The 3rd International Seminar of Science and Technology
ISST 2023 Vol 3 (2024) 01 005
Trends in Science and Technology for Sustainable Living
Faculty of Science and Technology
Universitas Terbuka

Survey of HPV genotypes using multiplex PCR and flow through hybridization in Jakarta, Indonesia

Jacksen*, Angelia Lailatul Jannah, Indah Saraswaty, Suwati, and Diki

Universitas Terbuka, Biology Department, South Tangerang, Banten, Indonesia, 15437

Abstract - This is a study to identify genotypes of Human Papilloma Virus (HPV) among women in Jakarta. The virus increases the risk of cervical cancer, which the second highest incident of cancer in Indonesia. Although there is vaccination program to prevent infection of HPV, it must be based on prior screening of recipients based on HPV genotypes. Each genotype has different potential causing premalignant lesion. Most previous studies are not defining the genotypes of HPV. Therefore, this study is conducted to survey genotypes of HPV using Multiplex PCR and Flow Through Hybridization. The Multiplex PCR and Flow Through Hybridization can detect 35 genotypes, consisting of 18 high risk and 17 low risk genotypes. Samples are patients who visit a clinic in Jakarta. Most of them are women who are already married and between 20 and above 50 years of age. Samples are collected between August to November 2022. There are 336 samples. The result shows that 53.57% of samples were detected positive for HPV. The most prevalent high-risk genotype is genotype 52 while the most prevalent low risk is genotype 11. There are 15,74% of cases of co-infection. The genotype 11 is the genotype that are the most often found in co-infection. The finding is not reported in previous studies. The Multiplex PCR and Flow Through Hybridization can detect genotypes of HPV.

Keywords: cancer, human papilloma virus, Multiplex PCR and Flow Through Hybridization

1 Introduction

Cervical cancer is the second largest number of cancer in Indonesia. This cancer ranks second after breast cancer (Riskesdas, 2018). While in global scale, the incidence is 13,3 cases per 100.000 women [12]. A progress in detection and prevention of cervical cancer will contribute to improvement of health among people. Cancer are malignant proliferation of cells. Each cells develop uncontrolled growth and division that eventually endanger other tissues and the whole body. As each cell are controlled by DNA, the role of genetic factor is crucial for development of cancer, including cervical cancer [4], [12]. Besides genetic constituents of patients, other factors including virus infection also increase possibility of cervical cancer incidence. Human Papilloma Virus (HPV) is the most common virus that infect patients prior to the development of this cancer [12]. Although many new techology provide possible treatment for cancer, such as targeted therapy and CRISPR [2], detection of HPV infection is crucial for reducing the incidence of cervical cancer [10].

*Corresponding author: jacksen.grameindo@gmail.com

Prevention of cervical cancer includes vaccination. Governments promote HPV vaccination to prevent this cancer for genotype 16 and 18. Despite vaccination program, detection of HPV genotype is important. There are 200 genotypes of HPV. Each genotype has different potential for causing cervical cancer. Therefore, detection of HPV genotype is a prerequisite prior to the vaccination. If a subject already has a certain genotype of HPV, the vaccination is less important. Previous studies of HPV genotypes are less specific. Most studies used RT-PCR, which can only detect genotype 6 and 18 only. Therefore, as study using different method of test, which is PCR- hibridyzation are required. This method can detect 35 genotypes, consist of 18 high risk and 17 low risk [14]. This study aims at indentify level of prevalence of each HPV genotypes to support HPV vaccination and detection program.

2 Research method

The method is Multiplex PCR and Flow Through Hybridization with a Hybrispot 12 Manual Vitro. Sample was taken using dry swab with Dacron and LBC (Liquid Based Cytology). The sample was put in a sterile tube. The Multiplex PCR and Flow Through Hybridization was using HPV DNA Genotyping that can detect 35 HPV Genotype. This research was carried out at the IMOQ Laboratory located in North Jakarta. Ethical clearance was obtained before the study. Sample collection starts from August to November 2022.

2.1 Research aim

HPV is a family of viruses consisting of more than 200 different types of viruses. Some types of HPV can cause infections of the skin, while others can cause infections of the genital tract and mucous membranes of the body, including the mouth and throat. Several types of HPV are common causes of sexually transmitted infections. Some types of HPV can also cause genital warts. However, several types of HPV have the potential to cause serious health problems, such as cervical cancer, vulvar cancer, vaginal cancer, penile cancer, and oropharyngeal. The aim of this HPV DNA genotyping study is critical in understanding HPV infection and its impact on human health. The results of this study may also help in the development of more effective prevention, diagnosis and treatment strategies.

2.2 Research procedure

HPV samples are taken from the patient's vagina by obstetrics and gynecology specialist or midwives. They use a vaginal speculum and then the sample media can use LBC (Liquid Based Cytology) and Cervical Swab or Sterile Dacron Dry Swab. HPV samples in LBC form can be transported at room temperature, while HPV samples in Sterile Dacron Dry Swabs can be transported at room temperature, transported in containers without media, or transported in NaCL 0.9%/PBS 1X. Next, it is processed at the IMOQ Laboratory using Hybrispot 12 Manual, Vitro using the Multiplex PCR and Flow Through Hybridization method.

The HPV Direct Flow CHIP kit methodology is based on the amplification of fragments in the L1 viral region of HPV by PCR, followed by hybridization into the membrane with a DNA- specific probe using DNA-Flow technology on the manual hybriSpot 12 platform. The biotinylated amplicons obtained in the PCR step are hybridized in a membrane containing a series of target- specific probes as well as amplification and hybridization control probes. DNA-Flow technology allows very fast binding of PCR products and their specific probes in a three-dimensional porous environment, compared to hybridization on conventional surfaces.

Once binding between a specific amplicon and the associated probe has occurred, the signal is visualized via an immunoenzymatic colorimetric reaction with Streptavidin−Phosphatase and chromogen (NBT- BCIP). The reaction produces an insoluble precipitate in the membrane at the position where hybridization has occurred. The results were analyzed automatically with hybriSoft™ software. PCR amplification can be performed directly from cell suspensions, fixed cells, or paraffin-embedded tissue sections. Therefore, it reduces sample handling time and getting the results.

3 Result and discussion

The result of 336 samples is presented as follows:

Table 1. Result of Whole HPV Genotypes Sampling

Specimen Identification	Total Sample	Explanation
HPV Positive	168	Genotypes identified outside of the 35 specific
HPV Positive (non specific)	12	The samples were dirty (blood) which caused PCR inhibitor and were not clean enough when washed using DNAse free water
HPV Negative	149	
PCR Inhibitor (blank) LBC, Dry Swab	4	Cotton swabs are not recommended because they are PCR inhibitors
PCR Inhibitor (blank) Dacron	3	
Total Sample	336	

Table 2. Grouping of High-Risk Genotypes Based On Age Group

Age group	Genotipe of High-Risk DNA																	
	16	**18**	**26**	**31**	**33**	**35**	**39**	**45**	**51**	**52**	**53**	**56**	**58**	**59**	**66**	**68**	**73**	**82**
20-25 years old			1					2	1	1	1	2	1	1				
26-30 years old	3	1			1		1	2		2		1	4	3	3		1	
31-35 years old							1	3		2	2		1	3	1		1	
35-40 years old	1	2						1	4	1	1	1		1		1		
40-45 years old	3	1			2				1	2		2	1	1	2	2		
46-50 years old	1	1	1		1	1		1		2			1		2		1	1
51-60 years old	5	6				1	1	1		6	1	1	1	2	2			1
Total Genotype	1	11	2	0	4	2	3	10	6	16	5	7	9	11	10	3	3	2

In Table 1, tt was shown that 53.57% of samples were detected positive for HPV, of which 50% of samples were positive for specific HPV genotypes. Besides, 3.57% of samples were positive for non-specific HPV genotypes, while 44.35% of samples were detected HPV negative. Meanwhile 2.08% of the samples were blank because there were PCR inhibitors. This result is higher than Liu

et. al [8]who found that total infection rate is 18.9%. This may be due to the patients do the test when they are required by the doctors to do the test.

Among the high-risk genotypes, it was found that all HPV genotypes were found in different age groups. For example, genotypes 52 and 59 are found in all age groups. The most obtained high-risk genotypes were 52 (16 cases) and 59 (11 cases) and 18 (11 cases), as shown in Table 2. Among low-risk genotypes, genotypes 6, 11, and 42 were found in all age groups. The most common low risk genotypes were genotype 11 (41 cases), and genotype 6 (24 cases). Genotype 69 was not found in this study.

As with the high-risk genotype, negative cases require further observation with different methods. Other different methods can result in the discovery of other genotypes that are not detected by one particular method, as reported by Indarti *et al* [7]. Among the high-risk genotypes, genotype 31 was not found in this study. These negative results require further observation, especially with a different method. Indarti *et al* research [7] found 33 HPV genotypes in patients diagnosed as negative, with a total of 83 samples using liquid based cytology (LBC) sample media. The unit used by Indarti *et al* [7] is the DiagCor GenoFlow Human Papilloma Virus Array Test (Geno Flow) with the Multiplex PCR and Flow Through Hybridization method with the ability to detect 33 HPV genotypes.

Table 3. Grouping of Low Risk Genotypes Based On Age Group

						Genotipe of Low Risk DNA									
Age group	**6**	**11**	**40**	**42**	**43**	**44/ 55**	**54**	**61**	**62/ 81**	**67**	**69**	**70**	**71**	**72**	**84**
20-25 years old	4	2		1	2		1		2			1			
26-30 years old	3	6		1		1			3			1			1
31-35 years old	5	8		2		3				2				2	1
36-40 years old	3	8	2	1	3			1	4				1	1	1
41-45 years old	1	5	1	2	1	4			1					1	
46-50 years old	2	6		3	1	1	2		1				1		
51-60 years old	6	6	1	4		2	4	2	3	1			2		2
Total Genotype	24	41	4	14	7	11	7	3	14	3	0	2	4	4	5

Age is a factor where the most HPV infection cases take place. The patient's age is between 20 – 50 years. All patients were grouped between 20-25, 26-30, 31-35, 36-40, 41-45, 46-50, and above 50 years. The results showed that there were 17 high risk genotypes and 14 low risk genotypes. In this study the authors conducted HPV DNA genotyping test with LBC and Sterile Dacron Swab samples from female patients aged 20 to 60 years who were sexually active. In order to detect cervical cancer, every woman should undergo cervical cancer screening beginning from 25 to 29 years old, based on the American Cancer Society (ACS) [5]. The vaginal examination should be every 3 years. At ages 30 to 60 years, cervical cancer testing is done every 3 to 5 years. Over the age of 65 years, cervical cancer screening in elderly women is carried out based on the doctor's decision.

The result of the study shows that women within 20-50 years old needs routing cervical examination. According to Fontham *et al.* [5] . in a study on data from 2012 to 2016, 5% of patients under the age of 30 were positive for cervical cancer. Another study by Cancer Search UK on data from 2016 to 2018, female patients under the age of 30 showed 13.5% of positive cases of cervical cancer, out of a total of 3,197 cases. WHO (World Health Organization) also recommends that women under the age of 30 who suffer from HIV/AIDS or have a weak immune system to undergo an HPV DNA Genotyping test with routine screening every 3 to 5 years. This certainly supports the HPV

Genotype Survey using Multiplex PCR and Flow Through Hybridization research in Jakarta sureyed respondents within age range of 20 to 60 years.

These results indicate that there are more HPV genotypes than Wulandari *et al* [14]. Meanwhile, this research only took samples in Jakarta, compared to Wulandari *et al* [14] who collected samples from Jakarta as well as Bogor, Bandung and Semarang and the number of samples almost doubled (> 800), using cervical swab sample media. These differences may also be caused by differences in sample processing methods. The author used a Hybrispot 12 manual-Vitro unit with the Multiplex PCR and Flow Through Hybridization method with the ability to detect 35 genotypes (18 HR HPV and 17 LR HPV) specifically and a non-specific Universal Probe HPV, while Wulandari *et al* [14] Used the Cobas® 6800 Platform for HPV identification and the INNO Lipa kit (Fujirebio) for HPV DNA genotype identification. The Cobas® 6800 tool for identifying HPV is an RT PCR method which has the ability to detect specific High Risk 16 and 18, as well as 12 non-specific HPV genotypes. In addition, INNO Lipa (Fujirebio) with a unit called Tendigo with the Line Probe Assay method with the ability to detect 32 genotypes (13 HR HPV specific, 6 HR HPV possible, and 13 LR HPV specific).

Table 4. High and low risk genotype in co-infection

No	Specimen code	Age (year)	HPV Identification	Genotype	
				High Risk (HR)	Low Risk (LR)
1	HPV-002	26		66	62/81
2	HPV-007	20		26, 51, 52, 53	62/81
3	HPV-008	22			6, 62/81, 70
4	HPV-012	26		33	58
5	HPV-014	26		45	42
6	HPV-021	30		45, 55, 66	
7	HPV-024	34			11, 43
8	HPV-027	39		18, 51	
9	HPV-029	38		56	6, 40
10	HPV-051	45		33, 52, 56, 68	44/55, 72
11	HPV-054	41			11, 40, 42
12	HPV-055	41		58, 66	11, 43
13	HPV-056	40		52	62/81, 71
14	HPV-061	40		68	6
15	HPV-062	40		53	40, 62/81
16	HPV-065	57		45	6
17	HPV-071	56		58	6, 40, 42
18	HPV-078	51		59	42
19	HPV-081	54			44/55, 54
20	HPV-089	27			6, 11
21	HPV-091	24			6, 43
22	HPV-094	23		59	11
23	HPV-096	27		16, 58, 59	
24	HPV-100	28		52, 73	
25	HPV-102	29			6, 44/55
26	HPV-107	33			11, 67
27	HPV-108	33			11, 67

No	Specimen code	Age (year)	HPV Identification	Genotype	
				High Risk (HR)	Low Risk (LR)
28	HPV-110	35		45	44/55, 72
29	HPV-112	35		45	43
30	HPV-113	32	Positive	45	43
31	HPV-115	33	Positive	66	6, 42
32	HPV-118	35	Positive		44/55, 72
33	HPV-119	34	Positive		11, 42, 84
34	HPV-120	38	Positive		11, 42, 84
35	HPV-121	35	Positive	58	
36	HPV-122	31	Positive	52, 73	44/55
37	HPV-125	36	Positive	52	11
38	HPV-126	46	Positive	52	73
39	HPV-129	44	Positive	51, 59	11
40	HPV-130	40	Positive	16, 51, 59	11
41	HPV-137	45	Positive	33	62/81
42	HPV-138	48	Positive	33	71
43	HPV-142	42	Positive	56	44/55
44	HPV-148	40	Positive	45, 51	11
45	HPV-149	46	Positive	35	6, 42
46	HPV-150	47	Positive		44/55
47	HPV-161	55	Positive		61, 71
48	HPV-162	51	Positive	18	42
49	HPV-166	50	Positive	26	11
50	HPV-173	52	Positive	53, 56	42, 44/55
51	HPV-180	55	Positive	66	6, 61
52	HPV-187	54	Positive	18	62/81

This study also found co-infection. In Table 4., a total of 336 samples showed HPV co-infection in 15,47% samples, with variations in High Risk and Low Risk HPV genotypes. According to Sobota et al. [13], the HPV co-infection categories were : 1) two or more HPV types from two or more species, 2) two or more HPV types from a single species, 3) two or more HPV types from a single high risk species (16, 18, 26, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82) . This study found that the highest co-infection in low risk is genotype 11. The genotype 11 infects other high risk (58.66, 59.52, 51.16, 45, and 26) genotype, and low risk (43, 40, 42, 6 , 67, and 84) geotypes. This result is different from Gospodinovic et al (2024) in Serbia , as well as Pisani and Cenci [10] in Italy who found that genotype 16 is the most prevalent genotype of co-infection.

HPV co-infection can cause persistent HPV infections over a longer period of time, as well as the risk of developing cancerous lesions. High-risk and low-risk HPV co-infection in immunosuppressed individuals may be due to more rapid viral replication or reactivation of latent infection (Setyowati, et al. 2020). The results are in line with Wulandari *et al* [14] which states that genotype 52 is the most frequently found genotype. These results also support Wulandari et. al [14] that the finding of different HPV genotypes requires evaluation of immunization policies based on vaccine coverage with different genotypes. Other study by Pisani and Cenci [10] in Italy found that (22.48%) have infection with two to five genotype of HPV. Therefore, Pisani and Cenci result of co-

infection percentage which is 22,48% is higher than this study which is only 15,74 % of samples have co-infection.

Table 5. Different co-infection type

No.	Co-infection Type	Co-Variate Genotype	Score of Co-infection
1.	Single Co-incection	Single genotype HR and LR	HPV-002, HPV-012, HPV-014, HPV-061, HPV-65, HPV-78, HPV-094, HPV-112, HPV-113, HPV-125, HPV-126, HPV-137, HPV-138, HPV-142, HPV-162, HPV-166, HPV-187 = 17 Spcimen
2.	Multiple Types Co-infection	Single genotype HR amd double LR	HPV-029, HPV-056, HPV-062, HPV-110, HPV-115 = 5 Specimen
3.	Multiple Types Co-infection	Single genotype LR and double HR	HPV-100, HPV-129, HPV-148, HPV-122 = 4 Specimen
4.	Multiple Types Co-infection	Double HR	HPV-127 = 1 Specimen
5.	Multiple Types Co-infection	Double LR	HPV-024, HPV-081, HPV-189, HPV-091, HPV-102, HPV-107, HPV-108, HPV-118, HPV-161= 9 Specimen
6.	Multiple Types Co-infection	Multiple genotype HR	HPV-021 = 1 Specimen
7.	Multiple Types Co-infection	Multiple genotype LR	HPV-008, HPV-054, HPV-119, HPV-120 = 4 Specimen
8.	Multiple Types Co-infection	Single LR and Multiple HR	HPV-007, HPV-130 = 2 Specimen
9.	Multiple Types Co-infection	Single HR and Multiple LR	HPV-071 = 1 Specimen
10.	Multiple Types Co-infection	Double LR and Multiple HR	HPV-051, HPV-096 = 2 Specimen
11.	Multiple Types Co-infection	Double HR and Double LR	HPV-055, HPV-149, HPV-173 = 3 Specimen
			Total of 49 Specimen

co-infection is a condition where a person is infected with two or more types of the HPV virus at the same time. HPV co-infection can cause various harms, including an increased risk of cancer. HPV co-infection can happen to anyone, for example HPV infection occurs when the virus enters a person's body through wounds, abrasions, or small tears in the skin, when a person has sexual relations with more than one partner or many partners (free sex), the condition a person has a weak immune system, having HIV/AIDS infection or anal sex.

The co-infection increase risk of cancer. There are several types of High Risk HPV genotypes that can cause cancer, including HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 58, 59, 66, 68, 73, 82. Co-infection with these types of HPV can increase a person's risk of developing cervical cancer, vulvar cancer, vaginal cancer, penile cancer, anal cancer and oropharyngeal cancer. Apart from cancer, HPV co-infection can also increase a person's risk of developing other sexually transmitted diseases (STDs), such as genital herpes, gonorrhea and syphilis. And also combined complications, for example HPV and HIV co-infections are often found together, and both increase the risk of sexually transmitted infections (STIs) by Onohuean *et al*.

A study by Sobota et al [13] shows that coinfection with HPV types from the same species can provide natural cross-protection against cervical cancer. However, co-infection with HPV types from different species was not associated with protection against cervical cancer. Meanwhile, Carrillo-García et al [1] has determined the frequency of HPV co-infection at various stages of cervical lesions in the development of cervical cancer Carillo-Garcia et. al also describe the impact of specific HPV type interactions on the risk of high-grade squamous intraepithelial lesions and cancer. They showed that co-infection with HPV16 and HPV68 increased the risk of developing high-grade squamous intraepithelial lesions and cervical cancer.

4 Conclusion

1 The most prevalent high risk genotype is genotype 52 while the most prevalent low risk is genotype 11. The percentage of infection is 53.57%

2 There are 15,74% of cases of co-infection. Genotype 11 is the genotype that are the most often found in co-infection. The finding is not reported in previous studies.

3 The Multiplex PCR and Flow Through Hybridization can detect genotypes of HPV. The detection of HPV genotypes, including co-infection is important for early detection of HPV infection. The detection of HPV genotype may support the doctors in determining future treatment.

5 Recommendation

1 The Hybrispot 12 Vitro manual test, can be the main alternative for preventing cervical cancer. The advantage of this method is that it can show the HPV genotype. The subject may have the right therapy before abnormal cervical cell changes occur.

2 Further research is needed to find out whether patients with HPV infection can undergo change from normal cervical cells into abnormal or precancerous cells. The follow-up research in question involves LBC + Cervical Swab samples from 1 patient to carry out HPV DNA Genotyping testing using the Hybrispot 12 manual platform with the Multiplex PCR and Flow Through Hybridization method. In addition, a cytology test to check the condition of cervical cells is required.

References

[1] Carrillo-García, A., Ponce-de-León-Rosales, S., Cantú-de-León, D., Fragoso-Ontiveros, V., Martínez-Ramírez, I., Orozco-Colín, A., ... Lizano, M. (2014). Impact of Human Papillomavirus Coinfections on the Risk of High-Grade Squamous Intraepithelial Lesion and Cervical Cancer. Gynecologic Oncology. doi: 10.1016/j.ygyno.2014.06.018.

[2] Diki, . D., Widowati, W., Afifah, E., Wahyuni, C. D., and Aviani, J. K. (2021). Development of CRISPR/Cas9 plasmid for gene editing of EGFRIII gene of Glioblastoma multiforme. In *The 1st International Seminar of Science and Technology for Society Development ISST 2021*.

[3] Diki, D., & Dwisatyadini, M. (2020). Curcumin Affecting Caspase 1 and Caspase 9 Increase and Cell Death in Cervical Cancer Cell Culture. In *The 3rd KOBI Congress, International and National Conferences Proceeding. Bengkulu University. Nov.*

[4] Diki, D. (1995). Analisis sitogenetika penderita Leukemia Mieloid Kronik (Skripsi). *Universitas Padjadjaran, Bandung*.

[5] Fontham. (2020). Cervical Cancer Screening for Individuals at Average Risk: 2020 Guideline Update from the American Cancer Society. American Cancer Society.

[6] Gospodinović, H., Grego, E., Jovanović, S., & Pavlović, L. (2024). Examination of the prevalence of high-risk human papillomavirus genotypes among women on the territory of Belgrade. *Vojnosanitetski pregled*, (00), 1-1.

[7] Indarti, J., Fernando, D., Fernando, F., Putri, R. A., Mahardika, A., and Ikhsan, M. (2019). Incidence of Positive Human Papillomavirus High Risk in Negative Cytology Result. *Indonesian Journal of Obstetrics and Gynecology*, 244-250.

[8] Liu, H., Xu, M., Dai, Y., Wang, L., Wang, W., & Ji, M. (2024). Prevalence and Genotype Spectrum of HPV Infection among Women in Xuhui District, Shanghai: Insights for Regional Cervical Cancer Screening Strategies.

[9] Mei Yang. Li li. Chunfan jiang. Min Zhou. Xiaogang Mao. Hui Xing. 2020. Co-Infection with Trichomonas Vaginalis Increases the Risk of Cervical Intraepithelial Neoplasia Grade 2–3 Among HPV 16 Positive Female: A Large Population-Based Study. Nine Clinical Research Centers in Central China. National Library Of Medicine - National Center for Biotechnology Information.

[10] Pisani, T., & Cenci, M. (2024). Prevalence of Multiple High Risk Human Papilloma Virus (HR-HPV) Infections in Cervical Cancer Screening in Lazio Region, Italy. *C ancer Diagnosis & Prognosis*, *4*(1), 42.

[11] Setyowatie, L., and Hidayah, N. (2020). Coinfection of High-Risk and Low-Risk Human Papilloma Virus in Urethra Condylomata Accuminata. Berkala Ilmu Kesehatan Kulit dan Kelamin, 32(3), 239-241.

[12] Singh, D., Vignat, J., Lorenzoni, V., Eslahi, M., Ginsburg, O., Lauby-Secretan, B., ... and Vaccarella, S. (2023). Global estimates of incidence and mortality of cervical cancer in 2020: a baseline analysis of the WHO Global Cervical Cancer Elimination Initiative. *The Lancet Global Health*, *11*(2), e197-e206.

[13] Sobota, R. S., Ramogola-Masire, D., Williams, S. M., and Zetola, N. M. (2014). Co-infection with HPV types from the same species provides natural cross-protection from progression to cervical cancer. BMC Infectious Diseases, 14, 177. doi: 10.1186/1471-2334-14-177.

[14] Wulandari D, Meidyandra RW, Andrijono (2023) Genotype profiles of high-risk human papillomavirus in women of reproductive age: A community-based study. PLoS ONE 18(7): e0287399. https://doi.org/10.1371/journal.pone.0287399