Prevalence of Anal Human Papillomavirus Infection in Asymptomatic Men Who Have Sex with Men in Khon Kaen, Thailand

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ABSTRACT

Human papillomavirus (HPV) infection is a common cause of sexual transmitted disease and common found in men having sex with men (MSM) who at particularly practice anoreceptive intercourse. Asymptomatic MSM with anal HPV infection could substantiate the importance of transmission between partners and also has been associated with anal cancer. This study aimed to assess the prevalence of anal HPV infection in MSM in Khon Kaen, Thailand and the associated risk was also evaluated. The demographic data and anal swabs were collected from 155 MSM. DNA was extracted and quantitated by GAPDH. HPV DNA was detected using GP5+/6+ primers and real-time PCR method and genotyped by reverse line blot hybridization. The result showed that HPV prevalence was 51% that consisted of 27.8% and 81% in low-risk and high-risk HPV type, respectively. HPV 16 is the most common followed by HPV 58 and HPV 18. Infection with multiple types was 49.4%. HPV infections were significantly associated with receptive anal intercourse and always condom usage. These results support the data of anal HPV infection in asymptomatic MSM and its importance to implement methods for prevention of HPV transmission and protection of anal cancer development.

Keywords: Human papillomavirus infection, Anal cancer, Men who have sex with men

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Introduction

Human papillomavirus (HPV) infection is the most common sexually transmitted infection worldwide (Denny *et al*., 2012) and can cause disease in men and women. Previous studies have demonstrated a high prevalence of HPV infection in males and HPV is responsible for important disease, particularly in men who have sex with men (MSM), an anal canal is the most common site of infection (Goldstone *et al*., 2011;Palefsky, 2007;Van der Snoek *et al*., 2003). This high HPV prevalence at anal site among MSM acts as a reservoir for transmission and is associated with an elevated anal cancer incidence estimated to be 44 times higher than that among the general population (Frisch *et al*., 2003). Mucosal HPV genotypes have been identified to more than 100 genotypes including low–risk and high-risk type according to their oncogenicity (Huang *et al*., 2004;Kawana *et al*., 2012). Although many HPV infections in men have been shown to be transient in nature (similar to HPV infections in women), a small percentage of high risk HPV infection is persist and can progress to genital warts, preneoplastic and malignant lesions of the anus, penis, and oropharynx; and recurrent respiratory papillomatosis (Van der Snoek *et al*., 2003). High risk HPV infections in MSM, and the prevalence of these infections have been rarely characterized in asymptomatic MSM in Thailand. The aim of this study is to prospectively detect the prevalence of HPV at anal canal in a population of asymptomatic sexually active MSM and also investigated the independent factors associated with HPV prevalence in this study population.

Objectives

The objectives of this study were to evaluate the prevalence of HPV infection among asymptomatic MSM in Khon Kaen, Thailand and to investigate the associated risk of HPV infection

Methods

Samples collection

The anal cell samples of 155 MSM were collected using swab and cells were suspended in formal saline tube.

DNA extraction

Anal cells were lysed by lysis buffer. Proteinase K was added and incubated at 60^{o}C for 30 min. Protein was precipitated with protein precipitation buffer. Isopropanol was used to separate DNA and the DNA pellet was washed with 70% ethanol. The DNA was stored at -20 ^{o}C. To confirm the success of the DNA extraction, GAPDH gene was investigated using real-time PCR.

HPV detection

HPV DNA was detected by SYBR-green real-time PCR. A final reaction volume of 10 ul containing 5 ul SsoAdvanced™ universal SYBR® green supermix (Bio-Rad), 10 uM of GP5+ (5’ TTTGTTACTGTGGTAGATACTAC) and GP6+ primers (5’-GAAAAATAAACTGTAAATCATAT) and 2 ul of template. The target DNA was amplified using real-time PCR condition (Applied Biosystems 7500 Fast Instrument) consisting denature step of 5 min at 95^{o}C and 45 cycles of 10 s at 95^{o}C and 30 s at 42^{o}C. The PCR products were subjected to melting curve analysis to determine the melting temperature (Tm). Gel electrophoresis was confirmed the real-time PCR product with 150 bp.

HPV genotyping

HPV positive samples were amplified by GP5/ GP6+ biotin labeled for genotyping by reverse line blot hybridization (RLBH) (Brule *et al*., 2002). Biodyne C blotting membrane (Pall Life Science) was activated in 16% (w/v) 1-ethy-3-(3-dimethylamino propyl) carbodimide (EDAC) solution (Sigma-Aldrich) for 10 min at room temperature, rinsed with distilled water and placed on mini blotter. Thirty-seven HPV type-specific 5'-amino linked oligonucleotides probes—including 13 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68); 12 low-risk HPV types (6, 11, 26, 40, 42, 43, 44, 53, 54, 61, 72 and 73); and, other HPV types (34, 55, 57, 66, 70, 82MM4, 83MM7, 84MM8, 82IS39, CP6108, 71CP8061 and 81CP8304)—were dropped on Biodyne C membrane through the wells of the mini blotter in parallel lines. Subsequently, biotin-labeled PCR products were added into the channels of the mini blotter perpendicular to the oligonucleotides probe lines, then hybridized and incubated with streptavidin-peroxidase-conjugate. The HPV types were detected using chemiluminescence (Natphopsuk *et al*., 2013)

Statistical analysis

The associations between HPV and many factors were analyzed using logistic regression. Adjusted hazard ratio (HR) and its 95% CI were calculated. Values of $p<0.05$ were considered statistically significant (STATA version 10.0).

Results

Total of 155 MSM were enrolled in this study. There was 51% positive for HPV DNA according to the melting temperature (Tm) in the range of 75–80 °C. Gel electrophoresis was used to confirm the presence of HPV amplicon at 150 bp (Figure 1).

The low-risk and high-risk HPV were 27.8% and 81%, respectively (Table 1). HPV 16 was most common (n=25) followed by HPV 58 (n=22), HPV 18 (n=13) and HPV 6 (n=9). HPV infection with only one type HPV was 36.7% with two types was 34.2% and more than two types were 15.2% (Table 2).

HPV infections were significant associated with receptive anal intercourse and always condom usage. This result showed that MSM with receptive anal intercourse have more risk of HPV infection (HR 2.6, 95% CI 1.29-5.2, P <0.05) even though they always used condom (HR 23.31, 95% CI 1.71- 3.41, P <0.05). There was no statistical different between HPV infection with aspect to age and number of partner in during 3 months of exposure (Table 3).

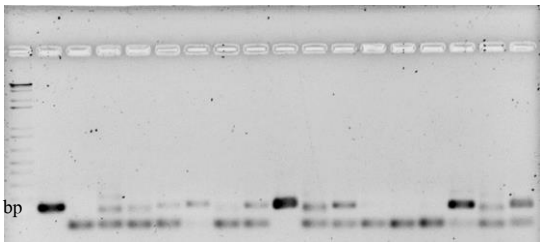

Figure 1 Gel electrophoresis of HPV DNA from samples. The real-time PCR products were separated on 2% agarose gel. Lane A contains the molecular weight marker. Lane B, J and P show the positive control (SiHa cell). Lanes D to I and K to R present the results of thirteen samples. Lane C is a negative control.

Discussion

This study investigated for HPV DNA and HPV genotyping at anal site of infection in asymptomatic MSM in Khon Kaen, Thailand using

Table 1 Prevalence of HPV infection in MSM, Khon Kaen, Thailand.

HPV infection	Cases, n (%)
Positive	79 (51)
Low-risk	22 (27.8)
High-risk	64 (81.0)
Other	3 (3.8)
Undetermined	10 (12.7)
Negative	76 (49)

real-time PCR and RLBH, respectively. The prevalence of HPV infection was 51%. The result found that MSM with receptive anal intercourse and used condom have high risk of HPV infection. This study demonstrated the detection of HPV DNA in anal cell samples using SYBR-green real-time PCR and Tm of positive HPV DNA was varied among 75-80˚C. Previous studied showed that the Tm in the range of 77–82 ˚C was considered to be positive for HPV-DNA (BioRad®, Hercules, CA, USA) (de Araujo *et al.*, 2009). From the result of this study suggested that the instrument for real-time PCR used has the effect to melting temperature of HPV DNA detection. A lot of HPV genotypes revealed the similar or different of Tm. Therefore, each instrument has been further optimized. In addition, gel electrophoresis as well as HPV genotyping was suggested to confirm HPV amplicon. The prevalence of anal HPV (51%) in asymptomatic MSM in Khon Kaen, Thailand was corresponded to previous reports that showed anal HPV infection of 71.4-85% in HIV-positive and 33.8-58.5% in HIV-negative MSM (Phanuphak *et al.*, 2013;Zhang *et al.*, 2014). However, in our study has a limitation in data of HIV data. HPV infection in MSM, the report showed that 36.7% were infected with at least one type of HPV and 51.9% were infected with multiple HPV types. HPV 16 was the most frequently detected as common type in the anus (de Pokomandy *et al.*, 2009;Nyitray *et al.*, 2011;Phanuphak *et al.*, 2013;Yu *et al.*, 2013).

Table 2 Distribution of HPV genotypes in MSM, Khon Kaen, Thailand

HPV genotypes	Cases, n (%)
Low-risk types	22 (27.8)
6	9 (11.4)
11	8 (10.1)
42	5 (6.3)
43	2 (2.5)
61	1 (1.3)
High-risk types	64 (81)
16	25 (31.6)
18	13 (16.5)
31	3 (3.8)
33, 35	5 (6.3)
39, 45, 52	4 (5.1)
56	8 (10.1)
58	22 (27.8)
59	2 (2.5)
Others types	
66, 70, 83MM7	1 (1.3)
Undetermined	10 (12.7)
Infected with one type	29 (36.7)
Multiple infection	37 (49.4)
Infected with 2 types	27 (34.2)
Infected with $\geq$ 3 types	12 (15.2)

Corresponding with this study, HPV 16 was the most common type and followed by HPV 58 and HPV 18 as well as high prevalence of multiple HPV infection (Table 2).

Table 3 Factors of infection with any human papillomavirus (HPV)

Factors	HPV status		HR (95% CI)	p-value
	Positive n= (79)	Negative n= (76)		
Age, n%				
< 30	44/55.7	42/55.3	1	-
≥ 30	35/44.3	34/44.7	0.98 (0.52-1.85)	0.957
Number of partners during 3 months, n%				
≤ 2	41/51.90	38/50.00	0.85 (0.45-1.63)	0.640
≥ 2	6/7.59	10/13.16	0.54 (0.19-1.57)	0.261
Out of data	32/40.51	28/36.84	1	-
Receptive anal intercourse, n%				
No	18/22.78	33/43.42	1	-
Yes	61/77.22	43/56.58	2.60 (1.29-5.20)	0.007
Use of condoms, n%				
Sometimes	9/11.39	26/34.21	1.40 (0.66-2.97)	0.373
Always	54/68.35	30/39.47	3.31 (1.71-6.41)	0.001
Non or out of data	16/20.25	20/26.32	1	-

However, the prevalence and genotype distribution of HPV varied broadly in different places (Forman *et al*., 2012). The result of this study showed that HPV infection was significantly associated with receptive anal intercourse and always condom usage (Table 3) corresponding with study of Chin-Hong et al. who showed that HPV infection was independently associated with receptive anal intercourse (odds ratio [OR], 2.0; P<.0001) (Chin-Hong *et al*., 2004). This result suggested that despite condom usage cannot completely prevent the infection of HPV. In this study, however, HPV was no association with age and number of partner in 3 months. In contrast to previous study showed that anal HPV infection was independently associated with >5 sex partners during the preceding 6 months (OR, 1.5; P<.0001) (Chin-Hong *et al*., 2004). Therefore**,** an expanding of period time in data collection has been influenced to HPV status. In our study has a limitation in some of data such as HIV status.

For conclusions, our study presents the prevalence of HPV and HPV genotypes in asymptomatic MSM in Khon Kaen, northeastern Thailand. High-risk HPV and multiple HPV infection are common. These data is a crucial for seeking methods to protect HPV transmission and anal cancer development.

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