Research Article

The Accuration of Liquid-Based Cytology and HPV DNA Test Combination as Precervical Cancer Lesion Screening

Akurasi Pemeriksaan Kombinasi Liquid-Based Cytology dan Tes DNA HPV sebagai Penapis Lesi Prakanker Serviks

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Abstract

Objective: To investigate the accuracy of liquid-based cytology, HPV DNA test, and the combination of liquid-based cytology and HPV DNA test, compared to histopathology as the gold standard of precervical cancer lesion screening.

Methods: This was a cross-sectional study. The medical records of patients who came to the Women's Health Clinic of Dr. Cipto Mangunkusumo Hospital during the period of July 2013 to December 2015 were evaluated.

Results: The high risk type HPV DNA is detected in 76% CIN 1, 88.46% CIN 2, and 84.21 CIN 3 in histopathology results. The accuracy of liquid-based cytology; sensitivity 88.54%, specificity 35.71%, PPV 75.89%, and NPV 57.69%. The accuracy of HPV DNA; sensitivity 81.25%, specificity 78.57%, PPV 89.66%, and NPV 64.71%. The accuracy of combination: sensitivity 94.79%, specificity 35.71%, PPV 77.12%, and NPV 75%.

Conclusion: The addition of HPV DNA test increased the sensitivity from 88.54% to 94.79% because of decreasing of false negative of liquid-based cytology. This thing has showed that the combination of liquid-based cytology and HPV DNA test could the one of the option of precervical cancer lesion screening method, especially in secondary or tertier health center in Indonesia.

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Keywords: accuracy test, HPV DNA, liquid-based cytology, precervical cancer lesion, precervical cancer lesion screening

Abstrak

***Tujuan**: Diketahuinya angka akurasi liquid-based cytology, DNA HPV, dan kombinasi keduanya dibandingkan dengan hasil histopatologi.*

***Metode**: Penelitian ini merupakan penelitian potong lintang dengan jumlah sampel 138 subjek pada Juli 2013 - Desember 2015 di RS Dr. Cipto Mangunkusumo Kencana.*

***Hasil**: DNA HPV tipe risiko tinggi terdapat pada 76% NIS 1, 88,46% NIS 2, dan 84,21% NIS 3 pada hasil histopatologi. Didapatkan akurasi pemeriksaan liquid-based cytology; sensitivitas 88,54%, spesifisitas 35,71%, NPP 75,89%, dan NPN 57,69%. Akurasi pemeriksaan DNA HPV; sensitivitas 81,25%, spesifisitas 78,57%, NPP 89,66%, dan NPN 64,71%. Sementara akurasi kombinasi keduanya adalah sensitivitas 94,79%, spesifisitas 35,71%, NPP 77,12%, dan NPN 75%.*

***Kesimpulan**: Penambahan pemeriksaan DNA HPV meningkatkan angka sensitivitas dari 88,54% menjadi 94,79% karena turunnya angka negatif palsu pemeriksaan LBC. Hal ini menjadikan kombinasi pemeriksaan liquid-based cytology dan DNA HPV dapat menjadi pilihan metode penapisan lesi pra-kanker serviks terutama pada fasilitas kesehatan sekunder ataupun tersier di Indonesia.*

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***Kata kunci**: DNA HPV, lesi prakanker serviks, liquid-based cytology, penapisan lesi prakanker serviks, uji akurasi*

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INTRODUCTION

Cervical cancer is the third most female malignancy in the world and the second most in Indonesia; The Globocan Project in 2012 stated that the incidence of cervical cancer was 20928 cases and mortality rate was 9498 cases annually.[1,2] Data from several hospitals in Jakarta reported the 5-year survival rate of cervical cancer stage I, II, II, and IV were 50%, 40%, 20%, and 0% respectively.[3] Both the incidence and mortality rate of cervical cancer were well-correlated with cervical cancer prevention program; particularly the precervical cancer lesion triage program such as visual inspection of acetic acid, cytology-based screening, colposcopy, and optoelectric.[4] The high incidence of cervical cancer in several developing countries, especially in Indonesia, was the result of inadequacy of screening programs in detecting the cervical cancer in its initial process, precervical cancer lesion. On the contrary, cytology-based screening have already been well established and organized to be a routine screening program of

precervical cancer lesion screening program in some developed countries. Cytology-based screening has already been modified to liquid-based cytology in which aimed to lower the un-satisfactory result and improve the sensitivity.[7] Previous studies showed that the sensitivity of liquid-based cytology varied from 76.2% to 96.24%.[5,6]

The HPV DNA test has a high sensitivity as a precervical cancer screening tool, especially in the above 30-year old female population, there of in several developed countries the HPV DNA test is used as a triage screening tools.[7,8] Some studies reported that HPV DNA test sensitivity was ranged 94.7% to 97.4%.[7,9] The additional HPV DNA test was alleged to be the co-testing which could be combined to the cytology screening in order to increase the sensitivity of precervical cancer screening program on account of its high sensitivity. The co-testing of liquid-based cytology and HPV DNA test intended to increase the sensitivity and lower the false negative rate in cytology-based screening tool. Furthermore, not with standing the high cost of both liquid-based cytology and HPV DNA test, this co-testing was allegedly reported to increase the sensitivity which crucial and important parameter for screening tool. To date, there were no data and report corresponded to this co-testing of liquid-based cytology and HPV DNA test in Indonesia. This study is expected to be the reference for some options in precervical cancer screening tool, especially in secondary or tertiary health care center.

METHODS

This cross-sectional study was carried out at the Women's Health Center Dr. Cipto Mangunkusumo Hospital, Jakarta from July 2013 to December 2015. We collected data from medical records of patients who went to the Women's Health clinic of Dr. Cipto Mangunkusumo Hospital from other health center referral or those who came by her own will to have a precervical cancer lesion screening program. The inclusion criteria were 20 - 65 years old and those who were sexually active. These patients were offered the co-testing of liquid-based cytology and HPV DNA test, which continued with colpos-copy. If the result of colposcopy was normal, they would not undergo biopsy (LEEP or LLETZ); meanwhile those with abnormal colposcopy results would undergo biopsy (LEEP or LLETZ). The cervical cancer result of histopathology was excluded in this study.

The data were run into sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) analysis for each examination consisting of liquid-based cytology, HPV DNA test, and histopathology. This analysis was performed through SPSS24.0 for Windows©.

RESULTS

There were 138 subjects recruiting to this study. All of subjects were ranged from 22 - 65 years old with mean age was 41.96 years old. Of these 138 subjects, 55 patients (39.9%) were parity of 3.

Table 1 pointed out that the results of liquid-based cytology of LSIL is at the most proportion sample with 33.3% and ASCH is the least proportion in this study with 3.6%. Aside from that, high-risk type HPV DNA was detected in 63% samples where it consisted of single DNA was detected in 34.1%, combination of high risk and low risk type with 1.4%, and the combination of high risk type with 27.5%; in which these high risk type HPV DNA, type 16 was the most proportion with 28.1%, followed by type 18 and type 52 (23.97% and 17.36 respectively).

Table 1. Characteristic of Liquid-Based Cytology, HPV DNA Test, and Histopathology Result

Examination	N (%)
Liquid-Based Cytology	
Negative	26 (18.8)
ASCUS	29 (21)
LSIL	46 (33.3)
HSIL	32 (23.2)
ASCH	5 (3.6)
HPV DNA Test	
Negative / Not Detected	44 (31.9)
Low Risk Type	7 (5.1)
High Risk Type	
Single	47 (34.1)
Combination of High Risk - Low risk	2 (1.4)
Combination of High Risk	38 (27.5)
Histopathology	
CIN1	50 (36.2)
CIN2	26 (18.8)

CIN3	19 (13.8)
Non CIN	43 (31.2)
Total	138 (100)

Of 50 samples of CIN 1, high risk HPV DNA was detected in 38 samples (76%); meanwhile of 26 samples of CIN 2, high risk HPV DNA was positive in 88.46%; and of 19 samples of CIN 3, there was 84.21% high risk HPV DNA (Table 2). Furthermore, high risk HPV DNA were detected in 62.07% of ASCUS, 67.4% of LSIL, 84.38% of HSIL, and100% of ASCH.

Table 2. Charateristic of HPV DNA Test and Histopathology

		DNA HPV			
		Not Detected	Low Risk	High Risk	Total
Histopathology	CIN 1	8	4	38	50
	CIN 2	3	-	23	26
	CIN 3	1	2	16	19
	Non CIN	32	1	10	43
Total		44	7	87	138

The accuracy and screening parameter of liquid-based cytology, HPV DNA test, and co-testing of liquid-based cytology and HPV DNA were analyzed by 2 x 2 table and resulted the accuracy parameter: sensitivity, specificity, positive predictive value, and negative predictive value. This study has results of sensitivity of liquid-based cytology, HPV DNA, and co-testing of liquid-based cytology and HPV DNA as 88.54%, 81.25%, and 94.79% respectively; and positive predictive value as 75.89%, 89.66%, and 77.12% respectively. On the other hand, there were poor specificity results (35.71%) in both liquid-based cytology and co-testing (Table 3).

DISCUSSION

From the diagnostic value we obtained, we concluded that the highest sensitivity is the co-testing of liquid-based cytology and HPV DNA (94.79%); compared to single screening method of liquid-based cytology (88.54%) and HPV DNA (81.25%); even though the sensitivity of 88.54% is decent as a precervical cancer screening tool. This sensitivity of liquid-based cytology was in accordance with other study conducted by Oh et al in 2002 and Beerman et al in 2009 which stated that the sensitivity of liquid-based cytology was 92% and 96.24%, respectively.[5,10] There was a wide range sensitivity of cytology-based screening as this procedure was operator-dependent, cytologist and pathologist. In this study, the operator bias was minimalized as the cytology sample was collected by the same experienced doctor. The poor specificity in liquid-based cytology (35.71%) is not consistent compared to the previous studies because the high false positive rate in this study which might be caused by error in interpreting the cytology result and the small proportion of non CIN result compared to CIN result. Nevertheless, this result not correlated to the purpose of this study which aims for screening the precervical cancer lesion as the specificity corresponds to the

Table 3. Accuracy of Liquid-Based Cytology, HPV DNA, and Co-Testing as Screening Tool

Diagnostic Parameter	Pre Cervical Cancer Lesion Screening		
	LBC	HPV DNA	LBC and HPV DNA
Sensitivity	88.54% (95% CI 80.42 - 94.14 %)	81.25% (95% CI 72 - 88.49 %)	94.79% (95% CI 88.26 - 98.29%)
Specificity	35.71% (95% CI 21.55 - 51.97%)	78.57% (95% CI 63.19 - 89.7%)	35.71% (95% CI 21.55 - 51.97%)
Positive Predictive Value	75.89% (95% CI 71.3 - 79.95%)	89.66% (95% CI 82.81 - 93.97%)	77.12% (95% CI 72.81 - 80.93%)
Negative Predictive Value	57.69% (95% CI 40.66 - 73.08%)	64.71% (95% CI 54.01 - 74.11%)	75% (95% CI 53.83 - 88.53%)
Positive Likelihood Ratio	1.38 (95% CI 1.09 - 1.75)	3.79 (95% CI 2.11 - 6.82)	1.47 (95% CI 1.17 - 1.86)
Negative Likelihood Ratio	0.32 (95% CI 0.16 - 0.64)	0.24 (95% CI 0.15 - 0.37)	0.15 (95% CI 0.06 - 0.38)

diagnostic purpose while sensitivity is the important value for the screening program to encompass as much as possible of the positive precervical cancer lesion patient.

In the HPV DNA, there was sensitivity of 81.25% and specificity of 78.57%. The sensitivity was slightly below the previous study of HPV DNA which the sensitivity of HPV DNA ranged 86 - 97.4%.[7,11-13] The discrepancy of sensitivity in this study might be caused by the proportion of Non NIS and NIS 1 is 67.4% while the NIS 2+ is 32.6%; this situation could conduce the lower sensitivity since HPV DNA reached the good sensitivity in NIS 2+ as the cut-off point.[7,8,14] Moreover, there was 7.9% samples with age below the 30 years old, where the HPV DNA is not recommended to be performed to women < 30 years old reckoned the high infection and regression rate in that population. In the perspective of liquid-based cytology, there were negative, ASCUS and LSIL result (72.14%) compared to 27.86% of HSIL; while Wheeler et al in 2014 reported the HPV DNA type 16 and 18 was detected respectively in 3.6% and 1.5% of negative result, 23.3% and 1.7% of ASCUS result, 32.5% and 19.6% of LSIL result; compared to 57.5% and 62.2% of ASCH, and 71.6% and 58.7% of result.[15] Furthermore, the low sensitivity of HPV DNA could be caused by high false negative which might be caused by several factors, for instance the detection assay which not covers some type of HPV DNA, low titer or copy in HPV, inadequacy of specimen including the DNA quality, cytology sample with low abnormal cell, and error of pathologist.[16]

The co-testing of these two screening methods increased the sensitivity as much as 6.25% and 13.54% of liquid-based cytology and HPV DNA, respectively. The increased sensitivity was also accompanied with decreased false negativity of these two screening methods. In the clinical practice, these parameters will implicate the longer period for the next follow-up screening. Low specificity in liquid-based cytology and co-testing correspond to the high false positive which might be caused by error in cytology result. One of the drawbacks of this study is the absence of notation of other risk factor in medical record to exclude the situation which might influence the false negative and false positive; and also other demographic factors.

CONCLUSIONS

This co-testing test improves the sensitivity as well as negative predictive value, and positive predictive value of liquid-based cytology and HPV DNA. This result implies that this co-testing test could be one of the options of precervical cancer lesion screening in secondary or tertiary health center in Indonesia reckoned of its high cost, approximately Rp 1.200.000,-. Further studies regarding the cost effectiveness in this co-testing test since, despite of its high cost, it will implicate the longer period for the next follow-up screening until 3 - 5 years follow up.

CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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