

## RESEARCH ARTICLE

# Human Papillomavirus Genotypes Associated with Mucopurulent Cervicitis and Cervical Cancer in Hangzhou, China

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## Abstract

**Background:** To investigate the infection status and predominant genotype distribution of human papillomavirus (HPV) infection among Chinese patients with mucopurulent cervicitis (MPC) or cervical cancer (CC) in Hangzhou. **Methods:** Initially, 217 cases of healthy cervix controls (n=50), acute MPC (n=89), and CC (n=78) were included; samples were collected between January 1, 2010, and January 30, 2013. Cervical specimens were screened for HPV using a nested polymerase chain reaction assay and DNA sequencing. **Results:** Overall prevalence of HPV infection was 16.7% in the control group, 51.9% in the MPC group, and 84.4% in the CC group. The predominant genotype detected in all 3 groups was the oncogenic variant HPV 16 (55.8%, 17.3%, and 6.3% in the CC, MPC and control specimens, respectively), HPV58 was the second most predominant HPV type in CC (9.1%), MPC (8.6%), and control group (4.2%). Most or all of the genotypes were oncogenic in the three groups. **Conclusions:** Infection with HPV was found to be prevalent among Chinese women with MPC or CC and oncogenic variants were in the majority. Therefore, peoples who suffered MPC with HPV DNA positive should be regularly followed-up, for prevention and early treatment of cervical cancer.

**Keywords:** Cervical cancer - human papillomavirus - mucopurulent cervicitis - oncogenic genotype

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## Introduction

Human papillomaviruses (HPVs), double-stranded and non-enveloped DNA viruses (7–8 kb long), are a group of remarkably diverse DNA viruses from the Papillomaviridae family, which are causally involved in the etiology of various benign and malignant neoplastic lesions of mucosal and skin epithelium. Different HPVs have been traditionally referred to as ‘types’, a type being a cloned full-length HPV genome, whose L1 nucleotide sequence is at least 10% dissimilar from that of any other papillomavirus type (Poljak and Kocjan, 2010). Currently, more than 200 different HPV types are officially recognized. Clinically, HPVs are commonly divided into two subtypes, the high-risk (HR) and low-risk (LR) type of HPV. The former is oncogenic potential, the latter is non-oncogenic. The HR genotypes includes HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 61 and 68, whereas the LR genotypes includes HPV6, 11, 30, 42, 43, 44, 53 and 64. Women and men involved in the transmission of HPVs can be both asymptomatic vectors and victims of these infections (Zhang et al., 2012; Liu et al., 2013).

Cervical cancer (CC) is a major fatal malignancy among women, causing about 275,000 deaths annually world-wide, most in developing countries. It is also a

highly preventable cancer if detected at its precancerous stages and treated by ablative procedures. In China, the age-standardized incidence and mortality rate are 9.6 and 4.3 per 100 000, respectively, while the mortality rate is even higher in some poor, and rural areas (Cai et al., 2013). It is now well established that infection with the high-risk human papillomavirus is a necessary cause of cervical cancer and this virus has been found in 99.7% of cervical tumors (Walboomers et al., 1999; Bosch et al., 2002). The distribution of type-specific HPV in invasive cervical cancer varies by geographic region. For example, HPV16 is slightly more prevalent in Europe and North America, HPV 31 is more prevalent in South/Central America, HPV 33 and 45 are more prevalent in Africa, and HPV 52 and 58 are more prevalent in Asia (Bao et al., 2008a).

Mucopurulent cervicitis (MPC) is a highly prevalent disease of the female reproductive tract. The diagnosis of mucopurulent cervicitis is based on two findings: visible mucopurulent (yellow) cervical secretions, and more than 10 PMNL/HPF in the cervical secretions (Willmott et al., 1988). The inflammatory condition of the cervix among patients with MPC is thought to be the consequence of infection with sexually acquired pathogens, such as Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG), Mycoplasma genitalium (MG),

Gardnerella vaginalis (GV) and Trichomonas vaginalis (TV), Mycoplasma hominis (MH), herpes simplex virus type 2 (HSV-2) (Paavonen et al., 1986). Furthermore, several studies have indicated that MPC may accompany infection with HPV (Liu et al., 2013).

This study was made in order to investigate the prevalence and distribution of HPV oncogenic genotypes in patients with MPC and cervical cancer in Hangzhou.

## Materials and Methods

The collection of specimen and the study protocol were approved by the Human Ethical Committee Institutional Review Board of Chinese Medicine Hospital of Hangzhou.

Hospital Cervical swabs of healthy controls were collected from women who attended for health examination, MPC swabs were collected from outpatients. All participants provided written informed consent. The cervical cancer tissue samples were obtained from the Department of Pathology. All cases were identified via standard histologic criteria at the Department of Obstetrics and Gynecology, Chinese Medicine Hospital of Hangzhou, Hangzhou, China.

A total of 217 women aged 20–66 years were initially included in the present study between January 1, 2010, and January 30, 2013: 78 in the CC group, 89 in the acute MPC group, and 50 in the healthy control group. Women in the MPC and control groups were examined for the presence of other sexually transmitted pathogens before the HPV analysis was performed. These pathogens included CT, NG, GV, MG, TV, MH, and HSV-2. Infection was detected by routine clinical microbiology methods.

DNA samples were extracted from the cervical swabs as previously described (Liu et al., 2013). Briefly, a cervical specimen was collected from each patient using a cotton swab and stored at  $-80^{\circ}\text{C}$ . For DNA preparation, cervical swabs were soaked in 2 mL of a 0.9% solution of sodium chloride for 2–5 hours at room temperature and then centrifuged at 5000 rpm for 10 minutes. The pelleted cells were resuspended in 200  $\mu\text{L}$  of TE buffer (10 mM Tris-HCl, pH 7.4; 1 mM EDTA, pH 8.0) and digested in a 50 mM solution of proteinase K (Invitrogen, Carlsbad, CA, USA) for 5–10 minutes at  $55^{\circ}\text{C}$ . The DNA was then precipitated with 100% ethanol and extracted using the DNeasy Tissue Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions.

Tissue samples from patients with CC were obtained during hysterectomy and stored at  $-80^{\circ}\text{C}$ . For DNA preparation, approximately 5–30 mg of frozen tissue was used for each DNA isolation procedure, which was performed using the DNeasy Tissue Kit.

The extracted DNA integrity was confirmed by standard PCR using  $\beta$ -globin primers (forward primer 5'-CAACTTCATCCA CGTTCACC-3' and reverse primer 5'-GAA GAGCCAAGGACAGGTAC-3').

DNA samples were stored at  $-20^{\circ}\text{C}$ . DNA samples from the MPC and control groups were screened for HPV infection by nested PCR using L1 MY09/11—GP5+/6+ consensus primers (Liu et al., 2013).

DNA samples from the CC group were screened by nested PCR with E6/E7 gene type-specific primers and

L1 MY09/11—GP5+/6+ consensus primers; all primers were synthesized in TaKaRa.

Samples that tested negative by nested PCR using the MY09/11—GP5+/6+ consensus primers were defined as "HPV infection negative." Samples that tested positive were subjected to direct DNA sequencing for HPV genotyping.

A  $\chi^2$  test was used to assess the significance of differences detected in the frequency of HPV infections among the 3 groups. A *P* value below 0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 16.0 (IBM, Armonk, NY, USA).

## Results

Of the 217 specimens collected from Chinese Medicine Hospital of Hangzhou, the mean age of patients in the CC group was 47 years (range 25–63 years); the mean age of women in the MPC and control groups was 38 years (range 20–66 years). In the MPC group, the rates of infection with CT, NG, MG, HSV-2, TV, GV, and MH were 20.2%, 12.0%, 9.3%, 5.5%, 3.2%, 4.0%, and 1.5%, respectively (data not shown). None of the healthy control individuals tested positive for the presence of these pathogens.

Of the 217 cervical samples initially processed, several tested negative for  $\beta$ -globin and were excluded from further analysis. That is, these  $\beta$ -globin-negative samples comprised 8 of the 89 patients with MPC (10.7%), 2 of the 50 control individuals (2.4%), and 1 of the 78 patients with CC (1.5%), resulting the remaining 206  $\beta$ -globin-

**Table 1. Prevalence of Human Papillomavirus Genotypes in the Study Population<sup>a,b</sup>**

Genotype	Histologic diagnosis			Total(n=206)
	Healthy cervix (n=48)	Acute MPC (n=81)	CC (n=77)	
Overall prevalence	8(16.7)	42(51.9)	65(84.4)	115(60.5)
HPV 6	0(0.0)	1(1.2)	0(0.0)	1(0.5)
HPV 11	1(2.1)	1(1.2)	0(0.0)	2(1.1)
HPV 16	3(6.3)	14(17.3)	43(55.8)	60(31.6)
HPV 18	0(0.0)	2(2.5)	4(5.2)	6(3.2)
HPV 29	0(0.0)	1(1.2)	0(0.0)	1(0.5)
HPV 31	0(0.0)	0(0.0)	1(1.3)	1(0.5)
HPV 33	0(0.0)	1(1.2)	3(3.9)	4(2.1)
HPV 35	0(0.0)	2(2.5)	1(1.3)	3(1.6)
HPV 39	0(0.0)	1(1.2)	5(6.5)	6(3.2)
HPV 43	0(0.0)	1(1.2)	0(0.0)	1(0.5)
HPV 45	0(0.0)	1(1.2)	0(0.0)	1(0.5)
HPV 52	1(2.1)	3(3.7)	2(2.6)	6(3.2)
HPV 53	0(0.0)	0(0.0)	2(2.6)	2(1.1)
HPV 58	2(4.2)	7(8.6)	7(9.1)	16(8.4)
HPV 59	0(0.0)	5(6.2)	6(7.8)	11(5.8)
HPV 66	1(2.1)	0(0.0)	0(0.0)	1(0.5)
HPV 67	0(0.0)	1(1.2)	0(0.0)	1(0.5)
HPV 68	0(0.0)	1(1.2)	4(5.2)	5(2.6)

CC, cervical cancer; HPV, human papillomavirus; MPC, mucopurulent cervicitis; <sup>a</sup>Values are given as number (percentage); <sup>b</sup>Because of the existence of multiple HPV infections, women with CC may be counted more than once. Oncogenic genotypes include HPV 16, 18, 31, 33, 35, 39, 45, 52, 53, 58, 59, and 68. Non-oncogenic genotypes include HPV 6, 11, 29, 43, 66 and 67

**Table 2. Single and Multiple Human Papillomavirus Infections Among Women with cervical Cancer (n=65)<sup>a</sup>**

Single infection		Dual infection		Triple infection	
Genotype	Incidence	Genotype	Incidence	Genotype	Incidence
HPV16	31(47.7)	HPV16+18	2(3.1)	HPV16+58+59	1(1.5)
HPV18	2(3.1)	HPV16+35	1(1.5)		
HPV31	1(1.5)	HPV16+39	3(4.6)		
HPV33	3(4.6)	HPV16+53	1(1.5)		
HPV39	2(3.1)	HPV16+59	2(3.1)		
HPV52	2(3.1)	HPV16+68	2(3.1)		
HPV53	1(1.5)				
HPV58	6(9.2)				
HPV59	3(4.6)				
HPV68	2(3.1)				
Total	53(81.5)		11(16.9)		1(1.5)

HPV, human papillomavirus; <sup>a</sup>Values are given as number (percentage)

positive specimens were tested for the presence of HPV DNA (Table 1).

Overall prevalence rates for HPV infection among patients with acute MPC were 51.9% (42/81) and the difference reached statistical significance ( $P<0.05$ ) compared with healthy control individuals (16.7%, 8/48). HPV 16 was the most prevalent genotype detected in the control group (6.3%); followed by HPV 58 (4.2%) and HPV 11 (2.1%), 52 (2.1%), 66 (2.1%).

Similarly, the oncogenic variants HPV 16 (17.3%) and HPV 58 (8.6%) were the most prevalent genotypes detected in the MPC group. The prevalence rates of other HPV genotypes are shown in Table 1. Eleven of the fifteen HPV genotypes detected in the MPC specimens were classed as oncogenic (HPV 16, 18, 33, 35, 39, 43, 45, 52, 58, 59, 68); the other 4 were classed as non-oncogenic (HPV 6, 11, 29, 67). Generally speaking, the prevalence of oncogenic genotypes detected in this group was 45.7% (37/81).

In the CC group, 65 (84.4%) of the samples were tested positive for HPV DNA by nested PCR using the E6/E7 gene type-specific primers. As can be seen from Table 1, HPV 16 was the most frequent genotype detected in this group, which occupied 55.8% of all positive samples, HPV 58 (9.1%) also was the second most predominant HPV type, which followed by HPV 59 (7.8%), HPV 39 (6.5%), HPV 18 (5.2%), HPV 68 (5.2%), and the rest were rarer. Among the CC specimens that tested negative with the E6/E7 gene type-specific primers, 2 (2.6%) tested positive using the L1 MY09/11—GP5+/6+ consensus primers, including 1 patient with HPV 31 and 1 patient with HPV 58.

Although there were three kinds of HPV infections (single, dual, and triple) among the CC group (Table 2), the majority of cases (n=53) were found to be single infections, indicating only a single genotype HPV can lead to CC.

## Discussion

In this study, we evaluated the prevalence of HPV

among patients with a cute MPC and CC, as well as among healthy volunteers in cervical samples using nested PCR. Our result showed that HPV infection is common in MPC and CC cases and measures should be taken to reduce the chance of HPV infection and especially to prevent the HPV lead to more CC cases.

Overall, the HPV infection rate (55.8%, 115/206) was very higher than that (16.5%) previously reported by Liu et al. (2011). Maybe because it was the methods and the objects were different. Quite a few of their samples were from infertility or pelvic inflammation patient. Furthermore, Hangzhou is a big, famous city in the east of China, with high population density, many mobile populations. With the development of rapid economy development and some women's thought are becoming more and more open; these factors are in favor of the HPV to spread.

A significantly higher rate of HPV infection was observed in the acute MPC group (51.9%) when compared with the reported 5.4% by Altuglu et al. (2002), who used Digene Hybrid Capture assay. The difference may partially be due to our method is more sensitive than that of they used. The prevalence of oncogenic HPV infection in this group was up to 88.1% (37/42), similar to that (77.5%) of Liu et al. (2013). HPV16, 58, 59-as oncogenic genotypes-were predominant in MPC group, indicative of MPC patients should be made aware of the danger of developing CC.

HPV is one of the most prevalent sexually transmitted viruses worldwide, and cervical infection with oncogenic genotypes, such as HPV 16, is generally accepted as the main cause of CC. The prevalence and distribution of oncogenic genotypes were also determined in 77 CC specimens. The overall prevalence of HPV infection in the CC group (84.4%) was similar to the rate of 85.9% reported in a meta-analysis of CC in Asia (Bao et al., 2008b). Like other regions worldwide, HPV16 was the predominant type (55.8%, 43/77) identified among CC patients in the present study; very similar to the previously reported rate in a meta-analysis of HPV in mainland China and Hong Kong (58.7%) (Bao et al., 2008a), but it was significantly lower than the rate found in Changchun, China (87.7%, 57/65) (Liu et al., 2013). As we know, except for HPV16 appears most frequently, the probability of other genotype can vary in different countries or areas. The second most prevalent genotype detected among the CC group in the present study was HPV 58 (9.1%, 7/77), the rate of which was obviously higher than that reported previously (1.5%, 1/65) (Liu et al., 2013). Very close to HPV 58 was HPV 59(7.8%, 6/77), followed by HPV39 (6.5%, 5/77). HPV 16, as the most frequent genotypes detected in the CC group, together with HPV 58, HPV 59 and HPV 39 accounted for 93.8% (61/65) of all HPV-positive cases.

Various HPV DNA tests have been introduced to meet the need for effective cervical cancer prevention screening in China (Lo et al., 2002; Qiao et al., 2008). However, these tests without a uniform standard frequently generate discordant results, whereas DNA sequencing is the generally accepted standard for molecular identification and genotyping of HPV (Ghosh et al., 2011; Liu et al.,

2013). So we selected the nested PCR with the currently accepted primers in this study.

In summary, HPV infection was detected in 84.4% of the CC specimens examined and in 51.9% of the MPC specimens. Considering there were different main genotypes in different area and the HPV threatens human health seriously, the patients with MPC should be closely monitored in order to avoid the MPC to develop into CC.

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The authors declare that they have no competing interests.

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