Nat. Volatiles & Essent. Oils, 2021; 8(4): 11154-11163

NVEO
Natural Volatiles &
Essential Oils

The Detection And Genotyping Of The Human Papillomavirus In Vietnam

Sang Tien Trieu[1*] , Khoa Van Tran[1] , Trang Thi Nguyen[2] , Giang Truong Ngo[1] , Tung Thanh Nguyen[1] , Hoang Minh Ngan[3] , Tien Anh Ngo[4*] , Vu Huong Ly[4] , Vu Van Tam[4]

[1] Department of Biology and Medical Genetic, Vietnam Military Medical University,Vietnam

[2] Department of Biomedical and Genetic, Hanoi Medical University, Vietnam

[3.] Lab IVF, 16A Private Hospital

[4] Laboratory of Applied Micro and Nanotechnology (LAMINATE), National Food Institute, Technical University of Denmark, 2800 Kgs, Lyngby, Denmark

Corresponding author: Sang Tien Trieu

Abstract

Background: Human papilloma viruses (HPVs) are the major etiological agents of cervical cancer which is a common woman's cancer type. More than 100 HPV types have been identified; approximately 70% are capable of infecting genital mucosa. A.

Materials and Methods: In the presented study we have used the Papanicolaou smear (PAP test) is used to detect epithelial abnormalities. More other the PCR-test assay combined with Reverse Dot Blot Hybridization has allowed for accurate detection of HPV and specific types of infection. Have been tested 1403 patient samples at Vietnam Military Medical University (Hanoi, Vietnam) to screen and identify HPV genotypes.

Results: Was detected, the 169 samples (12.03%) were positive and 152 samples (11.19%) had unidentified results of the PCR-test was noted positive samples. And the most frequently found three HPV subtypes were 18 (26.63%), 16 (17.58%), and 11 (13.59%). This group was followed by HPV subtypes 6 (9.78%), 81 (8.15%), 45 (5.43%) and 82 (3.26%). Other genotypes were detected at frequencies more over 4% and 6 genotypes occur only once.

Conclusion: The results have indicated the prevalence and diversity of HPV infection in specimens. It is extremely important is PCR-test and Reverse Dot Blot Hybridization using in the HPV diagnosis and strain determination.

Keywords: Human papilloma viruse (HPV), diagnosing, strain determination, polymerase chain reaction (PCR), Reverse Dot Blot Hybridization.

1. Introduction

The global tendency in health rescuing needs detailed and profound knowledge in health knowledge. Woman's health, and reproductive health, in particular, is one of the important study directions ways [1,2].

Human papilloma virus (HPV) is a DNA virus, belongs to papillomaviridae family, which is spread through skin-to-skin and most commonly sexually transmitted infection, include oral, vaginal or anal sex [3]. Sometimes, HPV can be transmitted from mother to infant, causing some genital or respiratory problem [4]. That may progress clinical manifestations such as focal epithelial hyperplasia (mucosal irritation, epithelial sclerosis, warts, and oral neoplasia). Typical symptoms of HPV patients are warts, especially genital warts that normally appear as small bumps, cluster of bumps or stem-like protrusions. They are variety in size, shape and color. In women, warts appear commonly in vulva. Besides, they can also present in anus area, cervix or inside the vagina. For men, the infected parts could be penis,

Recently, HPV strains have been established as causative agents of cervical cancer – the second most common causing woman cancer; approximately 70% of cervical cancers and 90% of genital warts [4-5]. Despite this fact, not all HPV types evolve to cancer. About 40 HPV types among more than 200 related virus can be spread through sexual contact while the rest that can cause non-genital warts are not sexually transmitted. Depending on benign or malignant lesions, various types of sexual transmitted HPV are divided into two types, high-risk and low-risk. High-risk group is directly related to cancer, especially cervical cancer. Other types of cancer caused by HPV can be listed as 95% cases of anal cancer, 75% cases of oropharyngeal cancer, 65% cases of vaginal cancer, 50% cases of vulvar cancer and 35% of penile cancer. Most of them are caused by HPV type 16 or 18. In contrast, low-risk group is low potential, mainly associated with benign lesions. Among the low-risk group, type 6 and 11 are most common ones [6-7].

To improve the management of patients at risk for cervical disease as well as prevention of invasive cervical cancer HPV detection and genotyping tests are needed. Furthermore, the better understanding of the high-risk type of HPV is necessary to evaluate the benefits of vaccines for the prevention of HPV infection. This can reduce the cervical cancer in the population [1,8-9]. Therefore, a number of molecular technologies have been developed for rapid and accurate HPV detection and genotyping such as nucleic acid-hybridization assays like the Southern blotting, in situ hybridization, dot-blot hybridization. Can be conducted signal amplification assays (eg., Carvista HPV, Hybrid Capture 2), and nucleic acids amplification assays (eg., Microarray, PapilloCheck, PCR, PCR-RFLP, real-time PCR, Abbott real-time, COBAS 4800 HPV, Genome sequencing, CLART HPV 2, INNO-LiPA, the Linear Array, Clinical Arrays HPV, MCHA, Pre Tect Proofer, APTIMA HPV Assay). These methods

indicated a powerful for detecting and genotyping HPV with great benefits such as Southern blot in the nucleic acid-hybridization assays is gold standard for HPV genomic analysis, Hybrid capture 2 and Cervista HPV in the signal amplification assays were approved by PDA (Food and Drug Administration) for quantitative HPV test with lower false-positive rate and high sensitivity to genotyping, and nucleic acids amplification assays showed a flexible technology for viral load and genotype with very high sensitivity and multiplex analysis. However, they also remain many weaknesses such as low sensitivity and time consuming in the nucleic acid-hybridization assays, impossible to recognize individual HPV types in the Hybrid capture 2, lower amplification signal of some HPV genotypes and high risk of false positives in the nucleic acids amplification assays [10-12].

Reverse Dot Blot Hybridization (RDBH) can accurately determine the infection and co-infection of the HPV type on the same specimen. This can be considered as advantage over sequencing assay. PCR reaction is able to recognize and extend primers in order to produce billion of copies from single double-stranded DNA within 30 cycles of amplification [(31)]. After PCR procedure, the positive products are used directly, again amplified the signal of the RDBH technique, that dramatically increases the sensitivity of the test, and reduces false-negative cases. Herein, we determined the prevalence of HPV infection and genotype distribution of 1403 patients by using PCR-RDBH. The results provide information for a more comprehensive view of HPV infection in the human population [13-15].

2. Materials and Methods

In this study, we used PCR combining with Reverse Dot Blot Hybridization (PCR-RDBH) to detect and identify genotypes of HPV. This assay offers superior advantages over other molecular biology techniques.

2.1. Cytological samples

This study was performed with 1403 cytological samples (patients' average age was 36.1 year, range: 17-83 years) in Vietnam Military Medical University (Hanoi, Vietnam) to detect HPV. Cytological samples were collected from patients with clinical symptoms of HPV or anamnesis of vaginal and cervical lesions. Samples were collected by using special tools to scrape or brush cells from the surface of the cervix, then preserved in PBS 1X solution in fridge.

2.2. Detecting and typing HPV genotype

2.2.1. HPV Extraction

All the samples collected from patients were centrifuged at 5000 g in 10 minutes and carefully removed the supernatant. The precipitation was collected. Using an aliquot (200 µm) of this precipitation, we extracted DNA with Kit of Viet A Corporation. DNA extracted was lyophilized and cryopreserved at -20 °C.

2.2.2. Primers and PCR-RDBH assays

To detect infection of HPV, we use general consensus primers MY09/MY11 with the sequence: (5'-GCG ACC CAA TGC AAA TTG GT- 3') and (5'- GAA GAG CCA AGG ACA GGT AC-3')[39]. This primers was used to amplify 2.5 µl of the extracted HPV DNA in 12.5 µl reaction. PCR was carried out in a thermal cycler under the following conditions: 95°C for 5 minutes, followed by denaturation at 94°C for 20 seconds, annealing at 55°C for 20 seconds, and extension at 72°C for 40 seconds for 40 cycles. PCR product is a 450 bp fragment in the corresponding part of the HPV L1, L2 gene.

Positive control accompanied with the commercial Kit (LightPower iVA HPV Genotype PCR-RDB Kit, Vietnam). PCRs products were detected by electrophoresising with 2% agarose gel in 30 minutes. The gel then was dyed with ethidium bromide 0.2 mg/ml (15 minutes) and observed by UV.

Samples, that were positively HPV examined were tested by RDBH to detect specific genotype of each sample. This Kit can identify 24 Human papilloma virus types : high-risk HPV genotypes consists of 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 50, 66, 68, 82 and low-risk HPV includes 6, 11, 42, 43, 61, 70, 71, 81. The positive signs were detected by colored experiment. Results were compared with hybrid membrane outline.

3. Results and discussions

2.3. Prevalence of HPV infection.

Between 2011 and April 2017, we collected 1403 cytological samples (patients' average age: 36.1 year, range: 17-83 years), and extracted DNA for the detection and genotyping of HPV based PCR-RDB method. All 24 HPV subtypes were detected and identified.

As shown in table 1, the overall HPV infection rate was 11.83% (166/1403). The HPV infection rates for different age group were 0.60% (<20 years old), 25.30% (20-29 years old), 36.14% (30-39 years old), 31.33 (40-49 years old), 4.82% (50-59 years old), and 1.81% (>60 years old).

The HPV infection rate indicated a parabolic curve, increasing with an increase in age from <20 years old to 30-39 years old, and then decreasing with a decrease in age from 40-49 years old to >60 years old (Table 1).

Figure 1 shows the distribution of the separated virus in genotypes. And the most frequently found three HPV subtypes were 18 (26.63%), 16 (17.58%), and 11 (13.59%). This group was followed by HPV subtypes 6 (9.78%), 81 (8.15%), 45 (5.43%) and 82 (3.26%).

Our results are slightly different in comparison with a research on women in Nigerian [16]. In Nigerian research, the most concentrated HPV infection group is from 20s years old to 30s years old while in our research, the proportion of infection patient in 20s years old is only ranked 3, even after the 40s years old group. This difference might be due to the culture and lifestyle and specially the age of marriage. However, the peak of HPV positive woman in our research is similar to a research in Southwest China, at 30-39 years [17]. In most age groups, the high-risk group accounts for the higher proportion. For the age group under 20, we only recorded one case of HPV infection so the risk classification may be misleading. This data points to the importance of testing for reproductive age women and promoting cervical cancer prevention.

2.4. HPV genotype

A specimen can be infected single-type HPV or co-infected with multiple genotypes at the same time. Table 2 showed 184 occurrences of genotypes/166 cases. There were un-typed 28 positive cases (16.97%) because these types are out of 24 determined types by Kit. As can be seen in figure 1, the co-infection rate (23.75%) is higher than single-type HPV (14.375%), especially some patients are infected up to 4 different types (1.25%). The most common co-infection type is that one between a high-risk and low-risk type (64.52%) with the most common one is HPV 16 and HPV 11 co-infection. Only one case in the study is co-infection between low-risk and low-risk types (HPV 6 and HPV 11). The co-infection rate was significantly higher than that of the sequential method. This is the advantage of Reverse Dot Blot Hybridation. This infection proportion result showed up that type 16 and 18 take the most percentage in the high-risk group and there were type 6 and 11 for the low-risk one, bears a similarity to the publiced information [18].

2.5. Relationship between region and proportion of high-risk and low-risk HPV types

Over the study area, HPV types in the high-risk group were higher (61,95%), with HPV 18 accounting for the highest proportion (26.63%), followed by HPV 16 (15.76%) compared to the low-risk group (32.61%) with the highest percentage (HPV 11) (13.59%), the un-typed positive case was 5.43%. The study data also bears a similarity in rates between high-risk and low-risk HPV cases in each region (Table 3). Particularly in the Hanoi area, the number of HPV 18 and HPV 16 cases, take the higher proportion, respectively 32.56% and 16.27% of total cases. This result is consistent with published Vietnam and abroad studies. So, the most common types were HPV16 and HPV18. This data points

to the need of accurately assessing the early detection of HPV infection and to research timely treatment to minimize the risk of cervical cancer. For screening and identification, the combination of PCR and Reverse Dot Blot Hybridation is an effective way to diagnose the potential for cervical cancer [16-17, 19-21].

4. Conclusions

The rate of HPV infection in the cases of gynecological examination at the Viet Nam Military Medical University from 2011 to April 2017 is 12.03%. In particular, the rate of infection of high risk group type is 61.95%, low risk is 32.61%, case of un-typed is 5.44%. Genotype HPV 16, 18 and 6, 11 make up the prevalence rate for the high-risk and low-risk group respectively. The percentage of one type infection is 61.875%, co-infection of two types is 19.375%, three types co-infection is 3.125%, and percentage of four types account for 1.250%. The PCR combining Reverse Dot Blot Hybridation method shows accurate results and should be widely applied.

5. References

1. Ayazbekov A, Nurkhasimova R, Kulbayeva S, Bolat K, Kurmanova AM, Yeskarayeva A, Sarbassova M, Kemelbekov KS. Features of Pregnancy, Childbirth and Postpartum Period of Young Mothers. Electron J Gen Med. 2020;17(6):em260. https://doi.org/10.29333/ejgm/8459

2. Ayazbekov A, Uteuliyev Y, Nurkhasimova R, Ibrayeva D, Khudaibergenova S, Kemelbekov K. Evaluation of women's health with intrauterine fetal death in the city of Turkestan for the years of 2013-2017. Annals of Tropical Medicine and Public Health. 2018;17:S804-S804

3. Cokkinides V, Albano J, Samuels A, Ward M, Thum J. American cancer society: Cancer facts and figures 2015. Atlanta: American Cancer Society. 2015.

4. Burd EM. Human papillomavirus and cervical cancer. Clinical microbiology reviews. 2003;16(1):1-17. https://doi.org/10.1128/CMR.16.1.1-17.2003

5. Faridi R, Zahra A, Khan K, Idrees M. Oncogenic potential of Human Papillomavirus (HPV) and its relation with cervical cancer. Virology Journal. 2011;8(1):1-8. https://doi.org/10.1186/1743-422X-8-269

6. Lowy DR, Schiller JT. Reducing HPV-associated cancer globally. Cancer Prevention Research (Philadelphia). 2012;5(1):18-23. doi: 10.1158/1940-6207.CAPR-11-0542

7. Centers for Disease Control and Prevention (CDC). Human papillomavirus-associated cancers-United States, 2004-2008. Morbidity and mortality weekly report. 2012;61:258-261. PMID: 22513527

8. Matsuo K, Chen L, Mandelbaum RS, Melamed A, Roman LD, Wright JD. Trachelectomy for reproductive-aged women with early-stage cervical cancer: minimally invasive surgery versus laparotomy. American journal of obstetrics and gynecology. 2019;220(5), 417-419. https://doi.org/10.1016/j.ajog.2019.02.038

9. Wild CP, Stewart BW, Wild C. World cancer report 2014: World Health Organization Geneva. 2014; Ch. 5.12.

10. Sun P, Song Y, Ruan G, Mao X, Kang Y, Dong B, Lin F. Clinical validation of the PCR-reverse dot blot human papillomavirus genotyping test in cervical lesions from Chinese women in the Fujian province: a hospital-based population study

11. Herrel NR1, Johnson NL, Cameron JE, Leigh J, Hagensee ME. Development and validation of a HPV-32 specific PCR assay. Virology journal. 2009;6(1):1-7. https://doi.org/10.1186/1743-422X-6-90

12. Bauer HM, Greer CE, Manos MM. Determination of genital HPV infection using consensus PCR. In: Herrington CS, McGee JO'D (ed). Diagnosis molecular pathology: a practical approach. Oxford: Oxford University Press, 1992; PP. 131-52

13. Zaravinos A, Mammas IN, Sourvinos G, Spandidos DA. Molecular detection methods of human papillomavirus (HPV). Int J Biol Markers. 2009;24:215–222.

14. Chan V, Yam I, Chen FE, Chan TK. A reverse dot-blot method for rapid detection of non-deletion α thalassaemia. British journal of haematology. 1999;104(3):513-515. https://doi.org/10.1046/j.1365-2141.1999.01221.x

15. Hsu YC, Yeh TJ, Chang YC. A new combination of RT-PCR and reverse dot blot hybridization for rapid detection and identification of potyviruses. Journal of Virological Methods. 2005;128(1-2):54-60. https://doi.org/10.1016/j.jviromet.2005.04.002

16. Akarolo-Anthony SN, Famooto AO, Dareng EO, Olaniyan OB, Offiong R, Wheeler CM, Adebamowo CA. Age-specific prevalence of human papilloma virus infection among Nigerian women. BMC public health. 2014;14(1):1-7. https://doi.org/10.1186/1471-2458-14-656

17. Tang Y, Zheng L, Yang S, Li B, Su H, Zhang LP. Epidemiology and genotype distribution of human papillomavirus (HPV) in Southwest China: a cross-sectional five years study in non-vaccinated women. Virology journal. 2017;14(1):1-10. https://doi.org/10.1186/s12985-017-0751-3

18. Wardak S. Human Papillomavirus (HPV) and cervical cancer. Medycyna doswiadczalna i mikrobiologia. 2016;68(1):73-84. PMID: 28146625

19. Nhung VT. Survey on HPV infection in women in Ho Chi Minh City by molecular biology techniques. Journal of Obstetrics and Gynecology. 2007;3-4.

20. Muñoz N, Franceschi S, Bosetti C, Moreno V, Herrero R, Smith JS,et al. Role of parity and human papillomavirus in cervical cancer: the IARC multicentric case-control study. The Lancet. 2002;359(9312):1093-1101. https://doi.org/10.1016/S0140-6736(02)08151-5

21. Muñoz N, Bosch FX, De Sanjosé S, Herrero R, Castellsagué X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. New England journal of medicine. 2003;348(6):518-527. doi: 10.1056/NEJMoa021641

Tables

Table 1 Propotion of HPV infection by age group (n=1403)

Age of patients, years	Total HPV infection	
	count	%
less than 20	1	0.60
20-29	42	25.30
30-39	60	36.14
40-49	52	31.33
50-59	8	4.82
60 and older	3	1.81

Table 2. Proportion of HPV infection by genotype

Genotype	Number	%
	High risk	
16	29	17.58
18	49	26. 63
45	10	5.43
58	4	2.17
31	3	1.63
51	0	0
59	0	0
82	6	3.26
33	3	1.63
52	2	1.09
66	0	0
35	4	2.17
53	3	1.63

68	1	0.54
39	0	0
56	0	0
Low risk		
6	18	9.78
11	25	13.59
42	1	0.54
43	1	0.54
61	0	0
70	0	0
71	0	0
81	15	8.15
Others	10	5.43
Total number	**184**	**100**

Table 3. Regional HPV infection proportion in region group in Viet Nam (cases)

Region	High-risk group		Low-risk group		Others		Total
	number	%	number	%	number	%	
Northwest	9	60.00	3	20.00	3	20.00	15
Northeast	11	45.83	4	16.67	9	37.50	24
Red River Delta	19	63.33	19	30.00	2	6.67	30
Ha Noi	61	55.96	43	39.45	5	4.59	109
Others	4	66.66	1	16.67	1	16.67	6

Figures

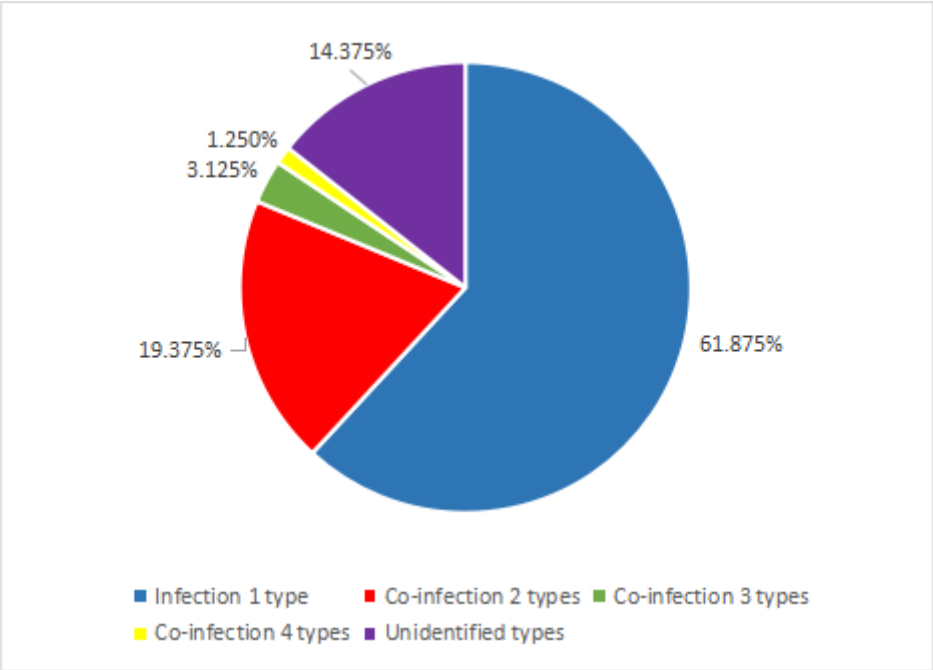

Figure 1. Frequency of appearance HPV infection and co-infection

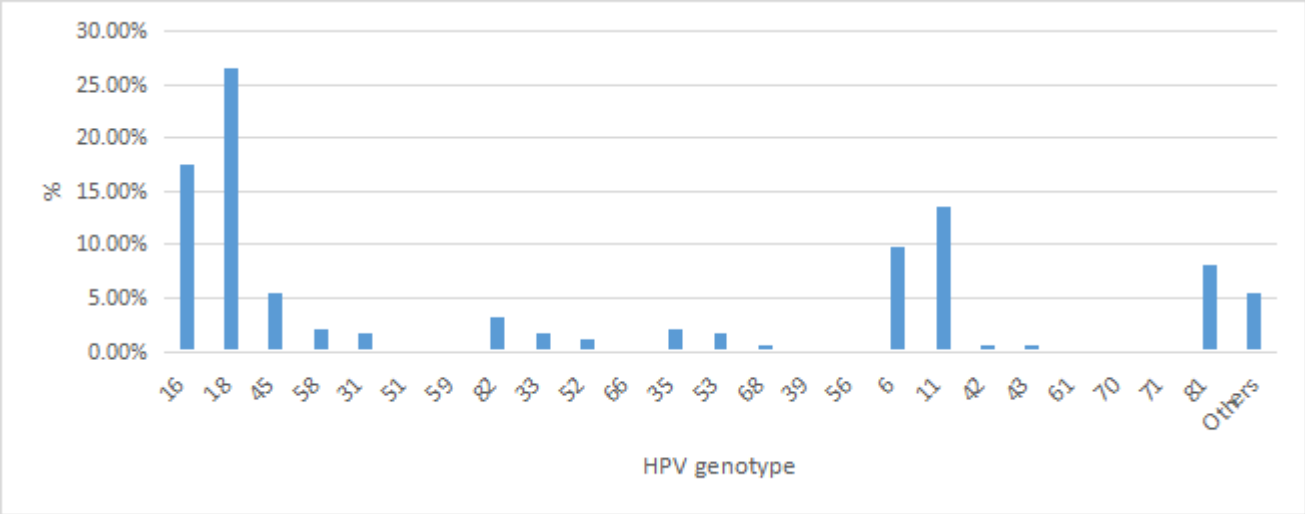

Figure 2. Prevalence of genotype-specific HPV infection in 1403 samples