RESEARCH COMMUNICATION

Prevalence of High-risk Human Papillomavirus Infection and Cytologic Result in Thailand

Sukumarn Sanersak Swangvaree[1], Phon Kongkaew[2], Parichat Rugsuj[2], Orasri Saruk[2]

Abstract

Cervical cancer is the second most common female cancer with a high mortality rate. The established cause is high-risk types of human papillomavirus (HPV) and a new modality for cervical cancer screening is the combination of cervical cytology with HPV testing. The aim of present study was to identify the prevalence of high-risk HPV infection, and cervical cytological profiles of healthy Thai women. This largest cross-sectional investigation of HPV testing so far with cytology screening in Thailand was conducted between April 2009 and March 2010, covering a total of 14,747 women. The correlation between HPV viral load and cytology was also assessed. The mean age of the study group was 46.4 years (range 20-77 years) and the prevalence of high risk HPV infection was 8.23%. In positive women, negative cytology was observed in 72.9% , and cytology abnormalities in 27.1%, as compared to 1.57% in HPV negative women. The highest prevalence of HPV infection was identified in the youngesy age group (≤30 years). The mean viral load was $6.06x10^5$ (range 5,040.13 to $1.05x10^7$)and HPV viral load titers were higher among in women with abnormal cytology.

Keywords: Cervical cancer screening - prevalence - human papillomavirus - cervical cytology - Thailand

Asian Pacific J Cancer Prev, **11**, 1465-1468

Introduction

Cervical cancer is the second most common cancer in women and is a common cause of cancer mortality worldwide (Munoz et al., 2000; Khuhaprema et al., 2007; Ferlay et al., 2010). The worldwide prevalence of human papilloma virus (HPV) infection ranges from 10 to 20% (Smith et al., 2008), and varies according to age and country (Wallboomers et al., 1999; Parkin et al., 2002). In Asian countries, the prevalence of high-risk HPV infection is low (Wang et al., 2003; Argyn et al., 2006; WHO/ ICO, 2007). Some studies show that high HPV viral load increases the risk of abnormal cervical lesions (Bosch et al., 2002; De Sanjose et al., 2007; Zhang et al., 2008).

In Thailand, cervical cancer is the most common malignancy of all female cancers. The estimated number of cases was reported to be about 74.9% of gynecological cancers (Cliffore et al., 2005). The incidence of cervical cancer is more than 6,000 per year, with a mortality rate of approximately 50% (Smith et al., 2008). Certain subtypes of human papillomavirus (HPV) play a role in the etiology of cervical cancer (Woodman et al., 2001). Currently, cervical cancer screening includes cytology, visual inspection with acetic acid and HPV testing. There are several methods of HPV testing such as hybridization, polymerase chain reaction, HPV integrated DNA and HPV mRNA (Huang et al., 2009). A recent meta-analysis (Josefsson et al., 2000) confirmed that the combination of HPV testing and cytology has the highest sensitivity and specificity of all screening modalities with sensitivity of 99.2% (95% CI: 97.4-100), and specificity of 87.3% (84.2-90.4%).

The aim of this study was to use HPV testing in combination with cytology screening in healthy women presenting for a gynecological check up at the National Cancer Institute, Thailand in order to describe the prevalence of HPV infection, and determine the correlation of viral load with cytology and age.

Materials and Methods

Between April 2009 and March 2010, 14,747 out of 43,590 women were screened for cervical cancer at the National Cancer Institute, Thailand using the combination of HPV testing and cytology. This study was approved by the institutional ethical committee.

The liquid-based technique (Thin-Prep system, Hologic, Marlborough, MA, USA) was used for cytology. The slides were examined by a cytologist and any abnormal cases were assessed by a pathologist at the pathological division. The cytologic results were reported using the Bethesda system classification 2001. Prior to HPV DNA testing, cervical smear specimen were collected for cytology with a Digene cervical sampler in Specimen Transport Medium. Abnormal cytology was defined as ASCUS or a higher grade of cell abnormality.

*[1]Gynecological Oncology Division, [2]Cytological Unit, Pathological Division, National Cancer Institute, Bangkok, Thailand *For correspondence: ssanersak@yahoo.com*

HPV testing was performed using the Hybrid Capture 2 (HC2) hybridization assay (Qiagen, Hilden, Germany). Thirteen types of high risk HPV were tested, including HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. The results were measured by comparing the light intensity compared with control (relative light units; RLUs). The positive test was defined by a RLU value equal to or greater than the positive control, which corresponds to 1 pg of HPV DNA/μl (about 5000 HPV viral copies). Thus RLU ratio to positive control is a semi-quantitative estimate of viral load (Carozzi et al., 2005).

The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS 11.0, SPSS Inc., Chicago, IL, USA). A Chi-square test was used to evaluate correlation between the viral load, cytology and age of the study group. A P value of less than 0.05 was considered statistically significant.

Results

A total of 14,747 women were screened for cervical cancer using cytology and HPV testing. The mean age was 46.39 years (range 20 to 77 years). The overall prevalence of HPV was 8.23% (Table 1). Normal cytology was observed in 96.3% of women and abnormal cytology was observed in 3.68% of women. Among woman with normal cytology, 6.22% were HPV positive. However, HPV positive was found in 60.7% of women with abnormal cytology, which 60.1% were atypical squamous cells (ASC), 74.73% were low-grade squamous intraepithelial lesions (LSIL), 97.6% were high-grade squamous intraepithelial lesions (HSIL) positive, whereas

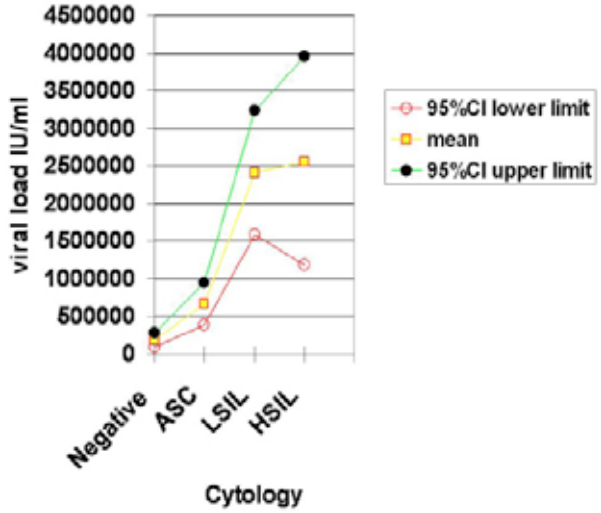

Figure 1. Viral Load in Patients with High Risk HPV According to Type of Cytology

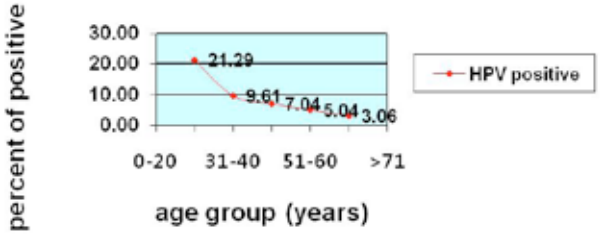

Figure 2. Age Distribution of Percent of Positive HPV Infection of Patients

Table 1. Cytology Results and High Risk HPV DNA Testing (HCII)

Cytology report	Total cases No (%)	HPV Result Positive (n) %		Negative (n) %	
Normal	14,205 (96.3%)	884	6.22	13,321	93.8
Abnormal	542 (3.68%)	329	60.7	213	39.3
Total	14,747 (100%)	1,213	8.23	13,534	91.8

(n) number of cases

Table 2. Correlation Between Age, Cytology and HPV Viral Load in HPV Positive Women

Category	Percent of cases	HPV (Mean)	Viral load (IU/mL) 95%CI	P value
Age (years)				0.047
≤ 30	4.59	$1.14*10^6$	$6.59\text{-}222.82*10^4$	
31-40	29.02	$8.03*10^5$	$48.11\text{-}112.51*10^4$	
41-50	43.63	$5.99*10^5$	$37.73\text{-}82.18*10^4$	
51-60	22.13	$2.65*10^5$	$9.95\text{-}43.06*10^4$	
61-70	0.63	$1.71*10^4$	$0.67\text{-}4.09x10^4$	
Cytology				0.012
Negative	68.27	$1.86x10^5$	$0.93\text{-}2.80x10^5$	
ASC	16.28	$6.69x10^5$	$3.88\text{-}9.51x10^5$	
LSIL	10.02	$2.41x10^6$	$15.84\text{-}32.29x10^5$	
HSIL	5.01	$2.56x10^6$	$11.79\text{-}39.55x10^5$	
AGC	0.42	$1.81x10^5$	$1.13\text{-}15.02x10^5$	

23.5% had an abnormal glandular lesion. The cytological finding of women with HPV infection were as follows: negative cytology 67.62%, ASC 15.59%, LSIL 10.19%, HSIL 6.00% and atypical glandular cells (AGC) 0.60%. A negative HPV test was reported in 91.77% of the women, comprising negative cytology 98.77%, ASC 0.80%, LSIL 0.27%, HSIL 0.01% and AGC 0.15%, respectively. The correlation between viral load and cytology is shown in Table 2 and Figure 1. Across the study population there was a wide range in the viral load. There was a significant correlation between viral load and severity of HPV on cytology (p=0.012). The higher viral load was significantly found among women with more severe cytology. The prevalence of HPV infection declined with increasing age. Women aged 21 to 30 years had the highest prevalence of HPV infection (Figure 2; 21.3%). All of the 7 women aged over 70 years old had negative HPV. Only one case of HPV infection was observed in those aged ≤20 years.

The overall mean HPV viral load in the women with HPV infection was 6.06x105 IU/mL (median 23,545.15 IU/mL). The minimum HPV viral load was 5040.13 IU/mL and the maximum was 1.04x107 IU/mL. The younger age group had a higher viral load than the older age group (Table 2; p=0.047).

Discussion

In the present study, overall HPV infection prevalence was 8.34%. The prevalence of HPV infection ranges from 10 to 35.4% in other countries (Bosch et al., 2002; Clifford et al., 2005; De Sanjose et al., 2007; Smith et al., 2008; Zhang et al., 2008). The prevalence of HPV infection varied according to age group. We found that women aged younger than 30 years old had the highest prevalence of HPV infection (21.29%). The prevalence of HPV infection was high in the younger age group and declined

with age, until 31-40 years, where it reached a plateau. Those aged older than 30 years had a low prevalence of HPV (<10%). This is a similar pattern to that observed in previous studies, and is a common shape for the age-prevalence curve for HPV infection (Cuzick et al., 2006). In addition, this study indicated that the younger age group had a significantly higher viral load than the older age group which is similar to previous reports (Zhang et al., 2008; Xu et al., 2009), However, Zhang et al. found no statistically significant difference of HPV infection between age groups.

In this study, HPV infection prevalence of women with normal cytology was 6.22% which is lower than that of previous reports in other countries. The prevalence of HPV infection among women with normal cytology ranged from 11.5 to 21.1% in Europe, Africa, and America. Shearman M. et al., 2003 demonstrated that HPV infection was found in12.7% of women with negative cytology, and 63.8% of those with abnormal cytology, of which 57.6% was ASC. Carestiato F. et al., 2006 reported a prevalence of HPV infection of 9.9% in women with negative cytology, 22.4%, 90.3%, and 90.4% in the cases with ASCUS, LSIL and HSIL, respectively. These findings show that the majority of the HPV positive women had negative cytology but those women with abnormal cytology had a higher rate of HPV infection than those with normal cytology. This result was explained by HPV infected women had spontaneously resolved about 80-90%. Only persistent HPV infective cases may be progression to pre-invasive and invasive cervical cancer (Insinga et al., 2009).

The correlation between viral load and cytology has been described in several studies (Carestiato et al., 2006; Xu et al., 2009). Carestiato et al. found high viral loads paralleled increases in SILs and the mean viral load ratio was related to cytological diagnosis. The viral load ratio was the amount of RLU of test by positive control. The mean viral load ratio of 923.1 represented HSIL cytology. In our study, there was a wide range in the viral load when assessed according to the cytological results. Those with less severe cytology had a lower viral load when compared with those with higher grade cytology abnormalities. In HSIL, the mean viral load was $2.56x10^6$ IU/ml (95% confidence interval, $1.179-3.955x10^6$). The correlation between viral load and severity of cytology was statistically significant indicating that those with a high viral load had a more abnormal cytology grade.

In conclusion, the majority of women in our study were HPV negative (91.77%), and only a very small proportion was found to have abnormal cytology (approximately 1%). This finding show that, the risk of an undetected abnormal lesion is very low when this combined screening modality is used in clinical practice. The prevalence of high-risk HPV infection in Thai women is lower than that reported worldwide. The findings in this study suggest that HPV viral load is useful for predicting severity of the cases with abnormal cytology results.

Acknowledgments

This study was supported by grants from The Oncological (Cancer) Society of Thailand under The Royal Patronage of Her Majesty the Queen and the National Cancer Institute, Thailand.

References

Arbyn M, Sasieni P, Meijer C, et al (2006). Clinical applications of HPV testing: a summary of meta-analyses. *Vaccine*, **24**, 78-89.

Bosch FX, Lorincz A, Munoz N, et al (2002). The causal relation between human papillomavirus and cervical cancer. *J Clin Pathol*, **55**, 244-65.

Carestiato F, Silva K, Dimetz T, et al (2006). Prevalence of human papillomavirus infection in the genital tract determined by hybrid capture assay. *BJID*, **10**, 331-6.

Carozzi F, Del Mistro A, Confortini M, et al (2005). Reproducibility of HPV DNA testing by Hybrid Capture 2 in a screening setting: intralaboratory and interlaboratory quality control in seven laboratories participating in the same clinical trial. *Am J Clin Pathol*, **124**, 1-6.

Clifford D, Gallus S, Herrero R, et al (2005). Worldwide distribution of human papillomavirus types in cytologically normal women in the international agency for research on cancer HPV prevalence surveys: a pooled analysis. *Lancet*, **366**, 991-8.

Cuzick J, Clavel C, Petry KU, et al (2006). Overview of the European and North American studies on HPV testing in primary cervical cancer screening. *Int J Cancer*, **119**, 1095-101.

De Sanjose S, Diaz M, Castellsague X, et al (2007). Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. *Lancet Infect Dis*, **7**, 453-9.

Ferlay J, Shin HR, Bray F, et al (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*, DOI:10.10002/ijc.25516. Date of access 4 October 2010.

Munoz N (2000). Human papillomavirus and cancer: the epidemiological evidence. *J Clin Virol*, **19**, 1-5.

Huang MZ, Li HB, Nie XM, et al (2009). Analysis of the dose-response relationship between high-risk human papillomavirus viral load an cervical lesions. *Trans R Soc Trop Med Hyg*, **103**, 779-84.

Insinga R, Dasbach E, Elbasha E (2009). Epidemiological natural history and clinical management of human papillomavirus (HPV) disease. *BMC Infect Dis*, **119**, 1-26.

Josefsson AM, Magnusson PK, Ylitalo N, et al (2000). Viral load of human papilloma virus 16 as a determinant for development of cervical carcinoma in situ: a nested case-control study. *Lancet*, **355**, 2189-93.

Khuhaprema T, Srivatanakul P, Sriplung H, et al (2007). Cancer in Thailand Vol.IV, 1998-2000. Bangkok Med Publ, Thailand, 54-5.

Parkin DM, Bray F, Ferlay J, et al (2002). Global cancer statistics. *CA Cancer J Clin*, **55**, 74-108.

Sherman M, Lorincz A, Scott D, et al (2003). Baseline cytology, human papillomavirus testing, and risk for cervical neoplasia: A 10-year cohort analysis. *J Natl Cancer Inst*, **95**, 46-52.

Smith JS, Melendy A, Rana RK, et al (2008). Age-specific prevalence of infection with human papillomavirus in females: a global review. *J Adolesc Hlth*, **43**, S5-25.

Wang S, Hildesheim A (2003). Viral and host factors in human papillomavirus persistence and progression. *J Natl Cancer Inst Monogr*, **31**, 35-40.

Wallboomers JM, Jacobs MV, Manos MM, et al (1999). Human papillomavirus is a necessary cause of invasive cervical

cancer worldwide. *J Pathol*, **189**, 12-9.
WHO/ICO Information Centre on HPV and Cervical Cancer (HPV Information Centre). Summary report on HPV and cervical cancer statistics in Thailand, 2007. [Date accessed]. Available at www. who. int/ hpvcentre Date of Access 1 June 2008.
Woodman CB, Collins S, Winter H, et al (2001). Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. *Lancet*, **357**, 1831-6.
Xu Y, Dotto J, Hui Y, et al (2009). High grade cervical intraepithelial neoplasia and viral load of high risk human papillomavirus: significant correlations in patients of 22 years old or younger. *Int J Clin Exp Pathol*, **2**, 169-75.
Zhang WY, Xue YZ, Chen M, et al (2008). Prevalence of high-risk human papillomavirus infection in different cervical lesion among organized health-examination women in shanghai, china. *Chin Med J*, **121**, 1578-82.