RESEARCH ARTICLE

Frequency and Type-distribution of Human Papillomavirus from Paraffin-embedded Blocks of High Grade Cervical Intraepithelial Neoplasia Lesions in Thailand

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Abstract

Cervical cancer is the most important female gynecological cancer, the second leading cause of cancer mortality in women worldwide and the second most common cancer in Thai women. The major cause of cervical cancer is persistent infection of human papillomavirus (HPV), leading to abnormal epithelial lesions, with progression to precancerous and invasive cancer. This study was conducted to investigate the frequency and type distribution of HPV in Thai women who had abnormal cytology. HPV detection from FFPE confirmed abnormal of high grade cervical intraepithelial lesions were for SPF-10-Innogenic Line Probe Assay. HPV-positivity was detected in 320/355 cases (90.14%) and HPV-negativity in 35/355 (9.86%). HPV-positive was found 147/320 cases (41.4%) of single infection, whereas 173/320 cases (48.7%) showed the multiple HPV infection. The most common seven types were HPV-16, -52, -18, -11, -51, -31 and -33, in that order. HPV 16 and 18, the important oncogenic HPV type, were observed in 64.8% of HSIL cases. Interestingly, a high proportion of multiple infections was found in this study and more than ten types could be detected in one case. Therefore, HPV infection screening program in women is essential, particularly in Thailand. Effective primary and secondary prevention campaigns that reinforce HPV screening for HPV detection and typing may be decrease the incidence and mortality of cervical cancer in the future and may lead to significantly improve the quality of life in Thai women.

Keywords: Human papillomavirus - frequency - type-distribution - cervical intraepithelial neoplasia - Thailand

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Introduction

Cervical cancer is the most common cause of cancer death in Thai woman. The incidence of new cases per years is 9999 and mortality is about 50% (Munoz, 2000). The etiology of this cancer is Human papillomavirus (HPV). High grade cervical lesion is precancerous lesion of cervical cancer that has abnormal tissue related to persistent HPV infection and can be developed to cervical cancer (Walboomers et al., 1999; Munoz, 2000). In recently more than 200 type of HPV have been report (Dowhanick et al., 1995; Villiers et al., 2004; Lurchachaiwong et al., 2009) and classified influence by carcinogenesis potential and about 40 types are correlated anogenital infection. Approximately 15 HPV type associate with high grade epithelial cervical neoplasia, invasive cervical and other anogenital cancer have been categorized as high-risk types such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82, whereas the HPV types which found primarily in genital wart or non-malignant lesion were designed as low-risk types such as HPV 6, 11, 40, 42, 43, 44, 54, 61, 72, 81, 83, 84; (IARC, 1995; Zur, 2002; Bharti et al., 2010). And there are some HPV types that can not be exactly classified into high-risk type such as HPV 26, 53 and 66, categorized as probable high-risk type (Bharti et al., 2010).

The most frequent of HPV infected in cervical cancer and high-grade cervical intraepithelial neoplasia (CIN) are HPV type 16, 18. The prevalent of high risk HPV in high-grade lesion is about 84.9% (84.1-85.7) (Dowhanick et al., 1995) and multiple infection of HPV is found about 20%-50% (Bhata et al., 2008; Ciapponi et al., 2011; Correnti et al., 2011). Currently, there are a lot of methods for the HPV detection and typing. Many of the efficiency techniques are based on signal and/or target amplification (Bharti et al., 2010; WHO, 2010). Recently, HPV detection methods are comprised direct hybridization (In situ hybridization and Hybrid Capture II), PCR amplification (Type specific and consensus sequence) and Emerging Technologies (Luminex XMap, Virul load/real time PCR, Pretect HPV-Proofer, DNA Chip, Gen-Probe Aptima, P16, Proecc) (Bharti et al., 2010). The Innogenic-Line –Probe-Assay (INNO-LiPA) is the new methods of PCR amplification, which can detect multiple HPV infection

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and high detection rate due to use short fragment of L1 base pair region (Bello et al., 2009).

The purpose of this study was to identify overall prevalence of HPV infection and distribution of multiple infections in paraffin-embeded cervical specimen of high-grade CIN by second generation of Innogenic-Line-Probe Assay using SPF-10 primers.

Materials and Methods

Subjects

Cervical specimens were collected by gynecologic oncologist, NCI, Thailand and sent to pathological diagnosis. All 355 paraffin-embedded specimens were newly diagnosed high grade cervical lesion form January 2008 to December 2011. This study was institute ethical committee approved.

DNA extraction

Sections of 5-10 μM thick were cut from Formalin-fixed Paraffin Embedded Tissue (FFPE) and collected in sterile micro-centrifuge tubes. The contaminant between each sample was eliminated by changing a cutter blade and careful microtome cleaning. Samples was de-paraffinized with xylene and rehydrated in absolute ethanol. The palette tissues were centrifuged and air-dried to ensure that no residual ethanol was present. And then DNA was extracted from the tissue using QIAamp DNA Mini kit (Qiagen) according to the manufacturer's protocol and stored at -20°C prior to the HPV amplification. DNA quality was checked by *β-globin*, house keeping gene, amplification using Polymerase Chain Reaction (PCR) under the following; initial denaturation at 94°C for 5 min, 40 cycles with the cycling of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min and then final extension at 72°C for 5 min. The primer sequences were as follows (Jacob et al., 1996): β-globin sense ACA CAA CTG TGT TCA CTA GC and β-globin antisense GAA ACC CAA GAG TCT TCT CT. The amplified products were subjected to electrophoresis and visualized by UV transilluminator. (SYNGENE, England)

HPV detection and genotyping

Human papillomavirus (HPV) DNA was detected by PCR amplification with a board-spectrum; SPF-10 biotinylated primer. The 65bp fragment in L1 region of HPV genome was investigated. PCR amplification is performed in a total volume of 50μl containing 40 μl Master Mix solution of INNO-LiPA HPV genotyping Extra Amp (Innogenetic, Belgium) and 10μl of extracted DNA. PCRs were run following profile; decontaminate of uracil containing DNA at 37°C for 10 min, initial denature 94°C for 10 min, 40 cycles of 94°C for 30 sec, 52°C for 45 sec, 72°C for 45 sec and then final incubate at 72°C for 5 min. The PCR product was processed immediately for genotyping or stored at -20°C for further study.

All of samples were genotyped using INNO-LiPA HPV Genotyping Extra (Innogenetic, Belgium) following the manufacturer's instruction. The principle of this genotyping kit is based on reverse hybridization assay and has designed for the identification of 28 different HPV type (HPV-6, -11, -16, -18, -26, -31, -33, -35, -39, -40, -43, -44, -45, -51, -52, -53, -54, -56, -58, -59, -66, -68, -69, -70, -73, -74 and -82) of the HPV by detection of specific sequence in the L1 region of the HPV genome. HPV genotyping was performed automatic on AutoBlot 300H (MedTec, USA). Briefly, 10 μl the PCR product of each sample was denatured with 10 μl denaturing solution and hybridized on the INNO-LiPA strip containing HPV specific probes, two HPV positive control and internal control; *HLA-DPB1* gene, reference lines. Hybrized strips were transferred onto AutoBlot 300H machine and incubated in hybridization buffer for 60 min at 49°C and following by stringent solution for 30 min on shaking platform. Detection was carried out using streptavidin-conjugated alkaline phosphatase and BCIP/NBT chromogen substrate solution at room temperature for 30 min, respectively. The reaction was stopped by aspiration of the substrate solution and washed with phosphate buffer. These strips should only be read when they were complete dry. The results can be interpreted visually with clear lines on the strip and scored by using the INNO-LiPA HPV genotyping Extra reading card which is indicated the position of each probe relative to the reference lines.

Results

Three hundred and fifty five high-grade cervical lesion specimens were collected. The mean age at diagnosis of patients is 45.37 years (24-78). The tissue specimens were received from cervical biopsy 11 cases (3.09%), large loop electrical excision procedure specimen 339 cases (95.5%), and hysterectomy 5 cases (1.4%).

The histology high grade cervical lesion was comprises squamous epithelial abnormality was 94.7% and glandular lesion was 5.3%. This study was found HPV positive from Innogenic line probe assay (INNO-LiPA) test is 90.14% of all specimens which single-type infection was 41.4% and multiple infection was 48.73% of all specimens and could be identified HPV type as shown in Table 1.

The multiple infection were comprised one type (45.9%), two type (14.7%), three type (12.5%), four type (9.7%), five type (4.4%), six type (4.7%), seven type (4.4%), eight type (1.3%), nine type (1.6%), ten type (0.9%).

Table 1. Distribution of Single and Multiple HPV Types Infection in High Grade Cervical Intraepithelial lesion (n=320)

	HPV type	Frequency (%)
Single type infection	16	44 (13.75)
	18	11 (3.44)
	31	7 (2.19)
	33	5 (0.94)
	39	1 (0.31)
	51	8 (2.50)
	52	61 (19.06)
	58	9 (2.81)
	Untypeable	1 (0.31)
Multiple types infection	2 types	47 (14.69)
	>3 types	126 (39.38)

Table 2. Distribution of Probable High Risk and Low Risk HPV Co-infection in High Grade Lesion of Cervical (n=320)

	HPV type	Frequency (%)
Probable high risk type	26	19 (5.94)
	53	24 (7.5)
	66	-
Low risk type	6	7 (2.19)
	11	75 (23.44)
	44	23 (7.19)
	54	3 (0.94)
	69	2 (0.63)
	70	18 (5.63)
	71	2 (0.63)
	73	3 (0.94)
	82	2 (0.63)
Untypable		**2 (0.63)**

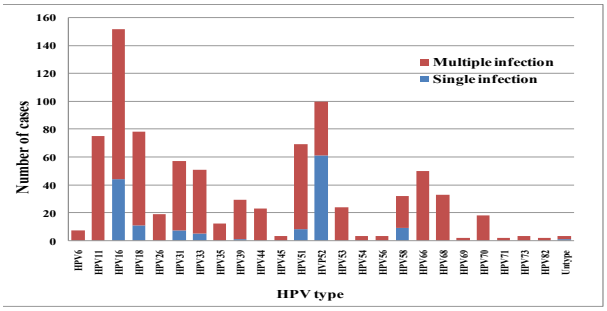

Figure 1. Proportion of Single and Multiple Infections of HPV Types in High Grade Intraepithelial Cervical Lesion

The common type of HPV infection were sequence by 16, 52, 18, 11, 51, 31, 33, 66, 68, 58, 39, 53, 44, 26, 70, 35, 6, 45, 54, 56, 73, 69, 71, 82, 40, 43, 59, 74. The proportions of HPV infection has shown in picture 1.

Interestingly, this study not found HPV 59 and 74 infection which are high risk HPV group and could be detected all of probable high risk and low risk type such as HPV 6, 11, 40, etc (Table 2) in term of multiple types infection with the other types.

Discussion

Human papillomavirus (HPV) testing and typing is important to present the prevalence and type distribution in patients or population. They also play important role in early detection of abnormalities cytological. The prevalence of HPV infection has been determined by using various techniques and the difference prevalence is geography and effect to screen the new cases of cervical cancer or prevention program, especially vaccine impact monitoring

(Dowhanick et al., 1995; IARC, 1995; Zur, 2002; Villiers et al., 2004; Bharti et al., 2010). Currently, there are many HPV testing methods to be developed. Specificity and sensitivity are quite variety in different method [9]. In this study, HPV infection was evaluated by SPF-10-LiPA (INNo-LiPA). The short fragment (65bp) of SPF-10 primers is appropriate amplified the formalin-fixed paraffin-embedded tissue (FFPE) which have a minimal volume and difficult to analyze (Gravitt et al., 2007; van Hamont et al., 2006; Bello et al., 2009).

From our results, HPV DNA and type distribution testing were investigated from three hundred and fifty five FFPE tissues with high grade cervical lesion of Thai women who attend the National Cancer Institute, Thailand. HPV DNA was detected in 90.14% (320/355) cases and non-infected showed the results as 9.86% (35/355). Of 320 HPV positive cases, 147 (41.4%) and 173 (48.73%) cases were infected with a single and multiple HPV types, respectively. The seven most frequent HPV types were 16, 52, 18, 11, 51, 31 and 33. Approximately 64.8% of cases are HPV-induced with HPV 16 and 18 as compares to the previous study by Correnti et al. (2011) reported that the frequency of high risk HPV genotype in low grade and high grade squamous intraepithelial lesions and cervical carcinoma among Venezuelan women, using Innogenesis genotyping extra procedure and 100 biopsy samples of HSIL were investigated. HPV DNA was detected in 95.0%, in which 87.3% and 3.2% were determined as high and low risk HPV type, respectively and 9.5% of total HSIL in this report was undefined HPV type. In addition, they could be detected both single (44.2%) and multiple (53%) infection and HPV 16 and 18 were the most common type among Venezuelan women, that was observed in 54.6% of HSIL cases. While, the meta-analysis of HPV prevalence and type distribution in women from south Asia was conducted by Bhatla et al. (2008) from nine publications, in order to estimate the impact of an HPV16/18 prophylactic vaccine in this region. A total of fifty-two of HSIL were subjected and HPV prevalence was 86.5%, which was sugge 69.2% of single type infection and 23.1% of multiple type infection.

In addition to the HPV prevalence of HR-HPV in severe cervical lesion study in Spain women (Mateos et al., 2011), 111/205 patients of available cytology results and histology confirmed with HSIL were observed in this study. The report showed that HPV 16 was the most prevalent HR-HPV, followed by HPV 31, 58, 52, 18 and 33, respectively. It has been indicated that HPV 16 and 18 account for 70.1% of all HSIL.

From a systematic review of seventy-nine studies of type specific HPV prevalence in cervical cancer and high grade lesions from 18 countries in Latin America and the Caribbean (Ciapponi et al., 2011), the results showed 82.5% of HSIL cases harbored in HR-HPV, in which 16.8% (12.9-21.2) was multiple type infection, 45.5% was infected with HPV 16 and 8.9% was HPV 18. The next five most common types were HPV 31, 58, 33, 45 and 52. The frequency of multiple HPV infection in this study was 12.6% (8.7-17.2). It was rather smaller than the other report.

In overall summarized of our study found that the HPV prevalence ratio in HSIL was not different from the other in analogous previous studies (Bhatla et al., 2008; Ciapponi et al., 2011; Correnti et al., 2011; Mateos et al., 2011), although the multiple HPV infection radio was higher than the others. The most six common HPV types in this study were HPV 16, 52, 18, 51, 31 and 33. There are small variation in prevalence and types-distribution sequence of HPV. The variety may be varies widely

between regions and the geographical variation is an important factor to consider in the implementation of vaccine campaigns in each region. Currently, a potential bivalent and quadric-valent vaccines show efficacy in the prevention of genital warts; HPV 6, 11 and oncogenic HPV 16, 18. It is a new opportunity to reduce the burden of HPV-related diseases, especially cervical cancer. However, the HPV vaccine was not appropriate design and more efficacies for Thai women population, which was infected with the other types of HPV, not only HPV 6, 11, 16 or 18. A development type-specific multivalent HPV vaccine may be provide more protection than the current vaccine and could be protected HSIL or CINII/III lesion. Interestingly, it may used for surrogate endpoint of impact monitoring effective of vaccine.

Moreover the data in this study showed the high frequency of HPV infection, especially multiple type infection ratios in Thai women which are interesting baseline data, although the sample size is small and not represented the overall prevalence in Thailand. Therefore, it is necessary to have a screening for HPV detecting and testing in normal population in Thailand, including used in clinical follow-up or treatment of precancerous lesion, in order to increase efficacy of protection and treatment of HPV-related disease in Thai women. The primary and secondary HPV-related disease screening campaign might be lead to significantly improve the quality of life. In addition, the effective implementation of the HPV vaccine and continued screening should dramatically reduce incidence or the mortality of HPV-related disease, especially cervical cancer in Thai women.

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