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ORIGINAL

Comparison of Detection of HPV DNA from Menstrual Blood in Menstrual Cup and Cervical Swab in Sexual Single Partner and Multi-Partner Women

Comparación de la detección del ADN del VPH a partir de la sangre menstrual en la copa menstrual y el hisopo cervical en mujeres con pareja sexual única y con varias parejas

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ABSTRACT

Introduction: Human Papillomavirus (HPV) is a virus found in the cervix of a sexually active woman. HPV enters micro lesions in the cervical epithelium binds to primary receptors in the membrane layer over time and becomes an invasive cancer. The invasive cancer process takes 5-10 years. Detection of HPV DNA has currently used cervical swab samples. Currently, HPV DNA can be detected in menstrual blood.
Objective: this study aimed to analyze the comparison of HPV DNA from menstrual blood in menstrual cups and cervical swabs and to analyze the risk factors associated with positive HPV DNA in single-partner and multi-partner women.
Method: cross-sectional method at one time with an analytical observational method using consecutive sampling, sample selection according to the researcher's criteria. The sample used was 44 women according to the inclusion and exclusion criteria. The Spearman statistical test was used to determine the comparison of results from detecting HPV DNA from menstrual blood and cervical swabs, determining the analysis of risk factors (age, occupation, parity, sexual partners, contraception, and smoking) related to positive HPV DNA in both groups using the chi-square test. and Spearman test according to the data scale.
Results: there was no difference in results between HPV DNA from menstrual blood in menstrual cups and cervical swabs in both the single-partner and multi-sexual partner groups of women with p= 0,209 and 0,301.
Conclusion: there is a comparison of HPV DNA detection from menstrual blood in menstrual cups and cervical swabs in single-partner and multi-sexual partner women. The accuracy of menstrual blood examination results is good in detecting HPV DNA.

Keywords: HPV DNA; Menstrual Blood; Cervical Swab; Women With Multiple Sexual Partners; Menstrual Cup.

RESUMEN

Introducción: el Virus del Papiloma Humano (VPH) es un virus que se encuentra en el cuello uterino de una mujer sexualmente activa. El VPH ingresa a las microlesiones en el epitelio cervical y con el tiempo se une a los receptores primarios en la capa de la membrana y se convierte en un cáncer invasivo. El proceso del cáncer invasivo tarda entre 5 y 10 años. Actualmente, la detección del ADN del VPH se realiza mediante muestras de hisopos cervicales. Actualmente, el ADN del VPH se puede detectar en la sangre menstrual.
Objetivo: este estudio tuvo como objetivo analizar la comparación del ADN del VPH de la sangre menstrual en copas menstruales e hisopos cervicales y analizar los factores de riesgo asociados con el ADN del VPH positivo en mujeres de pareja única y multipareja.
Método: método transversal a la vez con método observacional analítico mediante muestreo consecutivo,

selección de la muestra según criterio del investigador. La muestra utilizada fue de 44 mujeres según los criterios de inclusión y exclusión. Se utilizó la prueba estadística de Spearman para determinar la comparación de los resultados de la detección de ADN del VPH a partir de sangre menstrual e hisopado cervical, determinando el análisis de los factores de riesgo (edad, ocupación, paridad, parejas sexuales, anticoncepción y tabaquismo) relacionados con ADN del VPH positivo en ambos grupos utilizaron la prueba de chi-cuadrado. y prueba de Spearman según la escala de datos.
Resultados: no hubo diferencias en los resultados entre el ADN del VPH de la sangre menstrual en las copas menstruales y los hisopos cervicales en los grupos de mujeres de pareja única y multisexual con p = 0,209 y 0,301.
Conclusión: existe una comparación de la detección del ADN del VPH a partir de la sangre menstrual en copas menstruales y en hisopos cervicales en mujeres con pareja única y con pareja multisexual. La precisión de los resultados del análisis de sangre menstrual es buena para detectar el ADN del VPH.

Palabras clave: ADN del VPH; Sangre Menstrual; Hisopo Cervical; Mujeres con Múltiples Parejas Sexuales; Copa Menstrual.

INTRODUCTION

Human papillomavirus (HPV) is a sexually transmitted infection that causes various health complications, including cervical cancer. Cervical cancer, the fourth most common cancer in women worldwide, is primarily caused by infection with oncogenic HPV types 16 and 18, accounting for more than 70 % of cervical cancer diagnoses.[1] HPV is the most common viral infection of the reproductive tract, with the majority of sexually active women and men infected at some point in their lives.[2] While the majority of the infected population eventually recovers from the infection, persistent infection with certain types of HPV can lead to the development of cervical cancer.[3]

HPV has an exclusively intra-epithelial infection cycle and infects the squamous epithelium of the skin and mucosa, with a wide variety of genotypes known to infect the genital area.[4] In recent years, there has been increasing interest in understanding the potential presence of Human Papillomavirus (HPV) in menstrual blood, a topic that has significant implications for women's health and reproductive issues.[5] Emerging research suggests that menstrual blood may be a viable alternative to traditional screening methods, as it potentially contains traces of HPV.[6]

Several studies have detected the presence of Human papillomavirus in menstrual blood, implying that it would be useful for non-invasive screening for cervical cancer or pre-cancer. Previous research samples used sanitary napkins which were related to environmental empowerment issues regarding waste that was less biodegradable due to sanitary napkin waste. Menstrual cups can be used by women to reduce sanitary napkin waste. This tool is made from silicon which is used in the cervix, under the cervix, and functions to collect menstrual blood and is a comfortable tool, accepted by the public because it is safe and efficient to use for hygiene during menstruation. less likely to leak than sanitary napkins.[7,8] Previous research using liquid menstrual blood samples has also been conducted to detect Herpes Simplex Virus (HSV) from menstrual blood.[9]

A preliminary study was carried out by researchers on 13 May – 18 July 2023 using liquid menstrual blood and cervical swabs for cervical cancer screening in 5 samples of sexually active women in the city of Surabaya. The examination carried out at the Tropical Disease Center (TDC) laboratory obtained results that matched the results of samples from liquid menstrual blood and cervical swabs, namely 4 positive (+) out of 5 samples, thus early detection using the HPV DNA method from menstrual blood can be used to detect HPV DNA New method for cervical cancer screening.

METHOD

The research was carried out using a cross-sectional approach with an analytical observational method, namely measuring variables at the same time and the measurement results describe the conditions at that time.[10]

Respondent

Forty-four respondents were recruited into 2 groups, namely 21 groups of single-partner women and 23 sexual multi-partner women who were >18 years old, sexually active at least 3x a week, had regular menstruation, were willing to take part in the study, did not use an IUD, and were not diagnosed with cervical cancer in hospital data. Data collection was carried out by interviewing the researcher for 10 minutes with several questions related to risk factors (age, occupation, parity, sexual partners, contraception, and smoking). The research was carried out from November 2023 to March 2024 and received ethically appropriate information

from the Airlangga University Surabaya Hospital Committee which was prepared according to their choice and then saved the comparison in file form.

Menstrual Blood Collection in Menstrual Cup, DNA Extraction, and HPV DNA PCR

Samples were taken using a previously prepared sterile cotton, then specimens were taken from cervical swabs outside the menstrual period at the Pakis Community Health Center. The samples obtained were put into a mini tube containing 2 ml of PBS (phosphate-buffered saline).

On the 2nd day of the nearest menstrual cycle, respondents were instructed to use a menstrual cup, and a menstrual blood specimen was taken in a ±3cc menstrual cup stored in an ETA tube. Both samples will be sent to the Tropical Disease Center (TDC) for HPV DNA detection.

HPV DNA in cervical swabs and menstrual blood using the Reserve Hybridization (RH) Test (QIAGEN) method, DNA extracted samples in QIAamp Mini spin column, PCR using primers My11 and My09 in PCR master mix (Promega) tool used Qiagen DNA mini kit, and PCR with a Bio Product Select Cyler II machine.

Independent Variables

a. Sexual Partner Status: This variable categorizes women into two groups based on their sexual partner status - single-partner and multi-partner.

Measurement: Determined through self-reporting by the participants during the recruitment process.

b. Age: The age of the participants is an essential variable to assess its impact on HPV DNA detection.

Measurement: Recorded in years and categorized into age ranges (e.g., <20 years, 21-30 years, 31-40 years, 41-50 years).

c. Occupation: The occupation of the participants can be a factor influencing HPV DNA detection.

Measurement: Categorized into groups such as housewives, private/government employees, entrepreneurs, and prostitutes.

d. Parity: Parity refers to the number of times a woman has given birth.

Measurement: Categorized as 1 time, >1 time, or never based on the participants' reproductive history.

e. Contraception: The type of contraception used by the participants can impact HPV DNA detection.

Measurement: Categorized into methods like pill, inject, implant, condom, or no birth control.

f. Smoking: Smoking status is considered a risk factor for HPV-related diseases.

Measurement: Recorded as a binary variable (Yes/No) based on the participants' smoking habits.

Dependent Variables

a. HPV DNA Detection Results: The presence or absence of HPV DNA in menstrual blood and cervical swabs.

Measurement: Determined through PCR analysis of samples collected from menstrual cups and cervical swabs.

Control Variables

a. Menstrual Blood Collection Method: The method used to collect menstrual blood samples, such as using a menstrual cup.

Measurement: Specified as the technique employed during sample collection

b. DNA Extraction and PCR Analysis: The procedures followed for DNA extraction and PCR analysis of the samples.

Measurement: Recorded based on the specific protocols and tools utilized for DNA extraction and PCR.

The research was carried out using a cross-sectional approach with analytical observational methods, namely measuring variables at the same time and the measurement results describe the conditions at that time. The sampling technique was consecutive sampling, namely that respondents were selected according to inclusion and exclusion criteria. The SPSS statistical test uses Mann Whitney to compare the concordance of HPV DNA results from menstrual blood and cervical swabs in both single-partner and multi-sexual partner groups of women and to relate the risk factors of age, occupation, parity, sexual partners, contraception, and smoking-related to positive HPV DNA in both group and diagnostic test results for HPV DNA from both simples.

Protocol number for this research ethics committee letter: UA-02-23214.

RESULT

Respondent Characteristics

Twenty-one respondents as single partner women were recruited as samples.

Table 1. Results of HPV DNA detection in menstrual blood in women with single partners and sexual partners

PCR results of menstrual blood	Single-partner sexual	Multi-partner sexual	Amount
Positive	15 (71,4 %)	22 (95,7 %)	37 (84,1 %)
Negative	6 (28,6 %)	1 (4,3 %)	7 (15,9 %)
	21 (100 %)	23 (100 %)	44 (100 %)

Based on table 1, the results of HPV DNA detection from menstrual blood were 22 positive (95,7 %) and 1 negative (4,5 %) in multi-partners and there were 15 positive (71,4 %) and 6 negative (28,6 %) in the single-partner group.

Based on the investigation, the Mann-Whitney analysis output shows a significant value of 0,030, which means there is a significant difference between Based on Table 3, PCR detection results from cervical swabs, in the multi-partner group.

Table 2. Results of cervical swab HPV DNA detection in women with single partners and sexual partners

PCR results of menstrual blood	Single-partner sexual	Multi-partner sexual	Amount
Positive	11 (52,4 %)	20 (87 %)	31 (70,5 %)
Negative	10 (47,6 %)	3 (13 %)	13 (29,5 %)
	21 (100 %)	23 (100 %)	44 (100 %)

Based on table 2, PCR detection results from cervical swabs, in the multi-partner group there were 20 positive (87 %) and 3 negative (13 %), while there were 11 positive (52,4 %) and 10 negative (47,6 %) in the single-partner group.

Within the investigation, the results of the Mann-Whitney bivariate analysis of the Asymp value. Sig. (2-tailed) 0,013 < 0,05, so there is a significant difference between the results of cervical swabs from single-partner and multi-sexual partner women.

Based on the investigation, the results of the analysis of differences in HPV DNA detection from menstrual blood and cervical swabs in women with single sexual partners show the Asymp value. Sig. (2-tailed) = 0,209 > 0,05, which means that H0 is accepted or it can be concluded that there is no significant difference between the results of HPV DNA detection from menstrual blood samples in menstrual cups and cervical swabs in women with single sexual partners.

Based on the Mann-Whitney test, the results of the analysis of differences in the detection of HPV DNA from menstrual blood in menstrual cups and cervical swabs in women with single sexual partners show the Asymp value. Sig. (2-tailed) = 0,301 > 0,05, which means that H0 is accepted or it can be concluded that there is no significant difference between the results of HPV DNA detection from menstrual blood samples and cervical swabs in women with multiple sexual partners.

Table 3. Risk factors associated with positive HPV DNA in women with single and multi-partner sexual partners

Risk Factor	Single-partner Sexual Frequency ((n=10) (%))	Multi-partner Sexual Frequency ((n=20) (%))	p-value
Age Range			
a. <20 years	0 (0)	1 (5,0)	0,091
a. 21-30 years	1 (10,0)	10 (50,0)	
b. 31-40 years	5 (50,0)	3 (15,0)	
c. 41-50 years	4 (40,0)	6 (30,0)	
Occupation			
a. Housewife	8 (80,0)	0 (0)	
b. Private and government employees	0 (0)	0 (0)	0,001**
c. Entrepreneur	2 (20,0)	0 (0)	
d. Prostitute	0 (0)	20 (100)	
Parity			
a. 1 time	1 (10,0)	4 (20,0)	0,033*
b. >1 time	9 (90,0)	4 (20,0)	
c. never	0 (0)	12 (60,0))	

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Partner Sexual			
a. 1 pair	10 (100)	0 (0)	0,001**
b. >1 pair	0 (0)	20 (100)	
Contraception			
a. Pill	7 (70,0)	4 (20,0)	
b. Inject	2 (20,0)	7(35,0)	0,021**
c. Implant	1 (10,0)	0 (0)	
d. Condom	0 (0)	8 (40)	
e. No birth control	0 (0)	1 (5,0)	
Smoke			
a. Yes	1 (10,0)	15 (75,0)	0,001**
b. No	9 (90,0)	5 (25,0)	
Total	10 (100)	20 (100)	

** Spearman test(related)*
*** Chi-Square test (related)*

Based on table 3 risk factors with positive HPV DNA results were more common in women with multiple sexual partners, namely 20 respondents, at the age of 21-30 years there were 10 (50 %), all of them were prostitutes or commercial sex workers 20 (100 %), the majority were parity. 12 respondents (60 %) had never used barrier contraception, namely condoms, 8 respondents (40 %) and 15 out of 20 people (75 %) were active smokers.

Risk factors for single-partner women with positive HPV DNA results are 6 respondents (50 %) aged 41-50 years, 9 respondents (90 %) with parity > 1 time, 8 respondents (80 %) as housewives, 8 respondents (80 %), family planning users pills as many as 7 respondents (70 %) and the majority do not smoke 9 respondents (90 %).

Table 4. Diagnostic test results for detecting HPV DNA from menstrual blood in menstrual cups in single-partner and multi-partner women compared to cervical swabs as the WHO gold standard

	Gold Standard WHO		
	Swab +	**Swab -**	**Amount**
Menstrual blood+	30	7	37
Menstrual blood -	1	6	7
Amount	31	13	44
Sensitivity	96,77 %		
Specificity	46,15 %		
Positive predictive value	81,08 %		
Negative predictive value	85,71 %		
Accuracy	81,82 %		

Based on table 4, the diagnostic test results for detecting HPV DNA in menstrual blood compared with cervical swabs as the WHO gold standard showed a sensitivity of 96,77 %, specificity of 46,15 %, NPP of 81,08 %, NPN of 85,71 %, and accuracy of 81,82 %.

DISCUSSION

HPV can be detected in the menstrual blood of women with various clinical statuses of cervical disease. [11,12] Menstrual blood is a sample or specimen that is easy to collect yourself and is non-invasive. In this study, we wanted to show that HPV DNA can be detected in liquid menstrual blood in a general population group with normal cytology who were divided into two groups according to sexual activity and associated risk factors associated with positive HPV DNA. So, far this research is the first research in Indonesia to use menstrual blood to detect HPV DNA. This is different from previous research which collected menstrual blood samples from sanitary napkins which were collected and put in plastic ziplocks. Respondents in this study were instructed to use a menstrual cup to collect menstrual blood on day 2 for 2-4 hours, then hand it over to the laboratory staff and transfer it to an EDTA tube using a 3cc syringe. The EDTA tube containing blood is shaken 8 times to prevent blood clots. This sample collection provides convenience so that patients can collect samples in their free time according to laboratory operating hours.

Cervical cancer is a leading problem for women's health worldwide.[13] According to WHO (World Health Organization) in 2020, around 604,000 women were diagnosed with cervical cancer throughout the world and there were 342,000 deaths due to cervical cancer. Cervical cancer is the fourth most common in the world in women (WHO, 2022). Cervical cancer cases (36,633 cases) rank second after breast cancer (65,858 cases) (RI Ministry of Health, 2022). Data from the oncology polyclinic at RSUD Soetomo Surabaya, cervical cancer patient data for the period 2018-2022, there were 2,882 new cases and the highest number of stage IIIB sufferers was 1,465 cases (RSUD Dr. Soetomo Surabaya, 2022).

Cervical cancer is caused by infection with high-risk serotypes of Human Papillomavirus (HPV) which can cause the development of precancerous lesions and progress to invasive carcinoma if not treated medically and neoplasmic transformation takes years to become cancer. The main form of transmission of Human Papillomavirus (HPV) is sexual activity of any kind. The estimated overall risk for exposure to HPV infection is 15 % to 25 % for each new sexual partner and the risk is 28,5 % for single sexual partners (14). Women with multiple sexual partners have a high risk of sexually transmitted infections in HPV transmission because the main risk factors underlying the high-risk group include multiple sexual partners.[15]

PCR examination results showed that 22 of the 37 menstrual blood samples were positive for high-risk HPV infection in the multi-sexual partner group and 15 samples were positive for Hr-HPV infection in the single-sexual partner group. The results of this study are in line with research conducted by Wong et al., (2018) HPV DNA was detected in 83 % of women diagnosed with CIN and 4 % of normal sexually active women (single partners) due to the number of samples from the general population being smaller than the high-risk population.

The difference in the results of detecting HPV DNA from menstrual blood in the menstrual cup in the 2 groups (p=0,030) shows that there is a significant difference between the groups of women with single partners and multi-partner sexual partners. This research is in line with research by Kops *et al.*, (2021), the results of a sample of 5,268 women, 33,00 % (95 % CI 31,07–34,92) experienced multiple infections including one type of high-risk HPV present in 85,50 % from all infections. Young women who are or are not in a relationship and have had more than one sexual partner in the past year are more likely to experience multiple Hr-HPV infections.

Studies conducted by Wong *et al.*, (2018) to research conducted by Tsang *et al.*, (2024) have shown that the menstrual blood sample test is suitable for all menstruating women as a routine screening method, especially for symptomatic women who are reluctant to consult with a doctor due to embarrassment or illness and for women who require more frequent follow-up for successful treatment or recovery. Menstrual blood testing is an appropriate diagnostic specimen to consider for non-invasive detection of HPV DNA and genotyping using PCR.

The results of this study are consistent with research by Lee et al, (2016) which states that HPV testing with menstrual blood provides a new screening method modality that can significantly increase accessibility for cervical cancer screening.

The results of the PCR examination of cervical swab samples showed that 20 samples were positive for high-risk HPV infection from the multi-sexual partner group and in the single-sexual partner group, 11 samples were positive for Hr-HPV infection. The results of this study are in line with Sulistyawan's (2019) research that commercial sex workers are a high-risk factor for being infected with HPV DNA at 83 %.

The difference in the results of cervical swab HPV DNA detection in the 2 groups (p=0,013) shows that there is a significant difference between the groups of single-partner and multi-sexual partner women. Another study explains that multiple sexual partners are significantly associated with cervical intraepithelial neoplasia (CIN) or even cervical cancer. This suggests that women with multi-partner sexual behavior may be at higher risk of experiencing cervical abnormalities.[19]

A similar study found that the severity of bacterial vaginosis (BV) increased with the number of sexual partners, and the HPV-positive ratio also increased with the severity of BV.[20] This means that women with multi-partner sexual behavior are more likely to contract HPV, which is a significant risk factor for cervical cancer. Another study showed HPV 16 and related type a9 were most common in women in a population accounting for 38 % of samples in cervical cancer screening.[21]

The results of PCR examination of menstrual blood samples showed that analysis of HPV DNA detection results from menstrual blood in menstrual cups and cervical swabs (p=0,209) showed that there was no significant difference between detection results and menstrual blood in menstrual cups and cervical swabs in women with single sexual partners.

According to [22], in several studies using vaginal tampons will increase the detection of high Hr-HPV in respondents who use tampons for a long time (1-4 hours and overnight) but low detection in respondents who use tampons for long periods (1-4 hours and overnight). 10 seconds. This is the same as using a menstrual cup which can provide an opportunity to detect Hr-HPV in menstrual blood. Menstrual blood samples in this study were taken from a menstrual cup that was used for 2-4 hours and was installed in the respondent's vagina, directly collecting menstrual blood. This study used day 2 menstrual blood samples, there were differences in the PCR results of cervical swabs (code A) and menstrual blood (code C) in samples coded A6, C6, A14, C14, A15, C15, A18, C18, A19, and C19. The menstrual blood sample showed positive results while the cervical swab

showed negative results and samples coded A12, and C12 showed negative menstrual blood results and positive cervical swabs. In similar research such as samples coded A12 and C12, differences in results are influenced by the method of taking the sample, temperature, and method of storing the sample. Among women who tested positive for HPV, menstrual pads showed highly concordant results compared with samples collected by physicians.[23]

Different PCR tools will differentiate the types detected by research by [21] using PCR-Luminex which can detect HPV type 48, both HR-HPV and LR-HPV. Meanwhile, this study was able to detect HR-HPV types including types 16 and 18.

Analysis of HPV DNA detection results from menstrual blood in menstrual cups and cervical swabs (p=0,301) showed that there was no significant difference between detection results from menstrual blood in menstrual cups and cervical swabs in women with multiple sexual partners. The assertion is that HPV testing with menstrual blood provides a new screening modality that may significantly increase accessibility for cervical cancer screening.

There are differences in PCR results for samples coded A33, C33, and A37 and C37. The menstrual blood sample showed positive results while the cervical swab showed negative results. This is according to the findings of Lee et al, (2016) that the sensitivity, specificity, PPV, and NPV of the HR-HPV test with menstrual blood and the level of agreement for detecting HR-HPV were higher during the first day of menstruation compared to day 2 of menstruation. Viral load HR-HPV infection may decrease as menstruation progresses. Zhang et al, (2021) expressed a different opinion, namely that there was no statistically significant difference in the level of positive HR-HPV menstrual blood and several levels of positive HR-HPV menstrual blood on different menstrual days, from the first day of menstruation to the fifth day of menstruation were equivalent. Other differences in results were found in samples A33 and C33. The menstrual blood sample showed positive results while the cervical swab showed negative results. In line with research by [23] published in the Journal of Infectious Diseases comparing the acceptability and suitability of self-taken vaginal swab samples and menstrual blood samples. The study found that 94 % of participants preferred menstrual pads over clinician-collected samples, and concordance between menstrual pads and clinician-collected specimens was 94 % (95 % CI 83–98) for samples collected within 2 months overall. Another study is in line with the results of this study. The sensitivity of detecting HPV DNA in menstrual blood was higher than that obtained using self-collected vaginal swabs (89,8 % vs. 66,1 %).[24]

These studies suggest that menstrual blood may be a new method for detecting HPV, especially in places where cultural and social barriers are more common. However, further research is needed to validate this method on a larger scale and to overcome the limitations identified in this study especially when compared with cervical specimens collected by physicians.

This study's age correlation does not align with Yang *et al* (2020) research, which involved 10,086 women under 65 years old from Yangqu County. HPV genotypes were identified using standard HPV DNA testing. The overall prevalence of HPV infection was 8,92 %. High-risk HPV types were found in 8,80 % of cases, while low-risk types were found in 0,38 %. Single-genotype infections constituted 67,91 % of positive HPV cases. HPV-18 ranked as the 11th most common type among HPV-positive cases. Women aged 50 years and older had the highest HPV prevalence, while those under 30 years had the lowest. The distribution of HPV genotypes also varied across the age groups: under 30, 30-49, and 50 years and older

A different study conducted in China indicated that women under 30 years old had the lowest HPV infection rate, at 8,92 % overall (Liu, *et al.*, 2020). This implies that women in this age bracket have a lower likelihood of HPV infection compared to older age groups.

The risk factors for positive high-risk HPV infection differed significantly among parity groups in this study. Specifically, those with 2-3 parities showed an increased risk (>1 times) compared to those with single partners (91,7 %) and multi-partners (20 %). These findings are consistent with [25] research, which found that the risk of high-risk HPV infection in the ≥3 parities group was 1,228 times higher than in the <3 parities group. This suggests that higher parity may lead to more severe cervical injuries, identifying those with more than three parities as a critical population for preventing high-risk HPV infections.[26,27]

Another risk factor is the use of hormonal contraception in single partners who use pills, 8 respondents (66,7 %) and injections, 3 respondents (25 %), while in the multi-partner group, 4 respondents use pills (20 %) and injectables, 7 respondents (35 %). Ten years of hormonal contraceptive use from age 20 years is associated with an increased cumulative incidence of invasive cervical cancer at age 50 years of approximately 1 case per 1,000. Hormonal contraception has a very small negative impact on the absolute risk of cervical cancer.[28]

The majority of active smokers in the multi-partner group of women were 15 respondents (75 %). These results are by studies that show that women who smoke have a risk of developing cervical cancer 2 times greater than women who do not smoke because cigarettes contain carcinogenic ingredients. Research shows that women who smoke get nicotine and other dangerous substances in cigarettes in their cervical mucus. Apart from being carcinogenic, these substances can reduce the resistance of the cervix, making it easier for

infections to occur. The research results [29] show that smoking tobacco is associated with an increase in cervical cancer, and evidence-based tobacco control strategies can have an impact on reducing the spread of cervical cancer.[30]

The results of the diagnostic test for detecting HPV from menstrual blood and cervical swabs in the single-partner and multi-sexual partner groups showed a sensitivity of 96,77 %, specificity of 46,15 %, and a positive predictive value of 81,08 %, a negative predictive value (NPP) of 85,71 %. This specificity value is lower than the other values, this result is in line with previous research by Lee et al (2016) which obtained the specificity of the menstrual blood test for detecting CIN 3 of 45,5 %. Another study consistent with this study had a much higher sensitivity than the Pap test in detecting high-grade cervical intraepithelial neoplasia (HGCIN), its specificity was relatively lower.[17]

Research by Wong et al (2018) obtained sensitivity and NPN values of 83 % and 74 % for detecting CIN or HPV infection. Research that detects HPV in women with CIN or HSIL, in this study the samples used were women with normal cytology. The specificity and positive predictive value of menstrual blood from research by Lee et al, (2016) obtained 50 % and 61,5 % for detecting CIN 3 or worse on the first and second days of menstruation. Meanwhile, [18]found that the sensitivity and NPP were both 66,7 %

Similar studies reported the sensitivity results of menstrual blood, the study showed the diagnostic accuracy of menstrual blood in terms of sensitivity ranging from 82,8 % to 97,7 % and specificity ranging from 50,0 % to 98,0 % in the detection of cervical intraepithelial neoplasia or HPV infection.[6] In another study from,[22] cervical HPV detection carried out by doctors had 97,8 % agreement for detecting HPV. Although previous investigators have reported high levels of agreement for detecting HR-HPV from cervical and menstrual blood samples, respondents were advised to target HR-HPV or conduct the study in a case-control setting. Another study from the United States stated that women who were passive smokers had twice the risk of contracting HPV infection (OR: 2,45; 95 % CI = 1,34–4,48), compared to women who were not exposed, and women who were active smokers had a 3,5 times more likely to contract HPV (OR = 3,56; 95 % CI 1,23–10,30).[31] The limitations of this research in sampling are, that the menstrual cycle varies so that the time interval between taking cervical swab samples and menstrual blood exceeds the specified time of 1-35 days according to the normal menstrual cycle. This research does not carry out genotype checks so it cannot be known what type of HPV each person is. sample and blood count <1cc, liquid menstrual blood sampling is delayed and taken in the next cycle, thereby slowing down the research process. Meanwhile, the novelty of this research is that the population in this study used a general population compared with a high-risk population (prostitute women), liquid menstrual blood specimens in menstrual cups were used as research samples, the use of menstrual cups as a tool that plays a role in reducing sanitary napkin waste. more increasing.

CONCLUSIONS

The use of menstrual blood samples can be a new method for detecting HPV DNA as a cause of cervical cancer. This study concludes that there is no difference between the detection of HPV DNA from menstrual blood in menstrual cups and cervical swabs in single-partner and multi-partner women. Accuracy results from menstrual blood are good. Using a menstrual cup can pick up HPV from the cervix and vagina mixed with menstrual blood. Menstrual blood specimens can be used according to the woman's convenience because they are practical and time-saving.

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