Comparison of HPV DNA Screening using menstrual blood collected in sanitary pads versus cervical swabs in women with both single and multiple sexual partners

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ABSTRACT

Introduction: Persistent high-risk HPV infection is the main cause of cervical cancer. Sexually active women are at risk of HPV infection, especially if they have multiple sexual partners. To detect HPV, the HPV DNA test can be conducted using menstrual blood samples as an alternative to cervical cancer screening.

Objective: This study aims to analyze the comparison of results between HPV DNA screening with menstrual blood in sanitary pads and cervical swabs in women with single and multi-partner sexual.

Method: This study was an analytical observational study with a cross-sectional design. The sample used was 44 women (21 single partners and 23 multiple sexual partner) aged >18 years, sexually active, experiencing regular menstruation. Menstrual blood samples in sanitary pads and cervical swabs were examined PCR method. Statistical tests used Mann Whitney with a significance level of p≤0.05, diagnostic test and ROC description.

Results: HPV DNA was found in 47.6% (10/21) in menstrual blood and 47.8% (11/23) in cervical swabs with a significance value of p=1.00 in women with single sexual partners. HPV DNA was detected in 82.6% (19/23) in menstrual blood and 87% (20/23) in cervical swabs with a significance value of p=0.00 in women with multiple sexual partners. Menstrual blood in sanitary pads has a sensitivity value of 90.32%, specificity 92.31%, positive predictive value 96.35%, negative predictive value 80% and accuracy of 91.32% to detect HPV DNA.

Conclusions: The conclusion from this analysis shows that there is concordance between HPV DNA screening with menstrual blood in sanitary pads and cervical swabs with high accuracy values.

Keywords: Menstrual Blood; HPV Screening; Single Partner; Multipartner Sexual.

RESUMEN

Introducción: la infección persistente por VPH de alto riesgo es la principal causa de cáncer de cuello uterino. Las mujeres sexualmente activas corren el riesgo de contraer la infección por VPH, especialmente si tienen múltiples parejas sexuales. Para detectar el VPH, se puede realizar la prueba de ADN del VPH utilizando muestras de sangre menstrual como alternativa a la detección del cáncer de cuello uterino.

Objetivo: este estudio tiene como objetivo analizar la comparación de resultados entre el tamizaje de ADN del VPH con sangre menstrual en toallas sanitarias e hisopos cervicales en mujeres con pareja sexual única y multipareja.

Método: este estudio fue un estudio observacional analítico con un diseño transversal. La muestra utilizada fue de 44 mujeres (21 parejas solteras y 23 parejas sexuales múltiples) mayores de 18 años, sexualmente activas y que experimentaban menstruación regular. Se examinaron muestras de sangre menstrual en toallas
INTRODUCTION

Cervical cancer is the most frequently diagnosed gynecological cancer worldwide. According to data from the 2020 Globocan Burden of Cancer Study (Globocan), cervical cancer is the fourth leading cause of cancer death in women in 36 countries. Cervical cancer sufferers continue to increase, in 2012 there were 527 000 cases, increasing to 570 000 cases in 2018 and in 2020 reaching 604 127 cases with a death toll of 341 831 people. Data on new cervical cancer patients at Dr Soetomo Hospital Surabaya (2022) shows that of 464 cervical cancer patients, 64.2% of them were diagnosed with stage IIIB. This is very unfortunate because cervical cancer can be detected at the precancerous lesion stage by screening. 

Cervical cancer screening in Indonesia using the IVA method has not been running optimally. VIA examination coverage in 2019 - 2021 was only 6.83%. Lack of knowledge, awareness, fear, fatalism, shame, time and transportation constraints, and lack of husband’s support are some of the obstacles to implementing cervical cancer screening in Indonesia. Persistent High-risk Human Papillomavirus (HrHPV) infection is the main etiology of cervical cancer. Sexually active women are at risk of being infected with HPV. The risk increases with the number of sexual partners. Women with multiple sexual partners are a population at high risk of cervical cancer. Changing sexual partners causes the possibility of contracting sexually transmitted infections to increase, one of which is HPV.

HPV DNA testing has gradually become the main method for cervical cancer screening in recent years in several countries. Menstrual blood can be used as an alternative sample to detect HPV as an easy and convenient cervical cancer screening with sensitivity values ranging from 82.8% to 97.7% and specificity of 50% to 98% in cervical intraepithelial neoplasia or HPV infection detection. The results of a preliminary study on 5 female commercial sex workers in the city of Surabaya showed that 4 out of 5 menstrual blood samples on sanitary pads showed positive results for HPV, followed by the same results as cervical swab samples. These results indicate that menstrual blood can be used as material for HPV detection. Contrast to previous studies which detected HPV in menstrual blood in women who had been diagnosed with CIN or HSIL, in this study the samples used were women who had not undergone cytological examination.

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. The variable used is HPV DNA screening with blood menstruation in sanitary napkins with HPV DNA screening versus a swab on the cervix. First variable: results of HPV DNA examination with menstrual blood. Second variable: results of HPV DNA examination with cervical swab. Third variable: Cervical cancer screening results in women with single partners and sexual multipartners. Fourth variable is sensitivity, specificity, positive predictive value and negative predictive value of HPV DNA screening with menstrual blood. The sampling technique was carried out using consecutive sampling, that is, all women who were sexually active in the Pakis Health Center Working Area were selected based on inclusion and exclusion criteria, then 44 samples were taken randomly.

The research sample criteria were women aged ≥18 years, actively having sexual relations 1-3 times a week with 1 single partner or more than 1 partner for women with multiple sexual partners. Experience regular menstruation with a menstrual cycle ranging from 24-35 days and a menstrual period of 3-8 days, have not been diagnosed with cervical cancer with a history of VIA examination, pap smear, or positive HPV DNA, whether they are undergoing treatment or not and have a family history of cancer cervix.

The data used is primary data, namely the results of HPV DNA examination using the PCR method. Each respondent took 2 types of samples. First, taking a cervical swab sample with a sterile cotton swab by the
Pakis Health Center midwife. Swab samples were sent to the Tropical Disease Center (TDC) laboratory for HPV DNA examination using the PCR method. Second, taking menstrual blood samples. Respondents were given menstrual pads to use on the 2nd day of menstruation. The sanitary napkin that has been used for 4-5 hours is removed and placed in a plastic ziplock. Menstrual blood samples were sent to the Tropical Disease Center (TDC) laboratory for HPV DNA examination using the PCR method. HPV DNA examination samples were obtained from pieces of menstrual pads. The SPPS statistical test uses Mann Whitney to determine the differences between the two samples the level of significance in the data obtained is at a significance level of p≤0,05. (13) This research has received ethical approval from the Ethics Committee of Airlangga University Hospital, Surabaya.

RESULTS

Table 1. Receiver Operating Characteristic (ROC) of menstrual blood against cervical swab

<table>
<thead>
<tr>
<th>Variable</th>
<th>AUC</th>
<th>p-value</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menstrual blood</td>
<td>0,9132</td>
<td>0,000</td>
<td>Good accuracy</td>
</tr>
</tbody>
</table>

Table 1 explains that the ROC curve for menstrual blood has an Area Under Curve (AUC) area of 0,9132. The results of the ROC analysis were obtained p-value 0,000 if the p-value is less than 0,05, it can be concluded that the area under the ROC curve is significant and therefore there is evidence that the test carried out has good accuracy.

Table 2. Crosstab results of menstrual blood examination of cervical swab

<table>
<thead>
<tr>
<th>Menstrual blood</th>
<th>Cervical swab</th>
<th>Positive</th>
<th>Negative</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>28</td>
<td>1</td>
<td></td>
<td>29</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>12</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>13</td>
<td></td>
<td>44</td>
</tr>
</tbody>
</table>

Table 2 shows the results of the HPV DNA diagnostic test using menstrual blood against the gold standard (cervical swab). The number of true positives was 28, false positive 1, false negative 3, true negative 12.

Table 3. Results of the menstrual blood HPV DNA screening diagnostic test against cervical swab

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menstrual blood</td>
<td>90,32 %</td>
<td>92,31 %</td>
<td>96,55 %</td>
</tr>
</tbody>
</table>

Table 3 show that HPV screening diagnostic test with menstrual blood against swab cervical sensitivity, specificity, positive predictive values and negative predictive value respectively 90,32 %, 92,31 %, 96,55 % and 80 %.

Table 4. Results of PCR examination of menstrual blood simples

<table>
<thead>
<tr>
<th>PCR results of menstrual blood</th>
<th>Single-partner sexual</th>
<th>Multi-partner sexual</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>10 (47,6 %)</td>
<td>19 (82,6 %)</td>
<td>29 (65,9 %)</td>
</tr>
<tr>
<td>Negative</td>
<td>11 (52,4 %)</td>
<td>4 (17,4 %)</td>
<td>15 (34,1 %)</td>
</tr>
<tr>
<td>Amount</td>
<td>21 (100 %)</td>
<td>23 (100 %)</td>
<td>44 (100 %)</td>
</tr>
</tbody>
</table>

Table 4 shows that 29 samples (65,9 %) of menstrual blood PCR results were positive 15 samples (34,1 %) had negative results and no high-risk HPV infection was found.

Hypothesis testing between the variables menstrual blood, cervical swabs, and number of sexual partners was carried out using comparative test analysis. To determine the comparative analysis used, a normality distribution test, a Shapiro-Wilk test is necessary since the sample size consisted of 44 respondents (≤50).
Table 5 shows that the Shapiro-Wilk test for the menstrual blood variable has a significance level of $p = 0.000$, specifically $p \leq 0.05$, so it can be explained that this variable is not normally distributed. Analysis of differences in menstrual blood samples in sanitary pads in the two groups, namely the group of women with single sexual partners and the group of women with multiple sexual partners, used the Mann-Whitney non-parametric test. The conditions used are that a difference is meaningful if the $p$-value obtained is smaller than the significance level, namely $p \leq 0.05$.

Table 6 shows the significance value of $p = 0.016$, which means $p \leq 0.05$, so it can be explained that $H_0$ is declined, which means there is a difference in the results of HPV DNA screening of menstrual blood in sanitary pads for women with single and multi-partner sexual partners.

Table 7 shows that the PCR results of cervical swabs showed that 31 samples (70.5%) were positive 13 samples (29.5%) had negative results and no high-risk HPV infection was found.

Table 8 shows that the Shapiro-Wilk test for the swab variable has a significance level of $p = 0.000$, namely $p \leq 0.05$, so it can be explained that this variable is not normally distributed. For analysis of differences in cervical swab samples in the two groups, namely the group of women with single sexual partners and the group of women with multiple sexual partners, the non-parametric Mann-Whitney test was used. The conditions used are that a difference is meaningful if the obtained $p$-value is less than or equal to the significance level, specifically $p \leq 0.05$.

Table 9 displays the significance level is $p = 0.013$, indicating that $p \leq 0.05$. Therefore, it can be inferred that the null hypothesis ($H_0$) is rejected, suggesting a distinction in the outcomes of cervical swab HPV DNA screening between women with single and multi-partner sexual partner.

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Table 10. Results of Mann-Whitney analysis of menstrual blood samples and cervical swabs in the single sexual partner group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Significance</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single partner menstrual blood</td>
<td>1,000</td>
<td>H0 accepted</td>
</tr>
</tbody>
</table>

Table 10 presents the significance level as $p = 1,000$, indicating that $p > 0,05$. Therefore, it can be deduced that the null hypothesis (H0) is accepted, implying no distinction in outcomes between HPV DNA screening conducted with menstrual blood in sanitary pads and cervical swabs among women with single sexual partners.

Table 11. Results of Mann-Whitney analysis of menstrual blood samples and cervical swabs in the multi-sexual partner group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Significance</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multi-partner menstrual blood</td>
<td>1,000</td>
<td>H0 accepted</td>
</tr>
</tbody>
</table>

Table 11 demonstrates a significance value of $p = 1,000$, signifying $p > 0,05$. Consequently, it can be inferred that the null hypothesis (H0) is accepted indicating no disparity in outcomes between HPV DNA screening using menstrual blood from sanitary pads and cervical swabs among women with multiple sexual partners. Hypothesis testing between the variables menstrual blood and cervical swab and number of sexual partners was carried out using Spearman correlation test analysis.

**DISCUSSION**

ROC curve of menstrual blood against gold standard HPV DNA examination, namely cervical swab has a larger AUC area of 91,32 % and a significance value of 0,000 ($p < 0,05$). The area under the ROC curve (AUC) is a measure of how well a parameter can differentiate two diagnostic groups (diseased/normal). Based on the results of the ROC curve analysis, it shows that menstrual blood has a high accuracy value for detecting HPV. Research conducted by Zhang et al.\(^{(14)}\) demonstrated that menstrual blood is a feasible and accurate self-sample collection approach for cervical cancer screening with menstrual blood concordance rates and cervical swab is 92,7 %.

Results of HPV screening diagnostic tests using menstrual blood and cervical swabs in the group single-partner and multi-partner sexual sensitivity was found to be 90,32 %. High menstrual blood sensitivity results are possible because menstrual blood collected in sanitary pads can carry HPV in the cervix and vagina. The specificity of menstrual blood was found to be 92,31 %. Systematic reviews by Chakravarti et al.\(^{(10)}\) stated that the diagnostic accuracy of menstrual blood in terms of specificity ranges from 50 % to 98 % for detecting CIN 2+ lesions.

The PPV value for menstrual blood was found to be 96,55 %, meaning that if the results of an HPV DNA examination using a menstrual blood sample are declared positive, then the probability of actually being infected with HPV is 96,55 %. Negative predictive value (NPV) is the proportion of samples whose examination results are negative and who really do not suffer from the disease. The NPV value of menstrual blood is found to be 80 %, meaning that if the results of an HPV DNA examination using a menstrual blood sample are declared negative, then the possibility of actually not being infected with HPV is 80 %.

Previous research by Lee N et al.\(^{(26)}\) obtained sensitivity 87,5 %, specificity 50 %, PPV 61,5 % and NPV 83,8 % of menstrual blood tests for detecting CIN 3 or worse.\(^{(15)}\) Similar research by Wong JPH et al.\(^{(16)}\) obtained sensitivity and NPV values of 83 % and 74 % for detecting CIN or HPV infection.\(^{(15)}\) Contrast to the previous studies which detected HPV in women who had been diagnosed with CIN or HSIL, in this study the samples used were women who had not had a cytology examination. The review supports the use of menstrual blood as a screening tool for cervical cancer especially in developing countries where women are reluctant to participate in cervical cancer screening due to issues such as embarrassment and discomfort.

The results of the PCR examination of menstrual blood samples showed that 29 samples (65,9 %) were positive for high-risk HPV infection, with 19 samples from the multipartner sexual group. The results of this study are in line with research conducted by Wong et al.\(^{(15)}\) HPV DNA was detected in 83 % of women diagnosed with CIN and 4 % of normal sexually active women. The sample size of single sexual partner women in the study was not large enough, because only 4 % of single partner women with normal cytology findings had HPV DNA in menstrual blood.\(^{(8)}\) The results of the PCR examination of cervical swabs showed that 31 samples (70,5 %) were positive for high-risk HPV infection with 20 samples of them from the multipartner sexual group. The results of this study are in line with research conducted by Erawati et al.\(^{(16)}\) which showed that female prostitutes in Kediri City had a positive HPV prevalence of 68,1 %.

The observed discrepancy in HPV DNA screening results from menstrual blood collected in sanitary pads between the two groups ($p=0,016$) indicates a notable distinction between women with single and multiple sexual partners.
sexual partners.\(^{(17)}\) revealed a heightened prevalence of HPV infection, particularly with high-risk types such as HPV 16, 52, and 53, among female commercial sex workers. These workers face significant susceptibility to cervical and vaginal cancer development and may transmit infections to their clients, potentially contributing to a higher prevalence of HPV and HPV-related malignancies in the general population.\(^{(18)}\) Budukh et al.\(^{(20)}\) conducted a study demonstrating the viability of menstrual devices as an HPV testing tool, demonstrating a sensitivity of 83.3 % and a specificity of 95.1 % among patients diagnosed with CIN.\(^{(19)}\) The use of menstrual pads as a cervical cancer screening tool can provide comfort and convenience for participants and also reduce the costs of organizing screening clinics.\(^{(20)}\) Supported by other research by Wong et al.\(^{(16)}\) which states that menstrual blood has a sensitivity of 83 %, specificity of 98 %, positive predictive value of 99 %, and negative predictive value of 74 %.\(^{(15)}\)

The difference in the results of cervical swab HPV DNA screening in the 2 groups (p=0.013) shows that there is a significant difference between the group of women with single partners and multi-partner sexual partners. Multiple sexual partners have been reported as a risk factor for cervical cancer.\(^{(21)}\) Stated that the multipartner sexual group had a higher percentage of cervical conization pathology, especially CIN 2 and CIN 3. This is because the consequence of multi-partner sexualism is an imbalance in vaginal microecology, which theoretically directly causes Bacterial Vaginosis (BV). Dysregulated vaginal microbiota can increase the risk of transient and persistent HPV infections so BV and HPV together can increase the risk of CIN.\(^{(22)}\)

Analysis of the results of HPV DNA screening from menstrual blood in sanitary pads and cervical swabs (p=1,000) showed that there was no significant difference between the results of screening and menstrual blood in sanitary pads and cervical swabs in women with single sexual partners. The results of this study are consistent with the results of research conducted by Wong SCC et al.\(^{(23)}\) where the results of the study stated that screening for high-risk HPV using self-collected menstrual blood demonstrated a strong correlation (94 %) with traditional high-risk HPV testing, as reported by Farahmand M et al.\(^{(15)}\) with 98 % of the study participants confirming this correlation, stated that the advantages of using menstrual blood samples to detect HPV are that it is painless, not embarrassing and saves time. In addition, menstrual blood samples have the absolute advantage that they can be collected monthly in women before menopause. In a comparable investigation conducted by Naseri et al.\(^{(22)}\) it was reported that 94 % of respondents favored using menstrual pads over doctor-performed sampling if provided as an option for high-risk HPV screening.

Analysis of the results of HPV DNA screening from menstrual blood in sanitary pads and cervical swabs (p=1,000) showed that there was no significant difference between screening results and menstrual blood in sanitary pads and cervical swabs in women with multiple sexual partners. The results of this study are consistent with research by Lee N et al.\(^{(26)}\) which states that HPV testing with menstrual blood provides a new screening modality that can significantly increase accessibility for cervical cancer screening.\(^{(25)}\)

There are differences in PCR results for samples coded A1, B1, A28, B28 and A37, B37. The menstrual blood sample showed negative results while the cervical swab showed positive results. This is according to the findings of Lee N et al.\(^{(26)}\) 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.\(^{(27)}\) Zhang J et al.\(^{(14)}\) expressed a different opinion, namely that there was no statistically significant difference in the level of HR-HPV-positive menstrual blood between patients who provided sanitary pads for several days of menstruation and patients who provided only one sanitary pad.\(^{(28)}\) The HR-HPV positive rate of menstrual blood on different days of menstruation, from the first day of menstruation to the 5th day of menstruation was equal too.\(^{(29)}\)

Other differences in results were found in samples A33 and B33. The menstrual blood sample showed positive results while the cervical swab showed negative results. Eight instances were identified where modified menstrual pads detected high-risk HPV that went undetected by both self-collected vaginal swabs and samples collected by clinicians. This occurrence is feasible because high-risk HPV may reside beyond the cervix, inaccessible to both clinician-collected samples and self-taken vaginal swabs. However, it can be accessed through menstrual blood, which flows from the endometrium, cervix, and vagina.\(^{(31)}\)

CONCLUSIONS

The conclusion from this analysis shows that there is concordance between HPV DNA screening with menstrual blood in sanitary pads and cervical swabs with high accuracy values.

The limitations of the research in the article “Comparison of HPV DNA Screening using menstrual blood collected in sanitary pads versus cervical swabs in women with single and multiple sexual partners include:

1) Sample Size: the study had a relatively small sample size, which may limit the generalizability of the findings to a larger population.

2) Sampling Method: the use of consecutive sampling may introduce selection bias and limit the representativeness of the sample.

3) Distribution of Participants: the distribution of participants in terms of demographics, sexual

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behavior, and other relevant factors may not have been balanced between the groups, potentially impacting the results.

4) Non-Normal Distribution: the menstrual blood variable was found to not be normally distributed, which could affect the interpretation of the results.

5) Specific Population: the study focused on women in a specific geographic location and number of sexual partners, which may limit the generalizability of the findings to other populations.

6) Limited Scope: the study may not have considered all possible confounding variables or factors that could influence the results of HPV DNA screening using menstrual blood.

BIBLIOGRAPHIC REFERENCES


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Formal analysis: Nurul Avifah Rahman.
Acquisition of funds: Nurul Avifah Rahman.
Research: Nurul Avifah Rahman.
Methodology: Nurul Avifah Rahman, Pungky Mulawardhana, Puspa Wardhani.
Project management: Nurul Avifah Rahman, Pungky Mulawardhana, Puspa Wardhani.
Resources: Nurul Avifah Rahman, Pungky Mulawardhana, Puspa Wardhani.
Software: Nurul Avifah Rahman.
Supervision: Pungky Mulawardhana, Puspa Wardhani.
Validation: Pungky Mulawardhana, Puspa Wardhani.
Display: Pungky Mulawardhana, Puspa Wardhani.
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Writing - proofreading and editing: Nurul Avifah Rahman, Pungky Mulawardhana.