CLINICAL AND EXPERIMENTAL VACCINE RESEARCH

Clin Exp Vaccine Res 2022;11:141-148
https://doi.org/10.7774/cevr.2022.11.2.141
pISSN 2287-3651 • eISSN 2287-366X

Possible different genotypes for human papillomavirus vaccination in lower middle-income countries towards cervical cancer elimination in 2030: a cross-sectional study

Tofan Widya Utami[1], Andrijono Andrijono[1], Andi Putra[1], Junita Indarti[2], Gert Fleuren[3], Ekaterina Jordanova[3], Inas Humairah[1], Ahmad Utomo[4]

[1]Oncology Gynecology Division and [2]Social Obstetrics and Gynecology Division, Department of Obstetrics and Gynecology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia; [3]Department of Pathology, Leiden University Medical Center, Leiden, Netherlands; [4]Department of Research and Development, Dharmais Cancer Center, Jakarta, Indonesia

Received: December 19, 2021
Revised: January 24, 2022
Accepted: January 26, 2022

Corresponding author: Tofan Widya Utami, MD, PhD
Oncology Gynecology Division, Department of Obstetrics and Gynecology, Faculty of Medicine, University of Indonesia, Central Jakarta, 10430, Indonesia
Tel: +62-81808696983, Fax: +62-81905680569
E-mail: tofan.widya@ui.ac.id; tofanwidya@yahoo.com

No potential conflict of interest relevant to this article was reported.

This research is supported by the Department of Obstetrics and Gynecology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia. Subjects run in Leiden University Medical Center were funded by Female Cancer Foundation, while others were self-funded.

Purpose: Human papillomavirus (HPV) genotype and age distribution of HPV infection were crucial for the national vaccination and screening program planning. However, there was a limited study providing these data in the normal cervix population. This study aimed to explore the HPV genotypes profile of women with clinically normal cervix based on Visual Inspection of Acetic Acid (VIA) test.

Materials and Methods: A 7-year cross-sectional study was conducted from 2012 to 2018 in private and public health care centers in Jakarta. Subjects were recruited consecutively. Data were collected by anamnesis, VIA, and HPV DNA test using the polymerase chain reaction (PCR; SPF10-DEIA-LiPA25) method. HPV genotyping procedures include DNA extraction, PCR (SPF10-DEIA-LiPA25) using the HPV XpressMatrix kit (PT KalGen DNA, East Jakarta, Indonesia), and hybridization. The IBM SPSS ver. 20.0 (IBM Corp., Armonk, NY, USA) were used to analyze the data.

Results: A total of 1,397 subjects were collected. Positive HPV-DNA tests were found in 52 subjects (3.7%); 67% were single and 33% were multiple HPV infections. HPV 52 was the most frequently detected HPV genotype, followed by HPV 39, 16, 18 74, 44, 31, 54, and 66, respectively. The highest HPV infections in this population were in the 31–40 and 41–50 years old group.

Conclusion: This study suggested beneficial screening for women aged 31–50 years old. Instead of "original" nonavalent (HPV 16, 18, 6, 11, 31, 33, 45, 52, 58), the different "nonavalent" formula for HPV vaccines protecting against HPV 16, 18, 6, 11, 31, 39, 44, 52, 74 might be useful for Indonesian population. However, further multicenter studies with a huge sample size are still needed.

Keywords: Human papillomavirus, Vaccines, Cervix, Papillomavirus, Prevention

Introduction

Cervical cancer is one of the greatest threats to women's well-being, which places a tremendous financial burden and mental tension on affected individuals and families and increases national budget consumption for health [1,2]. Cervical cancer is confirmedly caused by persistent high-risk human papillomavirus (Hr-HPV) infection. This infection takes such a long time, approximately 3–17 years, to develop into cervical cancer. Human papillomavirus (HPV) status (positive versus negative), HPV genotypes, prevalence versus the incidence of detected HPV (length of contamination),

and high-grade cytology are the foremost imperative indicators of the chance of precancer at standard testing in a cervical cancer screening program based on HPV testing from age 30 years. Some studies determined that the highest risk HPV types through approximately 2 to 3 years after the standard screening is profoundly related to precancer lesions [3,4]. Therefore, we have a relatively long time to prevent HPV infection from becoming invasive by implementing the right vaccination and screening program [5].

HPV infection is prevalent in the first decade of sexual activity of young women [6]. The majority of cervical cancer cases involved the HPV-16 and 18 infections, and most of those cases come together with HPV 31, 33, 45, 52, and 58 as multiple infections [7,8]. Some studies conclude that the persistence of HPV infection, the severity of cervical pre-cancerous lesions, and the tendency to develop cervical cancer might be predicted by discovering the viral load and multiple HPV infection status of Hr-HPV. Furthermore, understanding HPV can provide a more insightful picture of the disease's natural history and cancer development associated with Hr-HPV [9-11].

In 2020, the World Health Organization (WHO) set a global strategy to accelerate cervical cancer elimination, with a goal of 90% of the population vaccinated, 70% of the population screened with a high-performance test, and 90% of women with precancer lesion and cervical cancer treated by 2030 [12]. Unfortunately, many lower middle-income countries (LMIC) and Indonesia do not yet have an established national vaccination program. Hence, a particular strategy is needed to build the program. While evidence-based clinical rules guarantee that the best healthcare conventions are accessible [13], there was a limited study about HPV genotype prevalence and distribution in the normal cervix population. Hence, the data on HPV genotype distribution and the age distribution of HPV infection were essential for the national vaccination and screening program [7]. This study is expected to provide an overview of the genotypes and age group distribution of HPV genotypes to support cervical cancer primary prevention, program development, and improve management algorithms [14].

Materials and Methods

This study is a cross-sectional study. The data were collected from January 2012 to July 2018. Subjects were reproductive age women admitted to Gyne-, Colposcopic, and Gyne-Oncologic Outpatient in both public and private clinics of the Obstetrics and Gynecology Department of Dr. Cipto Mangunkusumo General Hospital (RSCM), Primary Health Care (PHC), and other health care facilities appointed in "See and Treat" Female Cancer Program (FCP) Jakarta.

After passing the ethical review by the Ethics Committee of the Faculty of Medicine, University of Indonesia and RSCM, this study was conducted. Subjects' characteristics, VIA test results, HPV status, and HPV genotyping data were obtained consecutively in each study location. The inclusion eligibilities of the subjects were staying in Jakarta, would like to participate, and being married or sexually active. The exclusion eligibility were pregnant women, recently having a genital infection, cervical cancer, or a pre-cancerous lesion patient.

VIA negative patient was defined as an absence of white epithelial lesion (WEL) after 5% acetic acid application to the cervix. HPV DNA status was defined as a positive or negative HPV DNA test result which polymerase chain reaction (PCR) SPF10-DEIA-LiPA25 identified from cervical mucus of the women taken before the VIA test. The presence of one or more HPV DNA genotypes was considered as a positive result, while the absence of HPV DNA genotype was defined as a negative result.

The procedures were performed in the lithotomy position after informed consent involving the husband to explain the indications, procedure, possible side effects, and the treatment. The examiner was inserting a speculum into the vagina to reveal the cervix. Before the VIA test, the first inspection was carried out to assess whether the cervix was normal, with severe inflammation, suspicious pre-cancerous, or invasive cervical cancer. Furthermore, the examiner was applying a 5% acetic acid solution to the cervix, particularly at the transformation zone, including the squamocolumnar junction. The result was evaluated after 60 seconds. The result was considered a positive VIA based on the appearance of WEL. Otherwise, the absence of WEL was considered a negative VIA. Patients with negative VIA results then underwent the HPV-DNA test.

HPV DNA testing was performed by Leiden University Medical Center and KALGen Laboratory Jakarta. HPV genotyping procedures include DNA extraction, PCR (SPF10-DEIA-LiPA25), and hybridization. DNA extraction aimed to extract the virus in cells. In this step, the HPV XpressMatrix kit (PT KalGen DNA, East Jakarta, Indonesia) was used. This kit consists of solution A (alkaline cell lysis), solution B (buffer neutralizer), and solution C (buffer washer). PCR (SPF10-DEIA-LiPA25) aimed to amplify the L1 HPV region to detect 21 types of HPV subtypes, using GAPDH (glyceraldehyde 3-phosphate

 https://doi.org/10.7774/cevr.2022.11.2.141

dehydrogenase) as the gene of origin. The kit used for this step was the HPV XpressMatrix kit. Which consists of PCR mix, Polymerase Hot Start Taq DNA, and DNA template. PCR was performed using the following conditions: denaturation for 15 minutes at 95°C; denaturation for 15 seconds at 94°C, 40 cycles; followed by amplification at 55°C for the 30 seconds; extension for 1 minute at 72°C; and followed by extension for 5 minutes at 72°C.

Hybridization was used to identify 21 types of HPV subtypes. There were 15 Hr-HPV subtypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68) and 6 Lr-HPV subtypes (6, 11, 42, 43, 44, 81). In this step, DNA Xprex was used as a DNA hybridizer as a complement to HPV XpressMatrix, which used a controlled proportional integral derivative temperature accuracy that functions as a vacuum to remove residual waste after incubation and washing.

The size of this study was not based on power calculation. Instead, based on available data. The effect size of odds ratio 2 would be considered clinically significant. The odds ratio of 2 was considered as the minimum effect size.

The data were processed using IBM SPSS for Windows ver. 20.0 (IBM Corp., Armonk, NY, USA). Age categories are presented based on the median age. The data were analyzed using chi-square. Missing data were not included in the calculation.

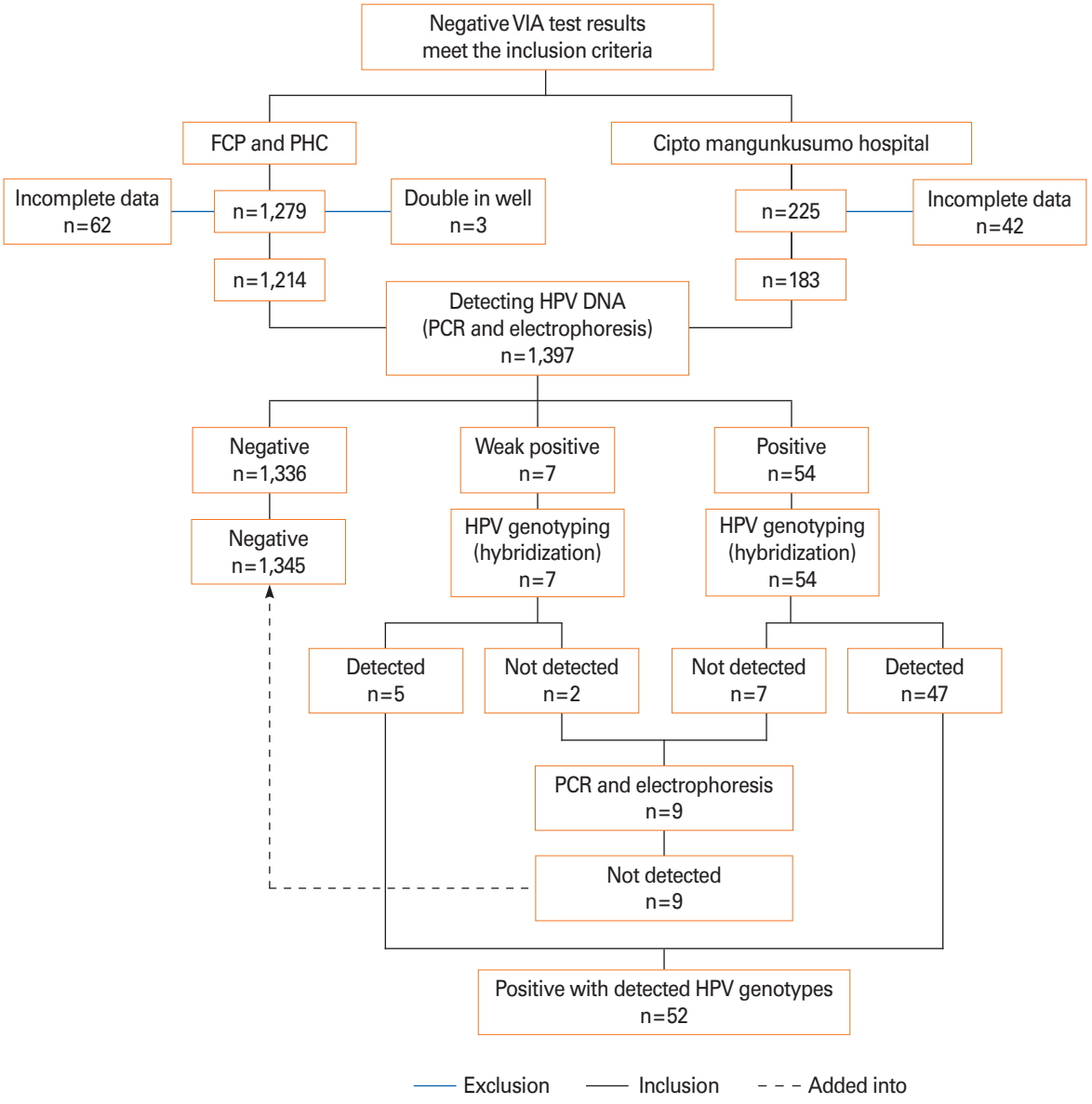

Fig. 1. Subjects' recruitment flowchart. VIA test, Visual Inspection of Acetic Acid test; FCP, Female Cancer Program; PHC, Primary Health Care; HPV, human papillomavirus; PCR, polymerase chain reaction.

This study was approved by the Institutional Review Board of University of Indonesia (no., 532/PT02.FK/ETIK/2011) and was undertaken with appropriate informed consent of participants or guardians.

Results

There have been 1,504 subjects who met the inclusion criteria, then 62 subjects from FCP and PHC and 42 subjects from Cipto Mangunkusumo Hospital were excluded due to incomplete data (age), while three subjects were excluded due to double subject data. Thus, 1,397 subjects with complete data were processed. Finally, 52 subjects were defined as HPV DNA positive groups with detected HPV genotypes described in Fig. 1.

The demographic characteristics of the subjects were described in Table 1. The median age of the subjects was 40 years old. The subjects consist of no subject in <20 years old group, 10 subjects (19.2%) in 20–30 years old group, 17 subjects (32.7%) in 31–40 years old and 41–50 years old group, and eight subjects (15.4%) in >50 years old group.

High-risk HPV infections were found in 55.8%, Lr-HPV infections were found in 13.4% of the HPV-DNA positive women, and unknown HPV type (HPV X) infections were found in 30.8% of the HPV-DNA positive women. Single infection was found in 35 subjects (67.3%), while multiple infections were found in 17 subjects (32.7%) (Table 2).

In Table 3, the HPV genotypes of each classification of HPV-

Table 1. Demographic characteristic (n=52)

Characteristic	Value
Age (yr)	40 (22–59)
<20	0
20–30	10 (19.2)
31–40	17 (32.7)
41–50	17 (32.7)
>50	8 (15.4)
Marital status	
Married once	42 (80.8)
Married more than once	3 (5.8)
Widow	7 (13.4)
Marital age (yr)	22 (15–48)
First menstruation age (yr)[a]	13 (10–16)
Contraceptive use[b]	
No history of contraception	25 (59.5)
Implant	1 (2.4)
Injected contraception	6 (14.3)
Intrauterine device	4 (9.5)
Oral contraceptive pills	4 (9.5)
Sterilization	2 (4.8)

Values are presented as median (minimum–maximum) for numeric variables for the distribution of abnormal data or number (%) for categorical variables.
[a]n=32; as many as 20 subjects missing data. [b]n=42; as many as 10 subjects missing data.

Table 2. HPV-DNA positive classification

Variable	No. (%)
HPV genotyping (n=52)	
High-risk HPV	29 (55.8)
Low-risk HPV	7 (13.4)
HPV X	16 (30.8)
No. of infections (n=52)	
Single infection	35 (67.3)
Multiple infections	17 (32.7)

HPV, human papillomavirus; HPV X, unknown HPV type.

Table 3. HPV genotypes of HPV-DNA positive (n=52)

Variable	No. (%)
High-risk	
16	5 (9.6)
52	5 (9.6)
31	1 (1.9)
39	1 (1.9)
51	1 (1.9)
66	1 (1.9)
18, 39	2 (3.8)
52, 44	1 (1.9)
52, 54	1 (1.9)
56, 74	1 (1.9)
66, HPV X	1 (1.9)
16,18, 31	1 (1.9)
18, 11, 39	1 (1.9)
31, 52, 54	1 (1.9)
39, 68, 73	1 (1.9)
51, 52, 53	1 (1.9)
52, 56, 74	1 (1.9)
16, 39, 52, 58	1 (1.9)
18, 39, 52, 58	1 (1.9)
73, 66, 39, 68	1 (1.9)
Low-risk	
74	3 (5.8)
6	1 (1.9)
44	1 (1.9)
44, HPV X	1 (1.9)
54, 69, 71, HPV X	1 (1.9)
Unknown type	
HPV X	16 (30.8)

HPV, human papillomavirus; HPV X, unknown HPV type.

 https://doi.org/10.7774/cevr.2022.11.2.141

Table 4. Frequency and age distribution of HPV genotypes

HPV genotypes	Frequency	Age group (yr)[a]			
		20–30	31–40	41–50	>50
HPV X	19	2 (2.4)	4 (4.8)	7 (8.4)	6 (7.2)
52	12	3 (3.6)	4 (4.8)	4 (4.8)	1 (1.2)
39	8	3 (3.6)	2 (2.4)	3 (3.6)	0
16	7	1 (1.2)	4 (4.8)	2 (2.4)	0
18	5	1 (1.2)	2 (2.4)	2 (2.4)	0
74	5	2 (2.4)	0	1 (1.2)	2 (2.4)
44	3	0	1 (1.2)	1 (1.2)	1 (1.2)
31	3	1 (1.2)	1 (1.2)	1 (1.2)	0
54	3	1 (1.2)	0	2 (2.4)	0
66	3	1 (1.2)	2 (2.4)	0	0
51	2	0	2 (2.4)	0	0
56	2	0	0	1 (1.2)	1 (1.2)
58	2	1 (1.2)	0	1 (1.2)	0
68	2	0	1 (1.2)	1 (1.2)	0
73	2	0	1 (1.2)	1 (1.2)	0
6	1	0	1 (1.2)	0	0
11	1	1 (1.2)	0	0	0
53	1	0	1 (1.2)	0	0
69	1	0	0	1 (1.2)	0
71	1	0	0	1 (1.2)	0

Values are presented as number (%).
HPV, human papillomavirus; HPV X, unknown HPV type.
[a]Percentage of all positive women, regardless single or multiple infections.

DNA positive were shown. The frequency of HPV X was 19, HPV 52 was 12, HPV 39 was 8, HPV 16 was 7, HPV 18 and 74 were 5, HPV 44, 31, 54, and 66 were 3, HPV 51, 56, 58, 68, and 73 were 2, and HPV 6, 11, 53, 69, and 71 were 1 (Table 4).

Discussion

We identified 52 women who tested positive, 67% were positive for a single HPV, and 33% were positive for multiple HPV infections. In this study, 55.8% of HPV infections were found as high-risk HPV infections, and the proportion of HPV 16 and 18 infections was 14.4%, regardless of single or multiple infections. HPV infections were highest in the 31–40 and 41–50 years old group (32.7%), followed by 20–30 (19.2%) and >50 years old group (15.4%). There is no positive HPV-DNA detected in the <20 years old group due to the lack of participants in this age group. In our study, the most frequent known type of HPV infection was HPV 52, 39, 16, 18, 74, 44, 31, 54, and 66. In our previous study, different results showed that HPV 31 infection was not frequent [15].

A persistent HPV infection has been known as the cause of cervical cancer [6]. In the northeastern part of Brazil, where cervical cancer incidence is also high, a study result concluded that 30% of 32 patients with abnormal colposcopic/cytologic changes were HPV-DNA positive [16]. However, the development of cervical pre-cancerous lesions and invasive cervical cancer is a multifactorial process. HPV infection alone may not be sufficient to transform normal into either pre-cancerous or cancerous lesions. Therefore, positive HPV results might also be found in normal cervix populations [6]. In this normal population study, 58% of HPV infections were high risk, and the proportion of HPV 16 and 18 infections was considered high (14.4% in total).

Several previous studies stated that HPV infection could occur even in normal cervical populations, either single or multiple. Cuschieri et al. [17] in 2004 observed HPV infections with multiple HR-HPV types were found in 3.4% of the negative neoplasm samples. A study in Malaysia of Hr-HPV genotypes showed that HPV-DNA existed in 84 (46.7%) of 200 samples collected in a health screening program from an ethnic group of Malay, Indians, and Chinese, where HPV-16, the high-risk oncogenic genotype, was the most frequently found (40%),

followed by HPV 18 (3.3%), HPV 31 (0.6%), HPV 33 (1.7%), and low-risk HPV (Lr-HPV) 87 (0.6%).

HPV consists of more than 200 identified genotypes which are grouped into high-risk types, such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68, and low risk, such as HPV 6, 11, 40, 42, 43, 44, 53, 54, 61, 72, 73, and 81, while several HPV genotypes remained untyped [17-19]. However, there is a limited study of single and multiple HPV infections in the normal cervix population. Also, the distinguishing factors of cervical cancer risk associated with infection with one or more HPV types have not yet been defined [20]. Li et al. [21] in Beijing, China, found that the incidence of CIN2+ was higher in patients with HPV 16 single infection than multiple infections of the same genotype HPV. In addition, the prevalence of multiple HPV infections in women with either normal or abnormal cytology was considered low [22].

The genotype of HPV and its combination that infects the cervix varies in several countries, but several meta-analyses confirmed the five most prevalent HPV strains in women with and without cervical neoplasia, including HPV 16, 18, 31, 52, and 58. In our study, the most frequent Hr-HPV infections were HPV 16 and 52. Several studies also support this finding. In Jakarta, Indonesia, Schellekens et al. [23] in 2004 found that a combination of HPV genotypes involved single and multiple HPV infections, which is the most frequent HPV infection were Hr-HPV 16 followed by Hr-HPV 18 and 52. In the same city, the study by Vet et al. [24] in 2008 showed that HPV genotype variants of pre-cancerous lesions were predominantly Hr-HPV 52 followed HPV 16 and 39, Lr-HPV 70 and 6, while Murdiyarso et al. [25] found that HPV 16, 18, 45, and 52 infections in patients with squamous cell carcinoma cytology were higher or equal than in the normal population.

It is well known that HPV genotype dispersion has geographical contrasts worldwide. Thus, there have been numerous studies in the world describing differences in the genotype of HPV in several countries. A huge-sized meta-analysis in Africa found that the most frequently HPV genotype contributed in African women with normal cervical cytology was HPV 16, 52, 35, 18, 58, 51, 45, 31, 53, and 56, respectively, in descending order [14]. A population-based cohort study identified HPV 16 as the most common high-risk type found in Hong Kong and HPV 52 in Guangzhou cohorts [26]. A study by Wolday et al. [22] in 2018 in Ethiopia concluded that the most frequent genotypes found were HPV 16 (44.1%), followed by HPV 35 and HPV 45 (each 6.2%), HPV 31 (4.4%), HPV 56 (3.7%), HPV 18 and HPV 59 (each 3.1%), HPV 58 (2.5%), and HPV 39 (1.9%), while the most common Hr-HPV infections among women with normal cytology were HPV 16 (20.3%), followed by HPV 35 (8.7%), HPV 56 and HPV 58 (each 5.8%), HPV 18, HPV 31, and HPV 39 (each 4.4%), HPV 45 (2.9%), and HPV 59 and HPV 68 (each 1.5%). HPV 52 had a high prevalence in an isolated Honduran community in Central America and was highly prevalent in Mexico [27,28]. Moreover, based on some studies, HPV 52 and 58 are known to have a higher prevalence in Asia than in other areas, while HPV 58 has a more significant proportion of the cervical cancer burden in Asia than elsewhere [29-32]. Different from other countries, in this study, we found that HPV 39, 44, and 74 also had a high incidence besides HPV 16, 18, and 52.

Different genotypes of HPV worldwide need to be taken into account for cervical cancer prevention and surveillance strategies, particularly in vaccination and screening programs. Currently the HPV vaccination regimen used is quadrivalent (4vHPV) which provides protection against HPV genotypes 6, 11, 16, and 18 and nonavalent (9vHPV) protecting against HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58 [33]. It can be seen that the nonavalent vaccines do not protect against the HPV 39, 74, and 44 genotypes, which had a higher frequency of infection in this study of 8/52, 5/52, and 3/52, respectively, compared to the genotypes protected by the nonavalent vaccination regimen that exists today.

The WHO's current recommendations on cervical cancer screening (2013) are age-based, but there are limited studies with age stratification in the population. The age stratification-based data were needed to determine the right age target for HPV-DNA testing or cervical cancer screening. However, most of the studies presented mean or median age, which did not help define the prevalence of HPV infection by age group [34]. Our study applied age-based stratification of the population, and we found that HPV infection was highest in 31–40 and 41–50 years old, suggesting that screening for women aged 31–50 years old would be significant for screening program outcomes.

An unsatisfactory Squamo-Columnar Junction (SCJ) can produce false-negative VIAs. Although most of the women over 50 years old have unsatisfactory SCJs, a critical marker in the VIA test, we include this population yet to see the number of satisfactory SCJs in this age group. The total of women over 50 years old in our study population was 228 subjects, including 149 subjects (65%) with satisfactory SCJ and 79 subjects (35%) with unsatisfactory SCJ. A total of 134 subjects (58.77%) from 228 subjects in the over 50 years old group were under

 https://doi.org/10.7774/cevr.2022.11.2.141

56 years old, including 101 subjects (75.73%) with satisfactory SCJ and 33 subjects (24.62%) with unsatisfactory SCJ. Among the subjects with unsatisfactory SCJ, there was no (0%) HPV-positive subject.

Our study provides age-based stratification. Thus, this study can be generalized to VIA negative population. However, the sample size of this study was limited. Furthermore, due to the lack of <20 years old group, we cannot describe HPV genotype in this age group. Despite these limitations, we believe this study can be generalized to Indonesia's population with a normal cervix.

In conclusion, our results suggest that HPV infection varies based on age group in women with normal cervix appearance, which is highest in 31–40 and 41–50 years old. Moreover, HPV 52, 39, 16, 18, 74, 44, 31, 54, and 66 are the most common HPV genotypes found in this study. Although in the clinically normal population, 58% of HPV infections were high risk, the proportion of HPV 16, as well as 18 infections, were 14.4% in total. This finding should become a special consideration for the densely populated LMICs. Moreover, HPV genotyping and age-based stratification data were critical for policy and strategy making and the management of cervical cancer screening and vaccination programs in Indonesian populations. Our findings suggested that screening for 31–50 years old women would be useful, and we proposed different formulas for more suitable "nonavalent" HPV vaccines, particularly in Indonesia as one of LMIC–middle-upper income countries, which is conferring protection against HPV 31, 39, 44, 52, and 74, instead of original nonavalent (HPV 31, 33, 45, 52, and 58) surely besides HPV 16, 18, 6, and 11. Further multicenter and population-based studies with huge sample size and well-distributed age groups are still needed.

ORCID

Tofan Widya Utami *https://orcid.org/0000-0002-0191-5902*
Andrijono Andrijono *https://orcid.org/0000-0001-9556-4404*
Andi Putra *https://orcid.org/0000-0001-5286-5346*
Gert Fleuren *https://orcid.org/0000-0002-9276-183X*
Ekaterina Jordanova *https://orcid.org/0000-0002-8121-1322*
Inas Humairah *https://orcid.org/0000-0002-5188-7508*
Ahmad Utomo *https://orcid.org/0000-0003-3092-3714*

References

1. Naizhaer G, Yuan J, Mijiti P, Aierken K, Abulizi G, Qiao Y. Evaluation of multiple screening methods for cervical cancers in rural areas of Xinjiang, China. Medicine (Baltimore) 2020;99:e19135.
2. Pan American Health Organization. Integrating HPV testing in cervical cancer screening programs: a manual for program managers. Washington (DC): Pan American Health Organization; 2016.
3. Demarco M, Hyun N, Carter-Pokras O, et al. A study of type-specific HPV natural history and implications for contemporary cervical cancer screening programs. EClinicalMedicine 2020;22:100293.
4. Hull R, Mbele M, Makhafola T, et al. Cervical cancer in low and middle-income countries. Oncol Lett 2020;20:2058-74.
5. Fontham ET, Wolf AM, Church TR, et al. Cervical cancer screening for individuals at average risk: 2020 guideline update from the American Cancer Society. CA Cancer J Clin 2020;70:321-46.
6. Crosbie EJ, Einstein MH, Franceschi S, Kitchener HC. Human papillomavirus and cervical cancer. Lancet 2013;382:889-99.
7. Sousa H, Tavares A, Campos C, et al. High-risk human papillomavirus genotype distribution in the Northern region of Portugal: data from regional cervical cancer screening program. Papillomavirus Res 2019;8:100179.
8. Hoque MR, Haque E, Karim MR. Cervical cancer in low-income countries: a Bangladeshi perspective. Int J Gynaecol Obstet 2021;152:19-25.
9. Wang Z, Gu Y, Wang H, et al. Distribution of cervical lesions in high-risk HPV (hr-HPV) positive women with ASC-US: a retrospective single-center study in China. Virol J 2020;17:185.
10. Oyervides-Munoz MA, Perez-Maya AA, Sanchez-Dominguez CN, et al. Multiple HPV infections and viral load association in persistent cervical lesions in Mexican women. Viruses 2020;12:380.
11. Moreno-Acosta P, Romero-Rojas A, Vial N, et al. Persistent high-risk HPV infection and molecular changes related to the development of cervical cancer. Case Rep Obstet Gynecol 2020;2020:6806857.
12. World Health Organization. Global strategy to accelerate the elimination of cervical cancer as a public health problem. Geneva: World Health Organization; 2020.
13. Zeferino LC, Bastos JB, Vale DB, et al. Guidelines for HPV-DNA testing for cervical cancer screening in Brazil. Rev Bras Ginecol Obstet 2018;40:360-8.

14. Ogembo RK, Gona PN, Seymour AJ, et al. Prevalence of human papillomavirus genotypes among African women with normal cervical cytology and neoplasia: a systematic review and meta-analysis. PLoS One 2015;10:e0122488.
15. Utami TW, Aziz MF, Purwoto G, et al. Prevalence of high-risk human papillomavirus (HPV) among negative visual inspection of acetic acid (VIA). Indones J Obstet Gynecol 2014;2:153-6.
16. Husain RS, Ramakrishnan V. Global variation of human papillomavirus genotypes and selected genes involved in cervical malignancies. Ann Glob Health 2015;81:675-83.
17. Cuschieri KS, Cubie HA, Whitley MW, et al. Multiple high risk HPV infections are common in cervical neoplasia and young women in a cervical screening population. J Clin Pathol 2004;57:68-72.
18. Hariri S, Dunne E, Saraiya M, Unger E, Markowitz L. Human papillomavirus. In: Roush SW, Baldy LM, Hall MA, editors. Manual for the surveillance of vaccine-preventable diseases. 5th ed. Atlanta (GA): Centers for Disease Control and Prevention; 2011. p. 1-11.
19. Leaungwutiwong P, Bamrungsak B, Jittmittraphap A, et al. Molecular genotyping of human papillomavirus l1 gene in low-risk and high-risk populations in Bangkok. Sex Transm Dis 2015;42:208-17.
20. Wu Z, Li TY, Jiang M, et al. Human papillomavirus (HPV) 16/18 E6 oncoprotein expression in infections with single and multiple genotypes. Cancer Prev Res (Phila) 2019;12:95-102.
21. Li M, Du X, Lu M, et al. Prevalence characteristics of single and multiple HPV infections in women with cervical cancer and precancerous lesions in Beijing, China. J Med Virol 2019;91:473-81.
22. Wolday D, Derese M, Gebressellassie S, et al. HPV genotype distribution among women with normal and abnormal cervical cytology presenting in a tertiary gynecology referral clinic in Ethiopia. Infect Agent Cancer 2018;13:28.
23. Schellekens MC, Dijkman A, Aziz MF, et al. Prevalence of single and multiple HPV types in cervical carcinomas in Jakarta, Indonesia. Gynecol Oncol 2004;93:49-53.
24. Vet JN, de Boer MA, van den Akker BE, et al. Prevalence of human papillomavirus in Indonesia: a population-based study in three regions. Br J Cancer 2008;99:214-8.
25. Murdiyarso LS, Kartawinata M, Jenie I, et al. Single and multiple high-risk and low-risk human papillomavirus association with cervical lesions of 11,224 women in Jakarta. Cancer Causes Control 2016;27:1371-9.
26. Liu SS, Chan KY, Leung RC, et al. Prevalence and risk factors of human papillomavirus (HPV) infection in southern Chinese women: a population-based study. PLoS One 2011;6:e19244.
27. Atkinson A, Studwell C, Bejarano S, et al. Rural distribution of human papilloma virus in low- and middle-income countries. Exp Mol Pathol 2018;104:146-50.
28. Jaisamrarn U, Castellsague X, Garland SM, et al. Natural history of progression of HPV infection to cervical lesion or clearance: analysis of the control arm of the large, randomised PATRICIA study. PLoS One 2013;8:e79260.
29. Mesher D, Soldan K, Lehtinen M, et al. Population-level effects of human papillomavirus vaccination programs on infections with nonvaccine genotypes. Emerg Infect Dis 2016;22:1732-40.
30. Romero-Morelos P, Uribe-Jimenez A, Bandala C, et al. Genotyping of the human papilloma virus in a group of Mexican women treated in a highly specialist hospital: multiple infections and their potential transcendence in the current vaccination programme. Med Clin (Barc) 2017;149:287-92.
31. Chan PK, Ho WC, Chan MC, et al. Meta-analysis on prevalence and attribution of human papillomavirus types 52 and 58 in cervical neoplasia worldwide. PLoS One 2014;9:e107573.
32. Clifford GM, Smith JS, Plummer M, Munoz N, Franceschi S. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. Br J Cancer 2003;88:63-73.
33. Toh ZQ, Kosasih J, Russell FM, Garland SM, Mulholland EK, Licciardi PV. Recombinant human papillomavirus nonavalent vaccine in the prevention of cancers caused by human papillomavirus. Infect Drug Resist 2019;12:1951-67.
34. World Health Organization. WHO guidelines for screening and treatment of precancerous lesions for cervical cancer prevention. Geneva: World Health Organization; 2013.