Pap Smear Accuracy in Detecting CIN I HPV

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ABSTRAK

Kanker serviks adalah penyakit ganas serviks uterus. Pap smear adalah suatu metode yang sederhana, murah dan tidak traumatik, dan dapat digunakan sebagai metode skrining di negara berkembang. Kelemahannya adalah bahwa ia memiliki sensitivitas rendah dan tidak dapat membedakan jenis HPV. Sebagai alternatif, telah dikembangkan pemeriksaan menggunakan capture hybrid 2. Tujuan dari penelitian ini adalah untuk menilai akurasi diagnostik deteksi pengujian HPV dengan metode Pap smear (tes Papanicolaou) dibandingkan dengan metode hybrid capture 2 pada Metode CIN I.: Penelitian ini menggunakan desain penelitian cross-sectional dilakukan di Klinik Rawat Jalan Onkologi RSUD Dr Soetomo. Pasien dengan CIN I di klinik onkologi Dr Soetomo diperiksa sediaan koilositosishistopathologik yang patognomonis untuk infeksi HPV pada sel leher rahim. Pada pasien dengan CIN I juga diperiksa hybrid capture 2 untuk mendeteksi HPV DNA. Hasilnya kemudian dibandingkan dan dianalisis untuk menilai akurasi statistik deteksi HPV dengan pap a. Pap smear sitologi CIN I menemukan 17 (47,2%) terinfeksi dengan HPV positif dan 19 (52,8%) dengan hasil negatif. Sedangkan metode pemeriksaan infeksi dengan menangkap hybrid 2 diperoleh 12 (33,3%) terinfeksi dengan HPV positif dan 24 (66,7%) menunjukkan hasil negatif. Perbandingan hasil infeksi HPV antara metode sitologi dengan menangkap hybrid 2 menunjukkan perbedaan yang signifikan (p = 0,001). Bila dibandingkan dengan pemeriksaan hybrid capture 2 pengujian HPV dengan metode Pap smear memiliki sensitivitas 91,7%, spesifisitas 75%, 64,7% nilai prediksi positif dan nilai prediksi negatif 94,7%. Sebagai kesimpulan, Pap smear memiliki kekhususan yang cukup tinggi dan akurasi yang lebih baik jika dibandingkan dengan HC-2 ***(MOG 2012;20:18-22)***

Kata kunci: *HPV, pap smear, hybrid capture 2*

ABSTRACT

Cervical cancer is a malignant disease of the cervix. Pap smear is a simple, cheap and not traumatic, and can be used as a screening method in developing countries. The weakness is that it has low sensitivity and is unable to distinguish the types of HPV. As an alternative, examination using hybrid capture 2 has been developed. The objective of this study was to assess the diagnostic accuracy of detection of HPV testing with Pap smear method (Papanicolaou test) compared to the method of hybrid capture 2 in CIN I. Methods: This study uses cross sectiona design research conducted at the Oncology Outgoing Clinic of RSUD Dr Soetomo. Patients with CIN I in Oncology Outgoing Clinic Dr. Soetomo hospital have been reviewed in preparations to re-look at the preparations koilositosishistopathologic picture which is patognomonis for HPV infection in cervical cells. In patients with CIN I were also examined hybrid capture 2 for detection of HPV DNA. The results were then compared and analyzed to assess the statistical accuracy of detection of HPV with a pap. Pap smear cytology CIN I found 17 (47.2%) infected with HPV positive and 19 (52.8%) with negative results. While the examination method of infection with the hybrid capture 2 obtained 12 (33.3%) infected with HPV positive and 24 (66.7%) showed negative results. Comparison of the results of HPV infection among cytology method with hybrid capture 2 shows a significant difference (p = 0.001). When compared with the examination of the hybrid capture 2 HPV testing with Pap smear method has a sensitivity of 91.7%, specificity 75%, 64.7% positive predictive value and negative predictive value 94.7%. In conclusion, HPV with a pap smear has a high enough specificity and better accuracy compared to HC-2. ***(MOG 2012;20:18-22).***

Keywords: *HPV, pap smear, hybrid capture 2.*

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INTRODUCTION

Cervical cancer is a malignant disease of the cervix of the uterus that can be derived from epithelial cells, fibroblasts, blood vessels and lymph either stand alone or mixed. This disease is a problem because of the incidence and death is likely to increase, especially the developing country. Cervical cancer incidence data from the Ministry of Health of the Republic of Indonesia is 60 per 100,000 individuals per year, while cervical cancer was ranked first with 26.2%. National incidence of cervical cancer most numerous on the island of Java, which is 89.48%. Cervical cancer is 65 to 77.7% of all gynecologic cancers. CIN is a picture of pre-cancerous changes and abnormal development (dysplasia) that occur in cervical epithelial lesions and were divided according to the degree of CIN I, II and III. Precancerous lesions/CIN is largely due to HPV infection, especially high-risk groups (16 and 18), but can also be caused by other HPV types that are not

potentially cause cervical cancer and some other sexually transmitted infections.

Diagnosis of CIN and HPV infection picture can be enforced by way of cytology or histopathology and molecular biology. In cytology of infection is made by a pap smear test is based on the discovery koilositosis, diskeratosis, parakeratosis, and abnormal basal cell condilomatosa. Advantages of this way is simple, cheap and not traumatic, and can be used as a screening method in developing countries, the weakness is the low sensitivity and is unable to distinguish types of HPV. HPV DNA testing can also be performed as a primary screening test, additional tests cytology, as a test of discrimination on the pap smear test results are questionable, and for evaluation of treatment outcomes in determining the prognosis of a dysplasia and dysplasia. (13) a very vital role of DNA tests HPV encouraged researchers to conduct research about comparative accuracy cytologic examination with examination of high risk HPV DNA in a low-grade precancerous lesions (CIN I)

MATERIAL AND METHODS

We conduct in oncology Outgoing Clinic of RSUD Dr. Soetomo for sampling pap smear and hybrid capture 2, Pathology Anatomy Laboratory of RSUD Dr. Soetomo for Pap Smear examination. Prodia Jakarta laboratory for examination hybrid capture 2. This study was conducted from May 2011. In this study the population are patients with CIN I was in Oncology Outgoing Clinic of Dr. Soetomo Hospital. Patients with CIN I in oncology outgoing clinic RSUD Dr. Soetomo was reviewed in preparation to re-look for koilositosis histopathologic picture which is patognomonis for HPV infection in cervical cells. In patients with CIN I we also examined hybrid capture 2 for detection of HPV DNA, which use the liquid hybridization technique for the detection of HPV DNA using 2 RNA probes. Probe B detects HPV types associated with cancer, including HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and probe A detects low risk HPV (HPV 6, 11, 42, 43, and 44). The results were then compared and analyzed statistics. Prior to sampling in patients with CIN I, we give information about the study to the patient (informed consent), then continued with the patient's signed declaration sheets untill they have understood and agree to participate in this study (informed consent). Patients will be kept confidential by giving the number and identity of the patient's initials instead. Statistical analysis using 2x2 tables to calculate sensitivity, specificity, positive predictive value and negative predictive value.

RESULT AND DISCUSSION

The research was conducted at the Oncology Outgoing Clinic of RSUD Dr. Soetomo Surabaya from December 2011 to March 2012. Sample obtained from a pap smear in the preparation of oncology outgoing clinic RSUD Dr. Soetomo Surabaya patients who met the study criteria. Subsequently conducted a search data report with the results of Pap smear of CIN I in Anatomical Pathology Laboratory. This preparation then performed re-examination of HPV infection by cytology and hybrid capture 2.

Sampling method in this study did not use sampling methods based on chance, but with the closest probability sampling with consecutive sampling which therefore the purpose of this study was to compare the presence of HPV infection by cytology and hybrid capture 2. The significance level used in this study is to say 0.05 means significant difference when the value of $p < 0.05$ and otherwise the difference is said to be meaningful if the value of $p = 0.05$

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Further cytologic examination to look for koilositosis as a patognomonis for HPV infection. While the examination of preparation using hybrid capture 2. The process begins with sampling, preparation of reagents, preparation of software, denaturation of DNA, preparation of control and calibrator hybrid capture 2, the probe solution preparation, hybridization, hybrid capture, detection of DNA-RNA hybrid using signal amplification (detection reagent one), washing, signal amplification detection using chemiluminescent sub-strate. The principle of hybrid capture 2 is performing DNA hybridization, the viral DNA will be bound by the probe, forming a bond with a DNA virus which is an RNA probe. Bond formed is called hybrid DNA: RNA. Hybrid DNA: RNA would be bound by specific antibody in the microplate wells. Association of antibodies to the hybrid DNA: RNA will react with alkaline phosphatase. It is detected by a chemiluminescent reaction that will result in signal amplification in the form of light emission. Light emission was measured by the luminometer produces the RLU (Relative Light Units). RLU value is what will determine whether or not patients were infected by HPV. Pap smear cytology CIN I found 17 (47.2%) infected with HPV positive and 19 (52.8%) with negative results (Fig. 1).

Comparison of the results of HPV infection in patients with CIN I cytology and hybrid capture method 2 obtained the same 11 samples showed the same positive results and 18 samples both showed negative results (Table 1) and obtained a positive result with the method of hybrid capture 2 but with negative cytology method.[6] In contrast obtained positive results with the method of

cytology but negative when examined by the method of hybrid capture 2. Comparison of the results of HPV infection among cytology method with hybrid capture 2 shows a significant difference (p = 0.001).

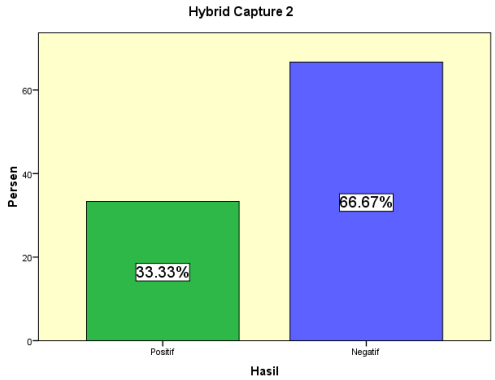

Figure 1. The result of hybrid capture 2 examination.

Table 1. Comparative examination of HPV infection between hybrid capture 2 method with pap smear.

Hybrid Capture 2	Cytology		Total
	Positive	Negative	
Positive	11	6	17
Negative	1	18	19
Total	12	24	36

When compared with the examination of the hybrid capture 2 HPV testing with Pap smear method has a sensitivity of 91.7%, specificity 75%, 64.7% positive predictive value and negative predictive value 94.7%.

This study aims to determine the ability of the hybrid capture system 2 to detect HPV in the sample. The working principle of hybrid capture 2 antibody was hybridized using chemiluminescent detection. Hybrid-ization between DNA viruses with RNA probes produced DNA-RNA hybrid is captured by the antibody in the microplate wells, which then reacts with a second antibody conjugated with alkaline phosphate that. Antibodies to these two acts as a signal amplification; more and more hybrid DNA-RNA that was caught on the wall plate capture, the more antibodies that can recognize both DNA-RNA hybrid.

Quantity of antibodies that bound to the DNA-RNA hybrid is measured by adding a chemiluminescent agent, or 1.2-dioxetan. The intensity of the emitted light indicates the presence or absence of target DNA in the sample. The light comes from dioxetan which has a short half-life and has an oxidation reaction of unstable intermediates. Alkaline phosphatase substrate dephos-phorilated adamantil-1,2-phosphate hydrolytic dioxetan form a metastable anion. This makes the nature of the metastable anion will fragmented to form methyl adamantanone and anion-moksibenzoat. Metal-anion excited moksibenzoat will emit light with a wavelength of 447 nm. Light resulting from the termination reaction by alkaline phosphatase chemiluminescent substrate is then detected by a luminometer and interpreted in units of RLU by the luminometer which is proportional to 1 pglmL positive control DNA of HPV types 16 and 5000 HPV genome. Determination of HPV DNA testing positive value based on comparison of samples with an average tripilicated RLU positive control (RLU/PC). If the ratio of RLU/PC (relative light unit/positive control) exceeds the threshold value is positive then the specimens tested positive for HPV DNA.

This study shows that an average of 40.25% (33.3% with the method of hybrid capture 2 and 47.2% with cytology method) contained HPV infection in patients with CIN I. An epidemiological study also showed a similar result that is 20 to 50% found HPV infection in patients with CIN I. (55) Other research conducted at the University of Zaragoza showed even greater results that found 66% or 80 of 120 patients with CIN I HPV infection.

This study showed that HPV testing with Pap smear method has a sensitivity of 91.7.7%, specificity 75%, 64.7% positive predictive value and negative predictive value 94.7%. Accuracy of the test or tests based on the sensitivity and specificity. Mean value of the sensitivity of a test ensures that the positive value is correct which produced positively to the opportunities that a small false negative value while the value of the specificity of a test is to ensure that the resulting negative value is a negative right to the opportunities that a small false positive values. False positive value means a positive HPV DNA test but after through other testing reveals no presence of HPV infection.

Determination of threshold concentrations of HPV DNA that have chance for making formation of cervical cancer is very important. Digene set a positive threshold value of 1.0 RLU/PC. Pap smear sensitivity and specificity are vary in detecting HPV. Study on 1200 women who underwent screening with the Pap smear and confirmed by colposcopy and biopsy showed a sensitivity of Pap smear 72%, while specificity rate was 90.2%. Other research in Cameroon on 4813 women who underwent screening with the Pap smear method showed sensitivity of 47.7% with a pap smear specific-ity of 94.2% and negative predictive values obtained (NPV/Negative Predictive Value) for pap smear was 87.8%. (59)

These studies showed that the detection of HPV with a pap smear still shows the variation of the diagnostic accuracy of 47.7% with a sensitivity range up to 72% and a specificity of 90.2% to 94.2%. In contrast studies of HPV detection by hybrid capture 2 method gives more consistent results than the method of pap smears. Research in Dublin Ireland in 299 subjects acquired HPV detection diagnostic test results with the hybrid capture 2, ie, sensitivity 83.7%, specificity 91.7%, and positive predictive value (PPV/Positive Predictive Value) 97.6%. A study meta-analysis of 11 cross-sectional study (cross-sectional studies) conducted in India and several countries in Africa that compared use of the method of pap smear and hybrid capture 2 for more than 58 679 women aged 25-64 years which showed that the sensitivity and specificity of pap smear 57% and 93% while the sensitivity and specificity of hybrid capture 2 62% and 94%.

CONCLUSION

HPV with a pap smear has a high specificity and better accuracy compared to HC-2.

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