

Original Article

Community-based prevalence versus hospital-based incidence of genital Human Papillomavirus infection in Central Vietnam

Vu Quoc Huy Nguyen¹, Viet Quynh Tram Ngo^{2,3}, Minh Tam Le¹, Hoang Bach Nguyen^{2,3}, Van Bao Thang Phan², Quang Vinh Truong¹, Ngoc Thanh Cao¹, Piero Cappuccinelli^{3,4}

¹ Department of Obstetrics and Gynecology, Hue University of Medicine and Pharmacy, Hue University, Hue, Viet Nam

² Department of Microbiology, Hue University of Medicine and Pharmacy, Hue University, Hue, Viet Nam

³ Carlo Urbani Center, Hue University of Medicine and Pharmacy, Hue University, Hue, Viet Nam

⁴ Department of Biomedical Sciences, University of Sassari, Sassari, Italy

Abstract

Introduction: This study aims to determine the genital HPV prevalence in reproductive-age women in Thua Thien Hue Province and comparison with HPV incidence in Hue University Hospital, Vietnam.

Methodology: Cross-sectional study on 1,034 women of reproductive age from 11 communes/wards of three districts representing three different geographic areas of Thua Thien Hue Province, Vietnam. The hospital-based group included 102 women with cervicitis and/or abnormal Pap smear result coming to Hue University Hospital. Extracting DNA from cervical samples, performing the real-time PCR for detecting HPV and the reverse dot-blot assay for HPV typing in HPV positive cases.

Results: In community, HPV prevalence was 0.9%. Mean-age of HPV positive group was 37.9 ± 6.2 years. The detected low-risk types were 6 and 11; high-risk types include 16, 18, 33, 45, 52, and 58. Single-type infection was found in 66.7% of cases. In hospital-based group, 41.2% of women have been infected with HPV, 6 different HPV types were detected. HPV18 was the most frequent high-risk type (33.3%), followed by HPV16 (15.1%); HPV6 was the most frequent among low-risk HPV types (31.2%). Single-type infection rate was 33.3%; 2 and 3 types co-infections were 28.6% and 38.1%, respectively.

Conclusions: Routine screening of high-risk HPV infection in women with symptomatic gynecologic infection and/or abnormal Pap smear appears to be benefit in early detection and prevention of cervical cancer, due to the high incidence of HPV infection.

Key words: HPV; real-time PCR; reverse dot-blot; genital tract infection.

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Introduction

According to World Health Organization, cervical cancer is the second most common cancer in women living in less developed regions with an estimated 445,000 new cases in 2012 (84% of the new cases worldwide); approximately 270,000 women died from cervical cancer, more than 85% of these deaths occurring in low- and middle-income countries [1]. In Vietnam, the incidence of cervical cancer is about 20-30 per 100,000 women/year; annually there are 5,000-6,000 women who will die from cervical cancer [2]. In contrast, cervical cancer incidence in developed countries is very low, thanks to the effective and efficient organized mass screening program and appropriate intervention after detection of abnormal cases [1].

Nowadays, HPV has been identified as the infectious agent having a causative role in the development of cervical cancer and precancerous lesions. High-risk HPV DNA have been identified in 99.9% of invasive cervical cancers specimens [3]. Data from medical literature with hundreds of observational studies and 6 randomized controlled trials recommend the importance role of HPV testing as a primary screening tool for early detection of cervical cancer precursors [4]; however local evidence from community-based HPV testing and the actual HPV infection prevalence are still needed, in order to assess the role of mass screening by HPV testing.

HPV is classified into 2 groups: low-risk and high-risk. Based on typing, the most common high-risk type was HPV16, followed by 52, 18, 31, 33, and 35. HR HPV was detected in 96.6% of CIN2, 95.5% of

CIN3/AIS specimens and 85.7% of specimens with invasive cancer [5]. Detection of HPV infection, especially in women with cervical lesions or abnormal Pap smear is very important in management of cervical intraepithelial neoplasia/pre-invasive cancer. Currently, there are different methods to identify HPV genotypes such as multiplex real-time PCR assay, PCR combined with blot hybridization, viral gene sequencing. This study aims to determine the community based prevalence of genital HPV and the HPV type in reproductive-age women, in comparison with HPV infection incidence in women coming to hospital because of gynecological symptoms / abnormal Pap smear in Thua Thien Hue, Vietnam.

Methodology

To investigate the community-based prevalence, a cross-sectional study on 1,034 women in reproductive age (15-49 years), married or have had sexual intercourse were enrolled after agreement on informed consent. Criteria of exclusion from study were: women having menses, pregnant women or in the post-partum period, having vaginal douching during 24 hours before examination, existing of cervical precancerous-cancerous lesion or history of pelvic radiation or after hysterectomy.

Sample size was calculated for rate estimate investigation:

$$n = Z_{\alpha/2}^2 \frac{p(1 - p)}{\Delta^2}$$

With the prevalence $p = 12\%$ [6], $\Delta = 0,02$, $\alpha = 0,05$, $Z_{\alpha/2} = 1,96$, minimum simple size is 1,015 women. In total, 1,100 women were invited for gynecologic examination.

The study has been carried-out in 3 districts, representative for geographic and socio-economic characteristics of Thua Thien - Hue Province: Hue city (408 samples), Nam Dong District (299 samples), and Phu Vang district (425 samples) with a total of 11 communes. From the eligible age group, 100 women per commune were randomly selected from the list of population provided by Commune Health Center; 66 women were excluded according to exclusion criteria, resulting in the final sample size of 1,034 women. All

individuals enrolled in the study were subjected to history taking, gynecologic examination and HPV testing.

In hospital based group, 102 women came to Hue University Hospital because of gynecologic disorders and diagnosed with infections and / or abnormal Pap smear. HPV testing for both groups were carried out from cervical samples, collected with the brush, transported to the lab within 48 hours in the 1.5 ml Eppendorf tube containing 400 μ L TE buffer and stored at -20°C until performing tests. DNA isolation and detecting HPV infection by a Taqman probe based real-time PCR assay were performed according to the manufacturer’s instructions (LightPoweriVA pDNA Extraction Kit and LightPoweriVA HPV Genotype rPCR Kit, Viet A Corporation, Ho Chi Minh City, Vietnam). The amplimers from HPV-positive samples were subsequently analyzed by reverse hybridization on the HPV reverse dot blot assay (LightPoweriVA HPV Genotype PCR-RDB, Viet A Corporation, Ho Chi Minh City, Vietnam). This assay comprises a membrane containing type-specific oligonucleotide probes, immobilized as dots. PCR products are hybridized at high stringency to these probes, generating a type-specific hybridization pattern. The LightPoweriVA HPV Genotype PCR-RDB permits specific detection of 24 HPV types. HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68 and HPV-81 were considered high -risk types, whereas HPV-6, 11, 40, 42, 43, 44, 54 and HPV-79 were considered low-risk types. Part of the β -globin gene was amplified from each sample as a positive control for DNA isolation. The HPV types are determined when the blue dots appear on the membrane corresponding to the type numbers according to the manufacturer’s instructions.

This study was approved by the ethical committees of the participating institutions.

Data were analyzed by the software SPSS 19.0.

Results

Distribution of HPV infection by geographic areas in community

Among 1,034 women having HPV testing, 9 cases were positive (0.9%), including 2 cases (22.2%) in Nam

Table 1. Geographic distribution of HPV positive cases from community.

Geographic area	No. samples	Positive	%
Mountainous area	299	2	22.2
Urban area	408	7	77.8
Coastal area	425	0	0.0
Total	1,034	9	100.0

Table 2. Women age and HPV infection.

Age groups	Community-based		Hospital-based	
	Total n (%)	HPV (+) n (%)	Total n (%)	HPV (+) n (%)
15-19	2 (0.2)	-	3 (2.9)	2 (66.7)
20-29	166 (16.1)	-	36 (35.3)	10 (27.8)
30-39	437 (42.2)	5 (0.5)	37 (36.3)	17 (46.0)
≥ 40	429 (41.5)	4 (0.4)	26 (25.5)	13 (50.0)
Total	1034	9 (0.9)	102	42 (41.2)

Dong district (mountainous area) and 7 cases (77.8%) in Hue City; no HPV positive case was detected in Phu Vang district, a coastal area (Table 1).

Women age and HPV infection

Regarding to age distribution as shown in Table 2, it is not different between under and over 40 year old in HPV infection rate both in population-based or hospital-based cohort.

Distribution of HPV types

In the community-based group, 9 cases were positive with real-time PCR for HPV; 8 HPV types were detected with RBD assay in 14 times. HPV-16 was the predominant one (3/10 detections) in the high-risk HPV group, followed by HPV-18 and HPV-58 (2/10 detections). In the hospital-based group, there were 6 high-risk HPV types detected in 93 times. HPV type 18 was the most common (33.3%), followed by type 16

(15.1%) and type 52 (14.3%). by type 6 (31.2%) (Table 3 and 4).

In the community-based group, up to 66.7% was single infection of HPV type, compared to 33.3% in the hospital-based group (p < 0.05). The remaining 33.3% was co-infected by 2 or 3 types in the community-based group. In the hospital-based, the incidence of co-infection by 2 and 3 HPV types was really high (28.6% and 21.4%); specially, there were 6 cases (14.3%) co-infected by 4 types and 1 case (2.4%) co-infected by 5 types.

Discussion

Cervical cancer is the second cancer in women worldwide [1]. Nowadays, HPV has been confirmed as the causative factor of cervical cancer development [3]. The increasing number of studies on relationship and causative effect between HPV and cervical cancer helped to clarify the pathologic role of viral DNA,

Table 3. Distribution of low-risk and high-risk HPV types.

HPV type	Community-based		Hospital-based	
	n	%	n	%
Low-risk				
HPV-6	2	14.3	29	31.2
HPV-11	2	14.3	12	12.9
High-risk				
HPV-16	3	21.4	14	15.1
HPV-18	2	14.3	31	33.3
HPV-33	1	7.1	0	-
HPV-45	1	7.1	0	-
HPV-51	0	-	1	1.1
HPV-52	1	7.1	6	14.3
HPV-58	2	14.3	0	-
Total	14	100	93	100

Table 4. Distribution of HPV infection by number of co-infected types.

Co-infection	Community-based		Hospital-based	
	n	%	n	%
Single HPV type	6	66.7	14	33.3
Two HPV types	1	11.1	12	28.6
Three HPV types	2	22.2	16	38.1
Total	9	100	42	100

allowing preventive approaches with more effectiveness to be introduced.

Carrying out survey, clinical examination and laboratory testing on the study sample which consists of 1,034 women from 3 areas representative for Thua Thien Hue province, we detected 9 positive cases with genital HPV DNA, resulting in the prevalence of 0.9%, among them, participants from Hue city occupied 77.8%, and those from mountainous area was 22.2%, no case have been detected from coastal area. These results were quite different from our initial expectation as well as those from other studies in Vietnam and abroad. The community-based study of Tho LT *et al.* in Hanoi found the prevalence of HPV infection of 5.13% [6]; in Ho Chi Minh City, the prevalence was as high as 12% [7]. Recent estimation on HPV prevalence in developing countries (15%) was much higher than our results [1]. HPV detection rate can be very different from one country to the other, even from one region to another one in the same country [8,9]. This variation can be explained by the differences in living environment, lifestyle and especially sexual behaviors. Based on the geographic perspective, HPV infection prevalence of the Hue City about 1.7% was similar to those from Duc NB (1.8%) [10] and not much lower than those from other study by Vinh LQ [11] done at 3 provinces, including Thua Thien Hue Province, with the HPV detection rate of 2.9%.

In hospital-based group, women with gynecologic infection and/or abnormal pap smear had up to 41.2% positive results of HPV infection. This result reaffirmed the important role of HPV screening in symptomatic gynecologic disorders coming to hospital. Our findings were in agreement with the findings of other authors [4,7,12,13]. It is clearly that the two different sources of data present important different infection rate, particularly in hospitalized women for gynecologic disorders, it is expected a far higher prevalence of HPV infection.

By evaluating characteristics of study sample, we would like to identify some factors related to the HPV infection rate. However, the low number of detected HPV positive cases did not allow the analysis with statistically significance. In community, HPV positive cases were seen in the group of women aged above 30 years (100%), mean age 37.9 ± 6.2 years, in comparison to the mean age of 37.3 ± 7.6 years of the negative group; similar outcomes were seen in other studies in Vietnam by Thanh PV and by Vu TN with conclusion of highest rate of HPV infection was women from 40-49 year of age [12,13]. In hospital group, HPV infection

was most common in reproductive-age women, around 20-39 year of age accounted for 64.3%.

This study in community found 9 different HPV types, among them 2 low-risk (HPV-6, HPV-11) and 6 high-risk types (HPV-16, 18, 33, 45, 52 and HPV-58), HPV-16, 18 and HPV-58 occurred in high percentage. Number of HPV types detected in our study was lower than those from other studies [7,14,15]. However, the predominant rates of some commonly seen low-risk and high-risk types were similar. The rate of 1-type infection was 66.7%, those of 2-types and 3-types co-infection were 11.1% and 22.2%, respectively. In hospital-based group, 41.2% of cases has been infected with HPV, 6 types of HPV was detected, the majority of high-risk HPV type took up 33.3%, belonged to HPV type 18, the second one was the HPV type 16 (15.1%) and the HPV type 6 dominated in the type of low-risk HPV (31.2%).

Results from many international studies showed the rate of hospital-based high-risk HPV types infection of about 70%, mainly by HPV-16 and HPV-18 [4,9]. The study in Ho Chi Minh City found that among HPV (+) cases, type 16 has had the highest rate (55.95%), type 18 occupied 36.11% and type 58 occupied 11.31% [16]. Another study by Vu TN found the rate of type 11 of 18.8%, type 16 of 22.6% [2]. Similar results were found in Hanoi by Vinh LQ *et al.* (2009), with type 18 (31.3%), type 58 (16.37%) and type 16 (14.6%) [14]. Despite the number of detected HPV positive cases in present study was not very high but from the rate perspective, type 16 still seen at highest percentage (33.3%), type 58 in 22.2%, type 18 and others in 11.1%.

Analysis on co-infection in hospital group, the proportion of infected cases with at least one type of HPV was 33.3%, 2 and 3 types were 28.6% and 21.4%, respectively. Besides, there were some cases coinfected with 4 or 5 types of HPV with low proportions. The persistent HPV infection was determined as direct cause of cervical cancer [3]. Lee *et al.* concluded that single type infection of HPV has relative risk of cervical cancer 19.9 times and co-infection of more than one type raise this risk of 31.8 times [17].

The limitations of this study include the rather small number of subjects involved in the hospital-based arm, and the technique of HPV testing, which was locally available and affordable but still not internationally standardized.

Conclusion

Human Papillomavirus infection prevalence among women in reproductive age in Thua Thien Hue province was found not to be high and has been observed mainly

in urban area. Most of positive cases were infected by high-risk types, namely HPV-16, 18, 33, 45, 52 and HPV-58, predominantly with single-type infection. HPV primary screening would not be feasible in our community using the low cost Taqman probe based real-time PCR assay. Routine screening of HPV infection in women with symptomatic gynecologic infection and/or abnormal Pap smear appears to be benefit in early detection, prevention of cervical cancer with high incidence of HPV infection.

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Corresponding author

Vu Quoc Huy Nguyen
 Department of Obstetrics and Gynecology, Hue University of
 Medicine and Pharmacy, Hue University
 6 Ngo Quyen Street
 Hue, Viet Nam
 Tel: +84.234.3822173
 Fax: +84.234.3826269
 Email: nvqhuy@huemed-univ.edu.vn

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