HPV GENOTYPES AND CYTOLOGIC ASSESSMENT IN WOMEN INFECTED WITH HUMAN PAPILLOMAVIRUS, HO CHI MINH CITY, VIETNAM

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Abstract. Up-to-date data on HPV infection and cervical abnormalities are essential for effective screening and prevention of cervical cancer. This study evaluated the association among cytologic abnormalities, HPV prevalence and genotype distribution among women undergoing cervical screening in Ho Chi Minh City, Vietnam. A descriptive, cross-sectional study was conducted in women (n = 589) 18 years of age and above from May to July 2022. Cytologic diagnosis was performed using the Papanicolaou test, and HPV genotypes were identified using quantitative PCR. The HPV prevalence was 9.8%, with 86% infected with a single genotype. The high-risk HPV genotypes, HPV-16 (22.5%) and HPV-58 (27.5%) were most prevalent. Cytologic abnormalities were observed in 5.9% (n = 35) of the cases, primarily atypical squamous cells of undetermined significance (4.1%) and low-grade squamous intraepithelial lesions (1.9%). HPV-16 and HPV-58 infections showed a significant association with abnormal cytologic findings. These findings demonstrate the importance of integrating HPV genotyping with cytologic screening in helping to improve the country's cervical cancer prevention programs.

Keywords: cervical cancer, cytologic cervical screening, human papillomavirus, HPV genotype, Pap smear

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INTRODUCTION

Cervical cancer is the fourth most common gynecologic cancer globally. In 2020, the disease affects over 604,000 people and results in over 341,000 deaths, with prevalence and mortality rates of 13.1 and 6.9 per 100,000 population respectively (Sung *et al*, 2021; Datkhile *et al*, 2022; UICC, 2020; Fowler *et al*, 2023). At least 85% of the cases occur in developing countries, probably due to limited public knowledge of its prevention and access to screening, diagnosis and treatment facilities (Sung *et al*, 2021; Zhang *et al*, 2021).

Human papillomavirus (HPV) is the dominant cause (over 90%) of anal and cervical cancer (Okunade, 2020; Szymonowicz and Chen, 2020). There are 200 recognized HPV genotypes, most being low-risk types, infections of which are non-oncogenic and last only 1-2 years (Chan *et al*, 2019; Okunade, 2020). High-risk types, in particular HPV-16 and -18, induce carcinogenic changes or precursor lesions in various organs, including the cervix (Wójcik *et al*, 2019; Chan *et al*, 2019; Okunade, 2020). Persistent infection with high-risk types is the most important factor in cancer development, as over 99% of cases of chronic high-risk HPV infections lead to cervical cancer.

Early screening, together with HPV vaccination and prevention, is crucial in the control of cervical cancer (Lee *et al*, 2016; Mullapally *et al*, 2022). Cytological diagnosis techniques, such as the Papanicolaou test (Pap smear) or liquid-based cytology, are the standard methods for cervical cancer screening and play an important role in reducing cancer prevalence; however, these techniques require highly trained cytologists, strict quality control and regular testing, which are costly and challenging for developing countries. Due to these limitations in the cytologic method, HPV genotyping techniques have emerged as a supplementary and alternative approach and given HPV's crucial role in the etiology of cervical cancer WHO recommends HPV genotyping as the primary screening method (WHO, 2021). HPV genotyping has the advantages of higher cost-effectiveness, superior sensitivity, better negative predictive power,

more reproducibility, and longer intervals between tests (Delpero and Selk, 2022). However, the technique carries a higher risk of overdiagnosis, leading to unnecessary harm and burden caused by the need to verify false-positive cases (Delpero and Selk, 2022; Melnikow *et al*, 2018). Co-testing of both approaches has been implemented to leverage their respective advantages and increase screening capability; studies were performed to evaluate and propose modifications to this combined approach. An analysis of pap smear and cytologic tests in Germany from 2015 to 2020 show the importance of co-test in the diagnostic routine in dysplasia units (Stuebs *et al*, 2022) and an extensive review of Swid and Monaco (2022) recommended leveraging both kind of tests in a flexible, case-by-case manner.

In Vietnam, cervical cancer is the most common gynecologic cancer, with a prevalence of 7.1 per 100,000 population. It is expected to be among the five most prevalent cancer types in the 2020s. Fortunately, HPV vaccination, prevention and education on sexual behavior have contributed greatly to the control of cervical cancer in Vietnam (Tran *et al*, 2020). However, despite government efforts, HPV vaccination coverage is still less than satisfactory, as only 12% of girls and young women 15-29 years of age have been vaccinated due to a lack of public awareness and limited access to public health facilities in remote and marginal areas. Pilot scale programs have achieved nearly 100% coverage; however, expansion of this success is challenging (La and Do, 2024). Prevalence and mortality have shown a steady decline although the trend is still slower than in some other countries in Asia such as South Korea and Thailand (Tran *et al*, 2020). Further intervention strategies are essential to prevent an increase in new cases (Thi Nguyen *et al*, 2019)

The majority of HPV genotypes found in Vietnam belong to the low-risk types; however, high-risk carcinogenesis types are detected among certain vulnerable groups such as female sex workers, raising the need for preventive as well as effective detection measures

(Pham *et al*, 2018; Le *et al*, 2021; Nguyen *et al*, 2021; Pham *et al*, 2022). Consistent with the global trend, HPV-16 and -18 are the most common high-risk genotypes in the country, but HPV-52 has emerged as a predominant high-risk type in many groups, such as female sex workers (Pham *et al*, 2018; Le *et al*, 2021; Nguyen *et al*, 2021; Pham *et al*, 2022), which will require obtaining comprehensive data of HPV genotypes for each vulnerable group to tailor appropriate HPV prevention and treatment strategies.

The establishment of the Papanicolaou (also known as Pap) smear screening in Vietnam played a significant role in the decrease in cervical cancer prevalence (Suba and Raab, 2012); however, its low sensitivity and detection of late stages of the disease have prompted the inclusion of HPV genotyping for early diagnosis (Cao *et al*, 2022). Our study aimed to evaluate HPV prevalence, genotype, and association with cytologic abnormality among women residing in Ho Chi Minh City, Vietnam. To achieve this goal, we performed cervical cancer screening in several polyclinics in Ho Chi Minh City, recorded the cervix cytologic result, HPV prevalence, and genotype of test women, and evaluated the association between HPV genotype and cytologic morphology. The result will provide a good assessment of the sensitivity and accuracy of the HPV testing method compared to cytologic analysis, contributing to the framework of HPV genotyping integration with cytologic screening in screening prevention programs.

MATERIALS AND METHODS

Study location, participants and design

The study was performed from May to July 2022 in three clinics in Ho Chi Minh City, namely Thuận Kiều Polyclinic, Hiền Đức Obstetrics and Gynecology Clinic and Dr. Trương Thị Thảo Clinic. A descriptive, cross-sectional approach was used. Participants (n = 589) were recruited from females >18 years of age attending cervical screening at these clinics. The required sample size was determined based on the formula (Sadiq *et al*, 2024):

$$n = (Z \times (1\text{-}a/2)^2 \times p \times (1\text{-}p))/d^2)$$

where n = required sample size;

α = type I error rate (0.05);

Z = critical value from the standard normal distribution (Z0.975 = 1.96);

p = estimated rate of HPV infection (0.165) (Nguyen et al, 2021); and

d = allowable margin of error of the estimate in the study (0.03)

A narrower margin tends to necessitate a larger sample size, whereas a broader margin can accommodate a smaller one. In this context, a 3% margin reflects the study's acceptance of the permissible error.

Exclusion criteria were (i) females experiencing menstruation, vaginosis, vaginal douching, recent vaginal medication use, sexual intercourse within the previous 48 hours, and chronic or acute illness; and (ii) females with a history of mental illness or communication disorders. Demographic information and medical history were collected. Cervical cell samples were obtained for Pap smears and HPV genotyping. Samples were processed and analyzed at HANHPHUCLAB (ISO 15189:2012, accreditation code VLAM-1.0730).

Cytologic diagnosis

A cytologic diagnosis was performed using a Papanicolaou test (Pap smear) adhering to the 2402/QĐ-BYT 2019 guideline of the Ministry of Health (Vietnam) (Vietnam Ministry of Health, 2021). Pap smear samples were stored for up to 24 hours before laboratory processing. A liquid-based cytology method was used for optimizing sample preparation based on the Max-prep kit (Corebiotech Co Ltd, Gwangju, South Korea). Samples of cervical cells were fixed on a microscope slide using cold acetone, and alcohol at different

concentrations (50, 70, 90, 95, and 100%) was used sequentially for dehydration. Cells were stained with Hematoxylin Harris dye for 60-90 seconds, then with Orange G dye for 1-3 minutes, and finally with Eosin Azure-50 dye for 1-4 minutes. Cytologic diagnosis was reported using the Bethesda 2014 system (Pangarkar, 2022), namely, negative (normal), atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL), and atypical glandular cells (AGC).

Quality control measures for the liquid-based Pap smear were performed in the pre-analytical, analytical and post-analytical phases. To ensure sample adequacy, a minimum of 5,000 cells were used for the liquid-based cytology according to instructions of the Max-prep kit (Corebiotech Co Ltd, Gwangju, South Korea). To minimize diagnostic errors and ensure reliability, cytologic abnormalities were validated by two qualified cytologists who independently reviewed the slides to confirm the diagnosis.

HPV genotyping

Quantitative (q)PCR was performed to genotype HPV. DNA from the samples was extracted using the SEEPREP 32™ automated extraction system (Seegene Inc, Seoul, South Korea) following the manufacturer's protocol. HPV DNA was detected using TopSENSI® HPV qPCR Kit SQH-103 (ABT Biological Solutions Co Ltd, Ho Chi Minh City, Vietnam), using FAM signal to detect HPV DNA and HEX signal as an internal control. HPV genotyping was performed using TopSENSI® HPV qPCR Kit SQH-103 and TopSENSI® HPV 6, 11, 16, 18 and High-Risk qPCR Kit SQH-006 (ABT Biological Solutions Co Ltd, Ho Chi Minh City, Vietnam). The former kit qualitatively detected DNA of 12 high-risk HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and the RNase P (RP) gene as the internal control. The latter kit specifically detected low-risk (HPV-6, HPV-11) and high-risk (HPV-16, HPV-18). Both TopSENSI® kits have no cross-reactivity with the host or other viral DNA. TopSENSI® HPV 6, 11, 16, and 18 qPCR Kit (ABT Biological

Solutions Co Ltd, Ho Chi Minh City, Vietnam) has a specificity and sensitivity of 100%, with a limit of detection of 19 copies per reaction, comparable to the standard Cobas® 4800 HPV Test (Roche Diagnostics, Rotkreuz, Switzerland). Moreover, the accuracy and precision of the HPV genotyping assays were confirmed (data not shown).

Data analysis

Descriptive statistics were used to analyze age groups, Pap smear cytological diagnosis results, HPV infection test results (positive or negative), and HPV genotypes. The results were reported as frequency or percentage. A p-value <0.05 is considered statistically significant using a Chi-square test. Data collection was conducted within a standardized framework in a continuous, non-interrupted study to minimize variations. Data were analyzed using a Stata 16.0 program (StataCorp LLC, College Station, TX).

Ethical declaration

The study protocol received ethical approval from the University of Medicine and Pharmacy at Ho Chi Minh City (UMP), Vietnam (IRB-VN01002/IORG0000860/FWA00023448), no. 476/HDD-DHYD, code 22313-DHYD. Prior written informed consent was obtained from each participant. Data were anonymized to protect the identity of the participants. The study did not affect the usual medical or treatment services of the three participating clinics.

RESULTS

Participants age distribution

The average age of the participants (n = 589) was 38.5 years, with the 30-39 years of age group (average age = 34.3 years) being the largest group (39.7%), followed by <30 years of age group (average age = 25.8 years, 24.1%), then 40-49 years of age group (average age = 43.7 years, 20.7%), and >50 years of age group (average age = 55.9, 15.7%) (Table 1).

HPV infection rate

HPV infection was detected in 58/589 (9.8%) participants. Among each age group, the HPV-positive prevalence was highest in the <30 years age group (13.5%), followed

Table 1

Distribution of HPV infection by age groups

Age group	Average age (years), mean ± SD	Frequency[1] *n* (%)	HPV prevalence (%) based on age group[2]	HPV prevalence (%) based on total number of participants[3]
<30 years	25.8 ± 2.7	104 (17.7)	14/104 (1)	2.4
30-39 years	34.3 ± 2.7	249 (42.2)	23/249 (9)	3.9
40-49 years	43.8 ± 2.7	143 (24.3)	12/143 (8)	2.0
>50 years	55.9 ± 5.9	93 (15.7)	9/93 (10)	1.5

[1]Calculation was based on total number of research participants (n = 589); [2]calculation was based on number of positive HPV and number of participants in the corresponding age group; [3]calculation was based on number of positive HPV and number of total participants in the study.

HPV: Human papillomavirus; SD: standard deviation

by >50 years of age group (9.7%), 30-39 years of age group (9.2%), and 40-49 years of age group (8.4%) (Table 1).

HPV genotypes

HPV genotypes were identified in 43 (7.3% of total participants) and unidentified in 15 (2.5% of total participants) positive cases (Fig 1A). Amongst the 43 identified cases, 34 (79%) had infection with a single high-risk genotype [HPV-16 (n = 9), HPV-18 (n = 1), HPV-31 (n = 1), HPV-33 (n = 2), HPV-35 (n = 3), HPV-39 (n = 7), HPV-45 (n = 3), HPV-51 (n = 1), HPV-52 (n = 4), HPV-58 (n = 11), HPV-59 (n = 1), and HPV-66 (n = 2); 4 cases (7%) with a single low-risk genotype [HPV-6 (n = 1) and HPV-11 (n = 3)], and 6 cases (14 %) with multiple genotypes [5 with two high-risk genotypes included HPV-52, HPV-33, HPV-35, HPV-39, HPV-51, HPV-66 and 1 case with both HPV-52 and HPV-11] (Fig 1B).

Among the age groups (n = 589), those <30 and 30-39 years of age had the same highest high-risk HPV infection prevalence (31%), followed by 40-49 years of age (22%), and then >50 years of age (16%) (Fig 1C).

Relationship between HPV infection and cervical cytologic diagnosis

The majority of cases had nonmalignant morphology (554/589 cases), of which 91.7% were negative with the HPV test. Among the atypical squamous cells of undetermined significance (ASC-US) cases (24/589), 75% had negative HPV tests and among low-grade squamous intraepithelial lesions (LSIL) cases (11/589), only 45.5% had negative HPV tests. A significant association was found between HPV infection and abnormal cytologic findings (p-value <0.001), highlighting the potential role of HPV in cervical lesions. HPV-16 accounted for 1.3 and 5.7% of the total normal and abnormal cytologic tests respectively, while HPV-58 comprised 1.4 and 8.6% of total normal and abnormal cytologic tests respectively. The occurrence of high-risk HPV-16 and -58 showed a strong association with abnormal cytologic results (p-value <0.05), highlighting their oncogenic potential.

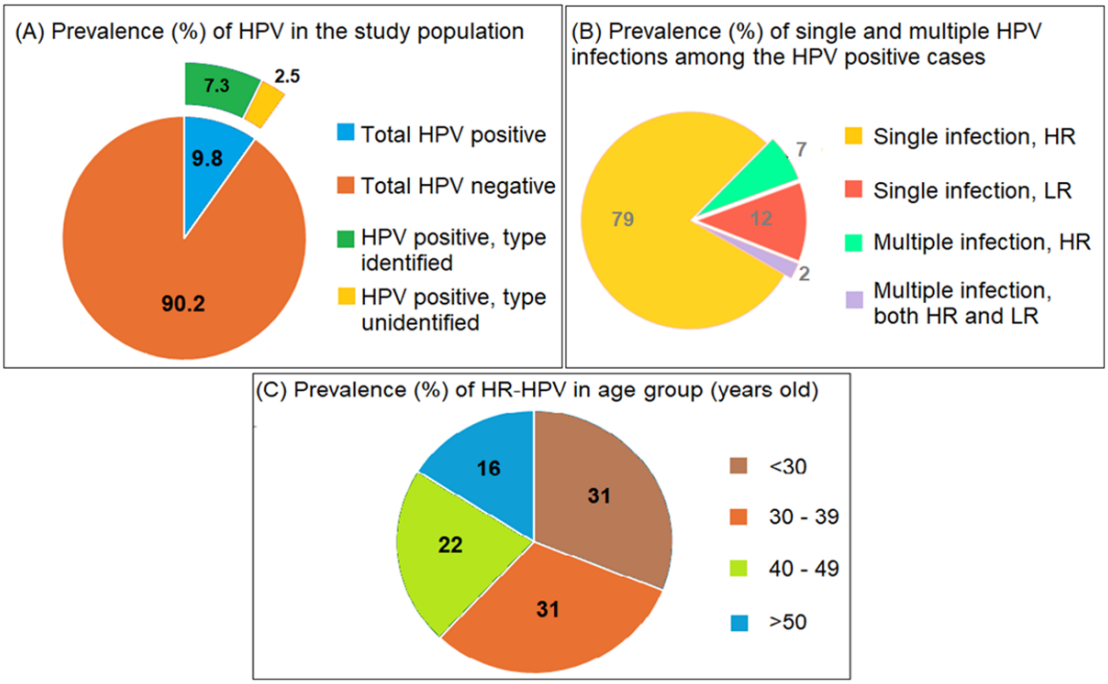

Fig 1 - HPV prevalence and types in the study population (n = 589)

(A) Prevalence (%) of HPV in the study population (n = 589); (B) prevalence (%) of single and multiple infections among identified HPV genotype cases (n = 43); (C) prevalence (%) of HR-HPV according to age groups (n = 45).

HPV: human papillomavirus; HR-HPV: high-risk human papillomavirus; LR: low-risk

Fig 2 shows microscopy images of cytologic test results in four cases. The cytological assessment of Pap smears, based on the Bethesda 2014 system, reveals a striking spectrum of HPV-associated cellular abnormalities, shedding light on the progression of cervical dysplasia. Notably, Panel A depicts atypical squamous cells (ASC-US) related to HPV-16, with nuclear enlargement and subtle hyperchromasia, signaling early cellular changes. Furthermore, Panel B illustrates

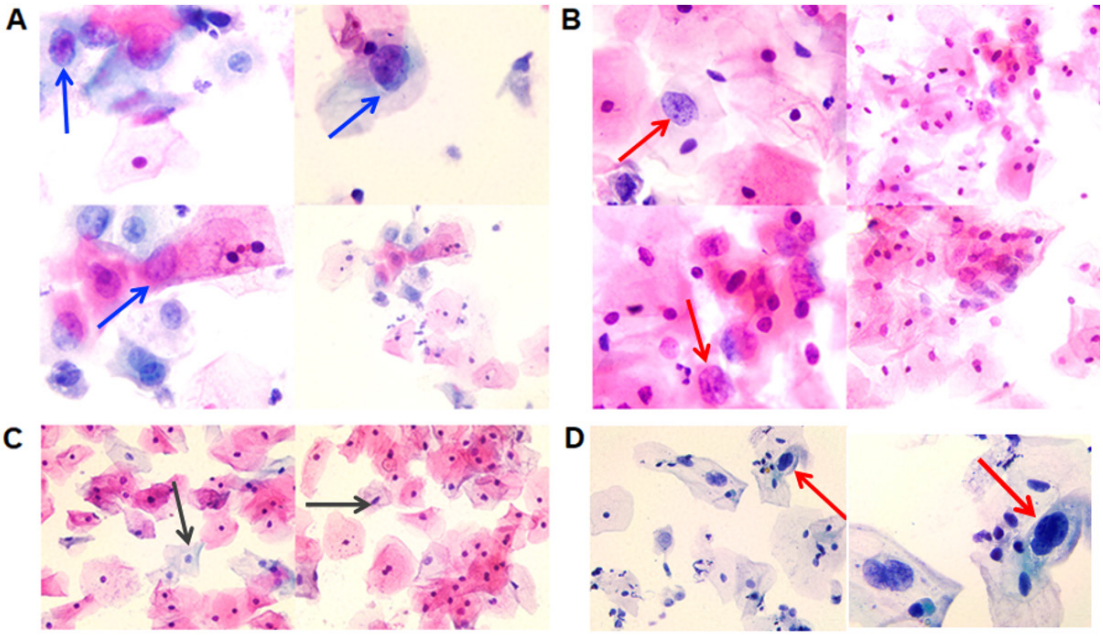

Fig 2 - Representative microscopic cytologic images of Papanicolaou tests from the study

(A) ASC-US with HPV-16 genotype; (B) LSIL with HPV-33 and -66 genotypes; (C) normal cytology with HPV-11 and -52 genotypes; (D) LSIL with HPV-58 genotype.

The blue arrow indicates cells in ACS-US with enlarged nuclei (2.5-3 times), minimal nuclear irregularities, and slight hyperchromasia. The red arrow highlights cells in LSIL with mild nuclear dyskaryosis, characterized by enlarged nuclei, irregular nuclear membranes, perinuclear halos, and irregular chromatin distribution. The black arrow represents superficial or intermediate squamous cells at the later stages of maturation, which appear uniform with small, pyknotic nuclei.

ACS-US: atypical squamous cells of undetermined significance; HPV: human papillomavirus; LSIL: low-grade squamous intraepithelial lesion

low-grade squamous intraepithelial lesions (LSIL) associated with HPV-33 and HPV-66, marked by mild nuclear dyskaryosis and disrupted chromatin, indicating early dysplasia. In contrast, Panel C shows normal cytology, despite HPV-11 and HPV-52 presence, with well-differentiated squamous cells, demonstrating that not all HPV infections lead to dysplasia. The final section of Panel D is devoted to LSIL connected to HPV-58, with atypical dyskaryosis and perinuclear halos. Together, these findings demonstrate the continuum of cytopathology from normal to dysplastic cells, emphasizing the crucial significance of HPV genotyping in improving diagnostic accuracy and guiding clinical management.

DISCUSSION

Participants age distribution

In our study, the average age of the participants (n = 589) was 38.5 years, mostly from the age group of 30-39 years. HPV infections in women, including the high-risk ones, can be observed as early as teenage age (Akinyi *et al*, 2024; Hu *et al*, 2024; Xu *et al*, 2023). It is essential, therefore, to have HPV and cancer screening as soon as possible. World Health Organization recommends that women 30-49 years of age should have cancer screening at least once (WHO, 2014). European countries, such as Belgium, France, Ireland, and Sweden, suggest Pap screening at 25 years of age, while the United States and South Korea propose 21 years of age (Schiffman *et al*, 2007). In Vietnam, the 1639/QD-BYT guideline of the Ministry of Health (Vietnam) recommends HPV screening in women as early as the age of 21, or after the first sexual intercourse (Vietnam Ministry of Health, 2021).

HPV prevalence

HPV is the most common viral infection of the reproductive tract and is also the dominant cause of cervical cancer (Okunade, 2020; Szymonowicz and Chen, 2020). The prevalence of HPV infection varies according to geography; in Southeast Asia, the average prevalence of HPV infection among

women without neoplasia is 14%, but remarkable differences exist both between regions and studies conducted within the same area (Bruni *et al*, 2010).

In our study, 58 participants from three clinics in Ho Chi Minh City were positive for HPV DNA, equivalent to a prevalence of 9.8%. This prevalence is lower than that reported at the University Medical Center HCMC, Ho Chi Minh City (16.5%) (Nguyen *et al*, 2021) and the Institute Pasteur in Ho Chi Minh City (48.7%) (Cao *et al*, 2022). There are no significant differences in location or period among these studies as they were all conducted in Ho Chi Minh City from 2019 to 2022. Different conditions in the medical facilities might be a reason for the differences in prevalence. Participants in the study at Institute Pasteur attended the facility for sexually transmitted diseases, whereas our participants and those at the University Medical Center HCMC attended the facilities for general gynecologic check-ups. Our study was also performed in private clinics instead of public hospitals, which resulted in a different population of attendees. Larger cohort research will be required to accurately estimate the women's HPV prevalence in Ho Chi Minh City.

Compared to other regions of the country, HPV prevalence in our study was higher than that of Luu and Le (2013), who reported a 3.3-9.3% HPV prevalence in representative provinces of northern, central and southern regions. An earlier study in various Bangkok hospitals reported an 8.7% HPV prevalence (Chansaenroj *et al*, 2010), and that among Indonesian women in urban areas during a similar period as ours reported a 5.3% prevalence (Handayani *et al*, 2020). In Cambodia, the prevalence is higher among participants of a factory workers' program (12%), higher than in a hospital-based program (5%) (Ueda *et al*, 2019). These differences in prevalence were probably due to differences in the geographical location of the studies and the participants' demographics. Most participants in our study might have attended previous HPV screening, while others attended clinics after signs

and symptoms of the infection were observed.

HPV genotypes

Among the high-risk genotype cases in our study, HPV-58 was the most prevalent, followed by HPV-16, -39, -52, -35, -45, -33, -66, -18, -31, -51, and -59. These results were not consistent with many other studies in Vietnam (Pham *et al*, 2018; Le *et al*, 2021; Nguyen *et al*, 2021; Pham *et al*, 2022; Tran and Le, 2021), which reported HPV-16, -18 and -52 as the most prevalent, and also similar to observations of cancer patients in Indonesia (Schellekens *et al*, 2004; Savira *et al*, 2022). Nonetheless, significant predominant HPV-58 prevalence was recently observed in certain populations of Vietnam (Nguyen *et al*, 2024), especially among women with normal cytology and high-grade lesions (HPV Information Centre, 2023; Le *et al*, 2024), and among sex workers (Pham *et al*, 2022). Studies in PR China including those from provinces neighboring Vietnam, such as Guangdong and Yunnan, also report significant HPV-58 prevalence and also of HPV-16, -18, and -52 (Baloch *et al*, 2017; Li *et al*, 2019b; Chen *et al*, 2022). The introduction of an HPV vaccine that provides protection and immunity against HPV-58 (such as the nonvalent vaccine) is therefore essential. Moreover, it would also be beneficial if a large cohort study is conducted in Ho Chi Minh City to explore occupational, cultural, and environmental factors in relationship with HPV lines present in neighboring countries, these factors played a crucial role in the transmissions, spreading, and the associated genetic relationship of HPV not only among provinces but also among nearby area of neighboring countries.

In our study, 70% of identified HPV genotypes were of the high-risk types, and women between under 30 and 39 years of age had the highest prevalence of high-risk HPV infection. A study in K Hospital (Cancer Hospital, Hanoi) also observed a similar oncogenic type prevalence (72.9%) (Tran and Le, 2021). Studies in neighboring countries, such as China, Indonesia and Thailand observed various prevalence of oncogenic HPV

genotypes (ranging from 44 to over 90%) (Chansaenroj *et al*, 2010; Baloch *et al*, 2017; Schellekens *et al*, 2004; Handayani *et al*, 2020; Savira *et al*, 2022). Notably, like our results, many studies in West Asia, China or Africa also observed the higher HPV and high-risk HPV prevalence in the 20-29, 30-39 and 40-49 age group, in which factors such as active sexual activities in younger women, hormonal changes post-menopause that compromised immunity, or the biasness due to higher awareness and initiative of young and middle-aged women in gynecological examination that skewed the detect incidences into them (Akinyi *et al*, 2024; Faqih *et al*, 2023; Hu *et al*, 2024; Xu *et al*, 2023).

Our study found that the majority (86%) of HPV-positive cases were infected with a single genotype, among which 92% were high-risk types. Six cases (14%) were identified as having multi-genotype HPV infections, 5 with high-risk types, and one with both high- and low-risk viruses. The predominance of infections with a single genotype was also observed in other studies in Vietnam (Nguyen *et al*, 2021; Tran and Le, 2021; Nguyen *et al*, 2024) and in neighboring countries (Schellekens *et al*, 2004; Baloch *et al*, 2017; Li *et al*, 2019b; Liao *et al*, 2020; Savira *et al*, 2022).

Cytologic test (Papanicolaou test)

Cells obtained from the cervix for cytologic examination (Pap test) were categorized into negative (normal), atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL), and atypical glandular cells (AGC). Women with LSIL usually have a higher chance of developing cervical cancer than normal ones, even though the risk is relatively low, and the stage would regress after 12 months in most cases (Kombe Kombe *et al*, 2021; Zhao *et al*, 2022). On the other hand, women with HSIL or AGC have a higher risk of oncologic development unless timely detection and follow-up treatment are performed (Kombe Kombe *et al*, 2021; Zhao *et al*, 2022).

Among the 589 participants in our study, 35 cases (5.9%) were

observed with abnormal results, 24 cases with ASC-US (4.1%), and 11 cases with LSIL (1.9%). Other studies also reported a cervical cell abnormality prevalence within the same range, with a notable presence of ASC-US and LSIL. A study in Hue Province reported a similar abnormality prevalence (5.4%), consisting of 0.3% HSIL, 1.0% AGUS, 1.1% LSIL, and 3.1% ASCUS (Nguyen *et al*, 2013). Another study in Thai Nguyen, Can Tho and Hue observes a significantly higher prevalence (272/2005 cases, 9.1%) of cervical cell abnormality, among which LSIL (3.9%), ASC (3.2%), and HSIL (2%) were predominant (Luu and Le, 2013). On the other hand, K Hospital (Cancer Hospital, Hanoi) reported only 2.1% abnormality (45/2149 cases), predominated by ASCUS and HSIL, followed by LSIL and carcinoma (Tran and Le, 2021). A study in neighboring Yunnan, PR China reported a 5.3% (42/793 cases) abnormality prevalence similar to our study, with 3% CIN 1 (LSIL) and 2.2% CIN2/3 (HSIL) (Baloch *et al*, 2017), while in Thailand, Chansaenroj *et al* (2010) observed a much lower prevalence (40/1662 cases, 2.4%), predominantly ASC-US, LSIL and HSIL, with only 5 cases having already developed carcinoma.

Relationship between HPV infection and cervical cytologic diagnosis

Our study observed a significant association between HPV infection and abnormal cytologic test results (p-value <0.001) and a strong association between high-risk HPV-16 and -58 infections HPV with abnormal cytologic results (p-value <0.05). Other studies also showed a significant correlation between high-risk HPV infection and cervical damage, such as the research of populations in Can Tho, Hue and Thai Nguyen, which observed a higher HPV infection in cervical abnormalities and even higher HPV prevalence in more severe cases (Luu and Le, 2013). K Hospital (Cancer Hospital, Hanoi) reported a strong correlation between HPV infection, especially the high-risk HPV-16 and 18, and abnormalities and carcinoma (Tran and Le, 2021). At the University of Medicine and Pharmacy in Ho Chi Minh City, the HPV infection

prevalence among women with ASCUS, LSI, and HSIL is 46.3, 85.7 and 100%, respectively, with significant (p-value <0.001) cytologic differences between HPV positive and negative cases, especially in the case of HPV-16 infection (Nguyen and Nguyen, 2018).

Studies in Indonesia also detected HPV-16 and -18 as the most predominant genotypes among cancer patients, with different prevalence and multiple infection combinations depending on cancer types (Schellekens *et al*, 2004; Savira *et al*, 2022). HPV-16 was identified as the most dominant type in cancer and precancerous patients in Beijing; moreover, infection and co-infection with HPV-16 increases the chance of more severe lesions and damage (Li *et al*, 2019b). In Yunnan, PR China Baloch *et al* (2017) noted higher high-risk HPV genotypes in more severe lesion cases, and HPV-16 is also the most frequent in the more advanced abnormality stage. A later meta-study conducted in PR China observed a significantly higher rate of high-risk HPV infection in women with symptomatic cervical lesions (25.7%) compared to healthy women (12.9%) (Li *et al*, 2019a).

It has long been demonstrated that the HPV DNA genotype test is a good indicator for the diagnosis of cervical abnormalities, including cancers (Delpero and Selk, 2022; Stuebs *et al*, 2022). In a Pap smear test together with an HPV-positive result, the presence of a high-risk HPV genotype is expected to increase the risk for cervical lesions. Thus, the Pap smear/HPV co-test is highly recommended and essential in cervical cancer screening (Stuebs *et al*, 2022). HPV-positive cases without abnormal cytologic changes or HPV-negative cases with precancerous lesions may be due to differences in immune responses among the participants and in virulence and infectivity among HPV types.

In conclusion, our study with 589 participating women from three private clinics in Ho Chi Minh City showed an HPV prevalence of 9.8%, with 7.3% having identified HPV genotypes. Among the infected cases, the majority (86%) had a single-genotype HPV infection,

in which high-risk genotypes accounted for 79%. The high-risk HPV-58 prevalence was highest, followed by HPV-16, then -39 and -52. The majority of cytology (Pap smear) tests were normal, while cytological abnormalities, atypical squamous cells of undetermined significance (ASC-US) and low-grade squamous intraepithelial lesions (LSIL), accounted for 4.1 and 1.9% of the tests respectively. A significant association was found between abnormal cytology results and high-risk HPV-16 and -58 infections. Such association demonstrates the feasibility of HPV testing as an indication of cervical abnormalities including carcinogenic ones, highlighting the need for HPV genotyping integration with cytologic screening in screening prevention programs.

ACKNOWLEDGEMENTS

The authors thank Thuận Kiều Polyclinic, Hiền Đức Obstetrics and Gynecology Clinic, and Dr. Trương Thị Thảo Gynecology clinic, HANHPHUCLAB, for their participation in the project.

CONFLICT OF INTEREST DISCLOSURE

The authors declare no conflict of interest.

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