer. Furthermore, the HPV as oncogenes or co-factors in the carcinogenesis of EC has been reported in the last 30 years (Petrick et al., 2014), especially combinative study of HPV infection rate on EC and cervical cancer tumor samples in geographic areas with a high incidence of EC (Zhang et al., 2010; Liu et al., 2014). According to epidemiologic classification, at least 15 oncogenic HPV types were found in cervical precancerous lesions and carcinoma, of which HPV16 and HPV18 were the most

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prevalent high-risk types worldwide, whereas HPV-58 has been found in a relatively higher proportion in eastern Asia (Chan et al., 2012). HPV prevalence of cervical smear in women shows different by geographic region varied from 6.7% to 29.6% found by population-based studies with different type-specific in China and 9.03% was found in Chaozhou population, which is closed to Shantou, with the most common type of HPV-52 and -58 (Lin et al., 2008). The incidence rate was 65.5% and 69.1% in tissues of ESCC cancerous and precancerous have been reported in Shantou (Shen et al., 2002). However, to our knowledge, no reliable epidemiologic data described HPV type-specific distributions in cervical precancerous lesions in Shantou have been reported. Current vaccines protect against 2 HPV types, HPV 16 and 18, which are associated with 70% of cervical cancers and 50% of high-grade cervical lesions. Perhaps, considering of geographic variation and type-specific HPV prevalence in women with cervical cytologic abnormalities could offer more benefit from cervical cancer (Clifford et al., 2005; Gage et al., 2013).

The aim of this present study was to address the description of HPV prevalence and type-specific distribution in women with cervical cytological abnormalities and to identify the most frequent multiple HPV types associated with different cervical lesions in Shantou population, which has high incidence of EC by a hospital-based study.

Materials and Methods

Study design and population

Shantou locates on the southeast coast of Guangdong province and is adjacent to Chaozhou, Jieyang city and southwestern Fujian province and Taiwan. Shantou City has a total area of 2, 064 square kilometer, with a population of about 5, 294, 400. The study group was consisted two kinds of consecutive participants who proceeded to routine screening or the Outpatient Gynaecological Clinic of the First Affiliated Hospital, Shantou University Medical College between September 2009 and August 2013. Eligible participants were identified from the database. Women were eligible if they had no previous diagnosis or treatment for cervical or vaginal disease, had not undergone hysterectomy and were not pregnant. All participants included in the study gave their written informed consent after discussing with Gyneacologists that there would be no implications to their health. The studied population was divided into 6 age groups according to the biological alterations in women's reproductive system (18–25 years, 26–35 years, 36-45 years, 45-55 year, 56-65 years and >65 years). Procedures for this study were approved by Research Ethics Boards at Shantou University Medical College.

Cervical specimen collection and HPV DNA extraction

Cervical exfoliated cell specimens were obtained by a gynecologist as part of routine investigative procedures at the hospital. Two separate cervical exfoliated cell specimens were collected independently for liquid-based cytological diagnosis and HPV DNA genotyping assays. For HPV DNA extraction, cells stored at a specimen transport medium (Hybribio Biotechnology Limited Corp., Chaozhou, China). High-quality DNA was yielded from lysis of cells by isolation of DNA, precipitation, and purification according manufacturer's instruction (Hybribio Biotechnology Limited Corp., Chaozhou, China).

Cytological diagnosis criteria

Cervical slides were prepared using a liquid-based cytologymethod. Cytological classifications of disease grade were made in conformity to the Bethesda 2001 criteria (TBS2001) (Solomon et al., 2002). The evaluation system included: negative (0), atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), squamous cell carcinoma (SCC) and atypical glandular cells (AGC). When a diagnosis of ASC-US was confirmed, the case was subclassified as ASC-US or ASC-H. The slides were evaluated for cervical cytology by three academic cytopathologists of the First Affiliated Hospital of Shantou University Medical College.

HPV genotyping

HPV DNA was amplified in a KP-TC48 (Chaozhou Hybribio Biotechnology, China) and genotyping for HPV was performed by flow-through hybridisation and gene chip by HybriMax (Chaozhou Hybribio Limited Corporation, Chaozhou, China). The gene chip contained type-specific oligonucleotides immobilised on a nylon membrane. The final results were detected by colourimetric change on the chip under direct visualisation. The chip allowed for the detection of the following 21 HPV genotypes: 15 high-risk (HR) types (HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -53, -56, -58, -59, -66 and -68), 6 low-risk (LR) types (HPV-6, -11, -42, -43, -44 and -CP8304 (81)).

Statistical analysis

Categorical variables were summarized by absolute frequencies and percentages, and continuous variables by means and Standard Error (SE). The Chi-square test and Fisher's exact test were used to compare proportions; the t-test was used to compare continuous variables. The data were analyzed by using SPSS 16 software.

Results

HPV prevalence in the study population

During the study period, total of 22, 989 consecutive women were subjected to gynecologic examination with cervical HPV-DNA testing. The final sample consisted of 22, 617 women with complete cytological and HPV-DNA testing results. Mean age of the study group was 36.41 ± 9.93 years. All of 21 different HPV types were identified in this study population. Overall, Table 1 showed the prevalence of any HPV including both low-risk and high-risk types in the study population was 19.76% (4468/22617), while high-risk HPV was positive in 16.97% (3837/22617) of all women in the study group. The 3 most prevalent HPV types were HPV-52 (4.27%,

Table 1. Human Papillomavirus Prevalence in General Population and Women with Normal Cytology

Positive	Total n=22617 (%)	Women with normal cytology n=22114 (%)
HPV	4468 (19.76)	4204 (19.01)
HR-HPV	3837 (16.97)	3584 (16.21)
LR-HPV	631 (2.79)	620 (2.80)
Single HPV	3215 (14.21)	3047 (13.78)
Multiple HPV	1253 (5.54)	1157 (5.23)
Multiple HR-HPV	904 (4.00)	820 (3.71)

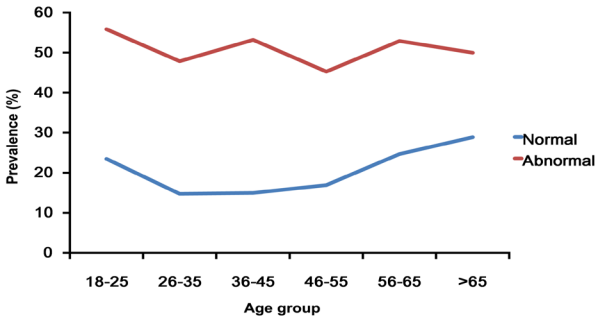

Figure 1. Age-Specific Prevalence of Human Papillomavirus in Women with Normal and Abnormal Cervical Cytology

965/22617), -16 (3.85%, 871/22617) and -58 (2.72%, 616/22617). Similarly, among women with HPV positive, HPV-52 was the most prevalent type (21.60%, 965/4468), followed by HPV-16 (19.49%, 871//4468), -58 (13.79%, 616/4468). Among a total of 22114 women with normal cytology, 13.78% (3047/22114) women had single-type infection, while 5.23% (1157/22114) women had multiple HPV infections. Within the normal cytology samples, the most common high-risk HPV types identified were HPV-52 (4.07%, 901/22114), -16 (3.63%, 802/22114) and 58 (2.46%, 545/22114) (Shown in Table 2).

HPV prevalence according to age

The 22617 women were classified by their age into 6 groups, and the age-specific prevalence of HR-HPV types were shown in Figure 1. A U-shaped age-specific prevalence curve was observed in HR-HPV positive women, specifically, prevalence decreased from 23.49% at age 18–25 years to a peak of 14.75% at age 26–35 years and 14.94% at age 36–45 years, then prevalence increased progressively from 16.91% at 46–55 years to 24.69 and 28.89% at ages 56–65 and > 65 years, respectively.

HPV prevalence according to cervical cytology abnormalities

Cervical cytological abnormalities were observed in 2.22% of women, of which 0.06% ASC-H, 1.55% ASC-US, 0.27% HSIL, 0.34% LSIL and 0.01% AGC. The mean age was 37.6years. As far as cytology was concerned, the overall HPV prevalence in women with cervical cytological abnormalities was 52.49%, and by lesion type was 71.73% (ASC-H), 42.0% (ASCU-S), 81.79% (HSIL) and 73.68% (ILSL). Furthermore, the proportion of samples containing at least one of the 15 HR-HPV was greater in SIL (76.64%) than in ASC (40.38%).The prevalence of single and multiple HR-HPV according to histological diagnosis are shown in Table 3. To our knowledge, the current study is the first study of the prevalence of HPV in women with cervical cytologic abnormalities, a large sample size in Guangdong with participation of more than 500 patients.

Table 2. Human Papillomavirus Type-specific Distribution in General Population and Women with Normal Cytology

Types	Total			Women with normal cytology		
	N	% of all (n = 22617)	% of HPV-positive (n=4468)	N	% of all (n=22114)	% of HPV-positive (n=4202)
High risk						
16	871	3.85	19.49	802	3.63	19.09
18	307	1.36	6.87	295	1.33	7.02
31	242	1.07	5.42	225	1.02	5.35
33	407	1.8	9.11	361	1.63	8.59
35	72	0.32	1.61	61	0.28	1.45
39	212	0.94	4.74	203	0.92	4.83
45	127	0.56	2.84	118	0.53	2.81
51	117	0.52	2.62	103	0.47	2.45
52	965	4.27	21.6	901	4.07	21.44
53	433	1.91	9.69	410	1.85	9.76
56	116	0.51	2.6	108	0.49	2.57
58	616	2.72	13.79	545	2.46	12.97
59	110	0.49	2.46	107	0.48	2.55
66	176	0.78	3.94	165	0.75	3.93
68	321	1.42	7.18	312	1.41	7.43
Low risk						
6	431	1.91	9.65	425	1.92	10.11
11	308	1.36	6.89	294	1.33	7
42	184	0.81	4.12	182	0.82	4.33
43	57	0.25	1.28	56	0.25	1.33
44	31	0.14	0.69	28	0.13	0.67
CP8304	225	0.99	5.04	225	1.02	5.35

HPV type-specific distribution according to cytological diagnosis

Table 4 showed the three most frequently detected HR-HPV types were HPV-58 (14.12%, 71/503), -16 (13.72%, 69/503) and 52 (12.72%, 64/503) in cervical abnormalities. HPV-58, -16 or -52 infections accounted for 40.56% (204/503) of all specimens with cervical cytological abnormalities, while the combined prevalence of HPV16/18 among HPV positive cases was only 16.10% (81/503). Among ASC-H cytology samples, HPV 16 was

Table 3. Human Papillomavirus Prevalence in Women with Cervical Cytology Abnormalities

Positive	Abnormal (n=503)	ASC-H (n=14)	ASCU-S (n=350)	HSIL (n=61)	LSIL (n=76)	AGC (n=2)
HPV	264 (52.49)	10 (71.43)	147 (42.00)	50 (81.97)	56 (73.68)	1 (50.00)
HR-HPV	253 (50.30)	10 (71.43)	137 (39.14)	49 (80.33)	56 (73.68)	1 (50.00)
LR- HPV	39 (7.75)	1 (7.14)	27 (7.17)	3 (4.92)	8 (10.53)	0 (0)
Multiple HPV	96 (19.09)	2 (14.29)	48 (13.71)	21 (34.43)	25 (32.89)	0 (0)
Multiple HR- HPV	84 (16.70)	1 (7.14)	40 (11.43)	21 (34.43)	22 (28.95)	0 (0)

Table 4. Human Papillomavirus Type-Specific Distribution in Women with Cervical Cytology Abnormalities

Types	ASC-H (n=14)	ASC-US (n=350)	HSIL (n=61)	LSIL (n=76)	AGC (n=2)	Total (n=503)
High risk						
16	6 (42.86)	29 (8.29)	22 (36.07)	12 (15.79)	1 (50.00)	69 (13.72)
18	0 (0)	9 (2.57)	1 (1.64)	2 (2.63)	0 (0)	12 (2.39)
31	1 (7.14)	11 (3.14)	3 (4.92)	2 (2.63)	0 (0)	17 (3.38)
33	0 (0)	23 (6.57)	12 (19.67)	11 (14.47)	0 (0)	46 (9.15)
35	0 (0)	9 (2.57)	2 (3.28)	0 (0)	0 (0)	11 (2.19)
39	1 (7.14)	6 (1.71)	0 (0)	2 (2.63)	0 (0)	9 (1.79)
45	0 (0)	3 (0.86)	2 (3.28)	4 (0)	0 (0)	9 (1.79)
51	0 (0)	3 (0.86)	3 (4.92)	8 (10.53)	0 (0)	14 (2.78)
52	0 (0)	36 (10.29)	9 (14.75)	19 (25)	0 (0)	64 (12.72)
53	1 (7.14)	12 (3.43)	3 (4.92)	7 (9.21)	0 (0)	23 (4.57)
56	0 (0)	6 (1.71)	1 (1.64)	1 (1.32)	0 (0)	8 (1.59)
58	1 (7.14)	35 (10.00)	16 (26.23)	19 (25)	0 (0)	71 (14.12)
59	0 (0)	3 (0.86)	0 (0)	0 (0)	0 (0)	3 (0.60)
66	1 (7.14)	6 (1.71)	3 (4.92)	1 (1.32)	0 (0)	11 (2.19)
68	0 (0)	6 (1.71)	0 (0)	3 (3.95)	0 (0)	9 (1.79)
Low risk						
6	0 (0)	4 (1.14)	1 (1.64)	1 (1.32)	0 (0)	6 (1.19)
11	0 (0)	8 (2.29)	1 (1.64)	5 (6.58)	0 (0)	14 (2.78)
42	0 (0)	2 (0.57)	0 (0)	0 (0)	0 (0)	2 (0.40)
43	0 (0)	1 (0.29)	0 (0)	0 (0)	0 (0)	1 (0.20)
44	1 (7.14)	2 (0.57)	0 (0)	0 (0)	0 (0)	3 (0.60)
CP8304	0 (0)	12 (3.43)	1 (1.64)	4 (5.26)	0 (0)	17 (3.38)

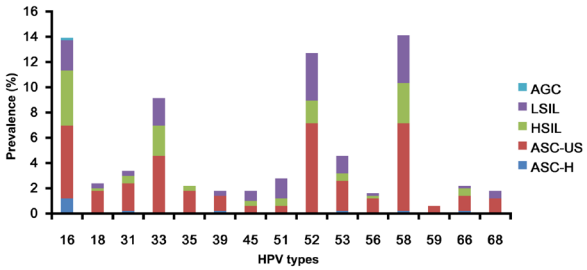

Figure 2. Human Papillomavirus Type-specific Distribution and Cervical Cytology Abnormalities

the most frequent type (42.86%, 6/14). HPV-52 has the highest frequencies (10.29%, 36/350) in the women with ASC-US, and followed by HPV-58 (10%, 35/350) and -16 (8.29%, 29/350). For women with HSIL, HPV-16 (36.07%, 22/61) was the most common type, followed by HPV-58 (26.23%, 16/61) and -33 (19.67%, 12/61). HPV-58 was the most common type in women with LSIL (19/76), followed by HPV-16 (12/76) and -33 (11/76). Furthermore, the percentage of cervical cytological abnormalities in individual HR-HPV type had been shown in Figure 2.

Multiple HR-HPV types in cervical cytological abnormalities

Overall, 16.70% (84/503) were infected with more than one HR-HPV type had been observed in women with cervical cytological abnormalities, which has significant difference compared with the prevalence in normal cytology (3.71%, 820/22114, $P < 0.001$). Furthermore, there was no significant difference on age between single (37.40±7.99 year) and multiple (36.98±9.41 years) HR-HPV infection women ($P = 0.714$). Of those multiple infection, 66.67% (56/84) were infected with two types, 23.81% (20/84) with three, 5.95% (5/84) with four types, 2.38% (2/84) with five and 1.19% with six types, respectively. Multiple HR-HPV type infections were 7.14% (1/14) in ASC-H, 11.43% (40/350) in ASC-US, 34.43% (21/61) in HSIL and 28.95% (22/76) in LSIL, respectively. Multiple HR-HPV positivity was significantly more common in SIL (31.39%, 43/137) than in ASC (11.26%, 41/364). Among cases with multiple infections, HPV-58/33 co-infection was the most frequent combination accounting for 20.23% (17/84) of the total multiple infections. This was followed by HPV-58/16 (14.29%; 12/84) and HPV58/52 co-infections (11.90%; 10/84). HPV-58/33 co-infection was the most combination for ASC-US (20.00%) and ISIL (18.18%), while HPV-58/16 co-infection was common for HSIL (28.57%, Table 5).

Table 5. Co-infections of Multiple High-risk Human Papillomavirus in Women with Cervical Cytology Abnormalities

Combination	ASC-H (n=1)	ASC-US (n=40)	HSIL (n=21)	LSIL (n=22)
HPV-58/16 (n=12)	1 (100%)	2 (5.00)	6 (28.57)	3 (13.64)
HPV-58/52 (n=10)	0 (0)	6 (15.00)	2 (9.52)	2 (9.09)
HPV-16/52 (n=8)	0 (0)	3 (7.50)	2 (9.52)	3 (13.64)
HPV-58/33 (n=17)	0 (0)	8 (20.00)	5 (23.81)	4 (18.18)
Other (n=37)	0 (0)	21 (52.50)	6 (28.57)	10 (45.45)

Discussion

The HPV type-specific in women with cervical cytologic abnormalities remains uncertain in Shantou, which is also an ESCC high-incidence area. To our knowledge, this is the first study to describe prevalence of HPV infection and its subtypes and their association with cervical cytological abnormalities by analyzing the whole spectrum of all 15 known HR-HPV types in a large scale population and in a representative set of women with cervical abnormalities in Shantou.

A population-based study in Chaozhou reported HPV prevalence of 7.89% among 48, 559 women age at 35-60 years (Chen, 2012). Our data obtained from Shantou city, which a more closely related population to Chaozhou from geographic location and population genetic background. The strength of this study was that the age for participants covered from 18-81 years. Generally, the vast majority of the HPV prevalence was 19.76% in total participants and was higher than 13.8% of a hospital-based study in Shenzhen near to Shantou (Wang et al., 2013). For age-specific distribution, a higher prevalence of HPV infection was observed in both young (18-25 years) and old (> 65 years) age groups in the general population, in agreement with the previous report in Hunan population, mid-south China (Li et al., 2013). Previous studies also reported types 52, 16, 58 have been fond as the 3 most common high-risk HPV type in Chaozhou population (Chen et al., 2012); HPV-52 was the most prevalent type (21.60%), followed by HPV-16 (19.49%), -58 (13.79%) in our study. Furthermore, our findings in the present study also obtained that 19.01% of women with normal cervical cytological, of whom 13.78% and 5.23% have single and multiple-infection, respectively. These data extend previous findings from age of participants and was initial estimation of the baseline burden of HPV infection in Shantou from hospital-based study.

Overall, the rate of abnormality was 2.22% found in our study, the HPV infection occurred in 52.49% of cases of those women with cervical cytological abnormalities, of which HSIL has the highest prevalence (81.97%), and regarding of the HPV type, HR-HPV was greater in SIL (76.64%) than in ASC (40.38%, $P < 0.001$). Multiple HR-HPV positivity was significantly more common in SIL (31.39%) than in ASC (11.26%, $P < 0.001$). Previous study in Chaozhou based on 99 women with cervical cytological abnormalities from 319 HPV positive women indicated the HPV-18, -16, and -58 were the most common types. However, the case number of cervical cytological abnormalities was small and limited with only 11 cases of HSIL observed (Chen et al., 2012). In the absence of information on type-specific HPV infection found in cervical cytological abnormalities in Shantou population, our data from representative set of 503 women with cervical abnormalities showed the 3 dominant HR-HPV types were HPV-58 (14.12%, 71/503), -16 (13.72%, 69/503) and 52 (12.72%, 64/503). HPV-58 had been found as a much higher proportion of cervical cancers in East Asia than other types (Chan et al., 2011). Similarly, the 3 most common types have been found in Korea population (Kim et al., 2013). HPV 58 and 52 were among the five most common types in invasive cervical carcinoma in eastern and southeastern Asia (Bao et al., 2008). A high prevalence of HPV-58 among CSCC has been reported in 33% in Shanxi and 28% in Shanghai China (Chan, 2012; Chan et al., 2013), as the second types with prevalence 21.8% in Western China, respectively (Li et al., 2012). Previous studies suggested that testing for individual HPV genotypes can improve risk stratification in women with minor cytologic abnormalities (Gage et al., 2013), and knowledge of the prevalence and type-specific HPV, either as single or multiple infections, in the cervical cytological abnormalities will be important for an appropriate intervention in cervical cancer prevention, and to understand the potential impact of HPV vaccines in the population. Interesting, the prevalence of genital HR-HPV infection in male sexual partners of HPV-positive women in Chaozhou was lower (5.32%) than that expected, and the concordance of HR-HPV between couples was extremely low (2.63%). These findings suggested that infected men consitute an important viral reservoir, contributing to transmission of HR-HPV to women and maintenance of infection, but HR-HPV infection may be less likely to persist in men than in women (Huang et al., 2013). Therefore, our data provide further information about the type prevalence in women with cervical cytological abnormalities in Shantou and might be used for the screening individuals with the high-risk type 58 in the men. Moreover, it will be important to monitor possible changes in HR-HPV type distributions in the vaccinated and HPV-screened population to better understand cross-protection and to identify putative new HR-HPV types once HPV16 and HPV18 are eliminated. Heterogeneity in HPV type-specific distribution in Shantou should be taken into account for the second-generation vaccines, speciallyHPV-58, and 52 may offer higher benefit for women in Shantou and neighborhood. Although the significance of HPV-58 in cervical cytological abnormalities is poorly understood, HPV-58 and 18 was associated with lack of treatment response (Munagala et al., 2009).

Combined the HPV type and cytologic abnormalities, HPV-16 was the predominant type in ASC-H (42.86%) and HSIL (36.07%), HSIL is preferentially caused by HPV16 and less often caused by other genotypes (Kovacic et al., 2006). Our results show some interesting differences and comprehensive spectrum with cervical cytological abnormalities data in Shantou, comparing Chaozhou. The most frequent types we detected in ASC-US were HPV-52 (10.29%, 36/350), followed by HPV-58 (10%, 35/350) and -16 (8.29%, 29/350), which differ from HPV-52 as the most common types detected in only ASC in Chaozhou. HPV-58 was the most common type in women with LSIL (19/76), followed by HPV-16 (12/76) and -33 (11/76), while HPV-16 had been found as the most common type in LSIL in Chaozhou. In ASC-US, HSIL and ISIL lesions, HR-HPV 33 had been found as the fourth predominant HR-HPV type. In some level, those data suggested the infective context is special in the women with cervical cytological abnormalities in Shantou.

From prevalence, 16.70% (84/503) of women with cervical cytological abnormalities contained two or more HR-HPV types. Multiple HR-HPV positivity was significantly more common in SIL (31.39%, 43/137) than in ASC (11.26%, 41/364), and the HSIL (34.43%) had highest multiple infections, comparing ASC and ILSL. In our population, HPV 58 was the most prevalent type, either as a single infection or combined with other genotypes. Multiple infections were most prevalent of HPV-58/33 co-infection, followed by HPV-58/16 (14.29%; 12/84) and HPV58/52 (11.90%; 10/84). Furthermore, HPV-58/33 co-infection was most combination for ASC-US (20.00%, 8/40) and ISIL (18.18%, 4/22), while HPV-58/16 co-infection was common for HSIL (28.57%). Multiple HPV infections correlated most prominently with lack of treatment compared with single type infection (Munagala et al., 2009). And several studies indicated that the cumulative burden of HPV multiple infections maybe a marker of differential immune response. Our data provided some evidence that risk in the context of HPV-58 may be particularly modulated by coinfections with other types in women with cervical cytological abnormalities. Co-infection with multiple HPV types had been observed more frequently among younger women and among those with cytologic abnormalities (Trottier et al., 2006). However, there was no significant difference on age between single (37.40±7.99 year) and multiple (36.98±9.41 years) HR-HPV infection women ($P = 0.714$).

Before we consider the implications of these results, we need to assess the potential limitations of this investigation. The main limitation of our study is that our study was a retrospective, hospital-based observational analysis, and study participants included two groups of population, gynecologic clinic visitors and health medical examination women. Therefore population profile may not represent those of the general population since the low participation might introduce a self-selection bias, Another limitation is that we chose HPV types that have been associated with cancer; we were not able to measure

all HPV subtypes. Consequently, we cannot rule out the possibility that unmeasured HPV types were confounding the associations reported. More studies will be necessary to examine these associations in study population.

In conclusion, in this large hospital-based study, we confirmed the HPV-52, -16 and -58 in women with normal cytology in Shantou; in addition, we comprehensively described HPV-58, -52 and -16 distributions in women with cytologic abnormalities, and particularly co-infection in women with ASC, HSIL, ISIL and multiple HR-HPV positive.

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