

Comparison of HPV Detection Using HC-II Method With PAP Smear Screening in Commercial Sex Workers in Kediri

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COMPARISON OF HPV DETECTION USING HC-II METHOD WITH PAP SMEAR SCREENING IN COMMERCIAL SEX WORKERS IN KEDIRI

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ABSTRACT

Female commercial sex workers are females that have multiple sexual partners and have high risk due to exposure to blood, semen, and vaginal discharge contaminated with microorganisms causing sexually transmitted disease such as infection caused by Human papillomavirus (HPV). This behavior creates a high susceptibility for commercial sex workers in obtaining HPV, which is the leading cause of cervical cancer. Cervical cancer is the most common cancer in females in Indonesia, which is why screening, especially for females with a high risk such as commercial sex workers, must be done. The purpose of this experiment was to compare the detection methods of HPV using Hybrid capture-II (HC-II) in order to find out high risks HPV types (type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) by Pap smear done in commercial sex workers in Campurejo Kediri Public Health Center. This study was a descriptive observational experiment with a cross-sectional method. The samples of this experiment were 47 female commercial sex workers, whose detection of HPV using HC-II method was done at the Clinical Pathology Laboratory of the Dr. Soetomo Hospital Surabaya, where 32 samples showed positive results (68.1%) and were infected with high-risk HPV and 15 negative results (31.9%), from the Pap smear three samples (6.4%) showed dysplasia (Cervical Intraepithelial Neoplasia/CIN 1) and 44 samples (93.6%) showed normal smears with inflammation or infection in the cervix. Statistically showed a significant difference between the results of HC-II and Papsmear ($p=0.000$).

Key words: Commercial sex worker. HPV, hybrid capture-II, Pap smear

INTRODUCTION

Female commercial sex workers are females who sells sexual activity for the purpose of getting a reward for their services.¹ Commercial sex workers change partners and have a high-risk sexual behavior, which causes a person to be exposed to blood, semen, vaginal discharge that have been contaminated with microorganisms causing Sexually Transmitted Disease (STD) such as bacteria, fungi, parasites, and virus such as Human Papilloma Virus (HPV). Commercial sex workers have higher risks to be infected with genital HPV.²

Human papillomavirus is a virus that is transmitted sexually³ and classified as a Papovirus, a DNA virus shaped icosahedral, with the size of 50 – 55 nm, 72 capsomers and 2-protein capsid.⁴ Two hundred types of HPV identified are classified into two types, HPV with a high-risk and HPV with a low-risks. High-risk HPV consists of 13 types, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.⁵ High-risks HPV has been prov-

en to cause cervical cancer and is associated with other epithelial carcinomas, such as rectal, vulva, vagina, penis, mouth, and throat.⁶

High-risk HPV can be found in 99.7% of invasive cervical cancer, 70% in precursor lesions of Cervical Intraepithelial Neoplasia/CIN 1 or Low-Grade Squamous Intraepithelial Lesions (LSIL), 80% in precursor lesion CIN2 or High-Grade Squamous Intraepithelial Lesion (HSIL) and 96% in precursor lesions CIN3 based on Polymerase Chain Reaction (PCR) results.⁷ Infections of multiple genotypes HPV of high-risk HPV are increased and the prognosis of cervical cancer worsens.⁸

There are two patterns of HPV infections, transient infections, and persistent infections. Transient infections usually happen at a young age, and 75% of HPV disappears in 1 – 2 years, so only 5% if not handled well, will cause persistent infection and cervical cancer.⁹

Cervical cancer is a malignant tumor that grows in the cervix or the lowest part of the womb that clings at the top of the vagina,¹⁰ the incidence of cervical cancer in Indonesia occupies the highest rank for ten types of cancer found in females, about 68.1%. High-risk HPV infection is one of the risk factors that has been proven to cause cervical cancer, other factors that influence cervical cancer in females, such as having multiple sexual partners, having sex at a young age, having active sex with a male who is at risk, smoking and number of pregnancies or inadequate labor management.¹¹

The incidence of cervical cancer can be decreased by screening, pre-cancer lesion therapy and diagnosing cervical cancer at an early stage, some methods that can be used as a screening method to prevent cervical cancer, such as Pap smear and Hybrid Capture-II (HC-II) method.^{12,13}

Molecular methods can detect asymptomatic HPV infection. One of the methods to detect HPV is HC-II. HC-II examines nuclei acid hybridization using Chemiluminescence microplate to identify 13 types of high-

vulnerable to infection and have multiple sexual partners. By knowing HPV as the leading cause of cervical cancer, detection of HPV for the screening of cervical cancer must be done.¹⁷

The purpose of this experiment was to compare the detection methods of HPV using HC-II with Pap smear examination especially in commercial sex workers that have a higher risk of obtaining cervical cancer.

METHODS

This experiment was a cross-sectional analytical observational type with the purpose to analyze the results of HPV detection method using HC-II with Pap smear. The samples were chosen by simple random sampling. Inclusion criteria were female sexual commercial workers from Kediri Social Services, who were not menstruating and had non-reactive ICT HIV rapid test result. The study was done in April to May 2017, with 47 samples.

Obtaining samples for HC-II examination was done with Hybrid Capture Cervical Sampler TM (Cytobrush)

Table 1. Reporting the results of Pap smear by Papanicolaou system, WHO, and Bethesda system in 2001⁵

Papanicolaou Sytem	WHO	Bethesda
Class I	Normal	Within normal limits
Class II	Atypical	Atypical Squamous Cell of Undetermined Significance (ASCUS)
Class III	Mild dysplasia	Low-Grade Intraepithelial Lesion (LGSIL)
	Moderate dysplasia	High-Grade Intraepithelial Lesion (HGSIL)
	Severe dysplasia	High-Grade Intraepithelial Lesion (HGSIL)
Class IV	In-situ carcinoma	High-Grade Intraepithelial Lesion (HGSIL)
Class V	squamous cell Carcinoma	Squamous Cell Carcinoma
	adenocarcinoma	Adenocarcinoma

risk HPV DNA qualitatively from a cervical specimen.¹⁴

The sensitivity of HC-II is 98%¹⁵ and the specificity is also 98%. This examination showed 90.8% cases of CIN3, 10% normal cervical epithelium and in a female with a SIL development of 15 – 28%.¹

Cervical cancer cases can be detected by known changes in the cervical area by Pap smear cytology examination. Pap smear is the mostly done screening method of cervical cancer due to its accuracy and low cost.¹⁰

Female commercial sex workers are females with a high-risk of obtaining cervical cancer because they are

the smear was then inserted into a cervical sample tube containing 1 mL of STM. Human papillomavirus examination using HC-II method was done in the Clinical Pathology laboratory of the Dr. Soetomo Hospital Surabaya, and the Pap smear examination was sent to Yayasan Kanker Indonesia Wilayah Kediri, the Anatomical Pathologist interpreted the results using WHO Bethesda system (Table 1). The sample collection was done by a midwife who was in charge of Sexually Transmitted Diseases at Campurejo Kediri Public Health Center.¹⁸

Results of the HC-II examination for detecting

HPV from a cervical smear was determined by comparing samples with the mean RLU/PC. Digene set the positive threshold 1.0 Relative Light Unit/Positive Control (RLU/PC). The diagnosis of Pap smear results was based on Papanicolaou classification and Bethesda system 2001. The data were analyzed based on the results of HC-II and Pap smear examination. The non-parametric Kruskal Wallis analytical study showed a difference in the results of HC-II method and Pap smear.

RESULTS AND DISCUSSION

During the sampling period of April to March 2017, there were 47 commercial sex workers from the social services of Kediri who fulfilled the inclusion criteria and not menstruating and has a non-reactive HIV ICT test. Characteristics can be seen in Table 1.

Table 2. Study sample characteristics

No	Characteristics	Total of positive (n)
1	Total of CSW (n)	47
2	Age	
	< 20 y.o	2
	20 – 30 y.o	8
	31 – 40 y.o	21
	> 40 y.o	16
3	Menarche < 12 y.o	29
4	First sexual intercourse < 17 y.o	30
5	Leucorrhoea	16
6	Smokes	22
7	Exposure to cigarette smoke > 1 hour /day	47
8	Marriage/partner > 1	47
9	Giving birth > 4	4
10	Use contraceptives > 5 years	18
	IUD	1
	Injections	8
	Pills	10
11	Menopause	7
12	Last education	8
13	Being a CSW > 1 years	43

Sampling was done in 47 participants of the study. From the 47 samples that were examined with the HPV HC-II detection method, there were 32 positive

Table 3. HC-II method and Pap smear results

NO	Examination	n Total	Positive Results		Negative Results	
			n	%	n	%
1	Hybrid capture -II	47	32	68.1	15	31.9
2	Pap smear	47	3	6.4	44	93.6

samples (68.1%) with a high-risk of HPV and 15 samples (31.9%) were negative, uninfected by high risk HPV. The positive results with high-risk HPV were samples infected with one or multiple types of 13 high-risk HPV, which were type 16, 18, 31, 35, 39, 45, 51, 52, 56, 58, 59, and 68.

Test results showed that commercial sex workers who were detected as positive HPV by HC-II method were many, as much as 32 samples (68%), this was appropriate with references that said that HPV was very infectious and transmission increased with the number of sexual partners and transmission between partners were about 60%. Commercial sex workers have a habit of changing sexual partners and have a high-risk to be infected with sexually transmitted diseases, especially high-risk genital HPV that can cause cervical cancer. The HC-II method is an antibody capture/solution hybridization/signal amplification assay that used qualitative chemiluminescence towards HPV DNA which can detect all genotypes of high-risk HPV in a person is suspected to have the HPV virus in his/her body. HC-II has been approved by the world and legalized by the Food and Drug Administration (FDA) USA.¹⁸

The results of this study did not differ much from the study done by Jia, who showed that female commercial sex workers in Northeast China had a positive HPV prevalence of 61.90%, as studied by Brown,²⁰ 66.8% in Peru and 77.4% in Belgium²¹ stated a positive result for HPV with HC-II. The results of the HC-II in this study were, consistent with the references indicating that female commercial sex workers had a high-risk to be infected with HPV,²² HPV was very infectious and its contagious was increases with the number of sexual partners, the transmission increased 60% between each partner,¹¹ this was related to commercial sex workers who have the behavior/hobby of changing sexual partners and causing a high-risk of obtaining STDs especially genital high-risk HPV that can cause cervical cancer.

Experiments with low results have been done in a few countries such as Madrid and Alicante in 2006 (31.5%) at Gaasian Spain in 2007 (7.5% - 35.5%) and Korea as high as 46.5%.²¹

The HC-II method is an antibody capture/solution hybridization/signal amplification assay that uses detection of qualitative chemiluminescence towards DNA HPV that can detect all genotypes of high-risk HPV in a person who is suspected to have the HPV virus in his/her body. HC-II has been recognized by the world and is legalized by FDA USA.¹⁸ The benefit of HC-II examination is that it can be examined in the early stages of HPV infection before the virus has caused changes in the cervix and hence cause cervical cancer.¹⁷ The sensitivity of HC-II is 98%¹⁵ and the specificity is 90.8%. The examinations can detect high-risk HPV in 90.8% of CIN3 cases,¹⁶ in 10% of normal cervical epithelial and 15 – 28% of females developing SIL.¹⁴

The weakness of the HC-II examination is that it can only detect high-risk HPV infection but cannot give information if pre-cancer lesions are already formed or not, hence the specificity of HC-II in a couple of studies are lower than Pap smear.²³

This study showed that the HC-II method developed by Digene was very potential to use in cervical dysplasia screening, combined with other examinations like Pap smear, Colposcopy, etc. HC-II is important in primary screening of females above 35 years old. HPV infection in the elderly female, can cause a persistent infection which if not detected and handled in the right way, may increase the risk of dysplasia or cervical cancer.¹²

The benefit of HC-II examination is that it examines an earlier condition where one maybe infected with high-risk HPV in their body before the virus can change the cervix and cause cervical cancer. The sensitivity of HC-II was 98 % whereas Pap smear was 51 – 76%. If compared to PCR, HC-II had a 92 – 94% consistency with cytology or histology examination, a shorter period of time, there was no or minimum contamination and contains a B probe that could detect 13 types of HR HPV DNA.¹⁹

The results of Pap smear examination in this study were read with Bethesda 2001 classification, Papanicolaou classification found a normal smear, Negative Intraepithelial Lesion of Malignancy (NILM), vaginal bacterial infection, *Trichomonas vaginalis* infection, fungi infection, and LSIL. Two samples (4.3%) showed normal smears (class I), 42 samples (89.4%) showed a class II Pap smear result that indicated inflammation and microorganic infection. Three samples (6.4%)

showed dysplasia or Low S Intraepithel Lesion (LSIL) caused by HPV.

Twenty-three class II Pap smear results showed NILM with endocervical cells, metaplasia cells, intermediate squamous superficial cells, there was no endocervical cells or PMN inflammation of Para basal cells; 4 samples showed inflammation with *Trichomonas vaginalis* infection and intermediate, Parabasal squamous superficial cells, there were no endocervical cells and PMN inflammation cells; and 8 samples showed *Candidiasis* infection with spores and hyphae, also intermediate, Parabasal squamous superficial cells and endocervical cells and PMN inflammation cells. The results of the samples that showed dysplasia, LSIL showed endocervical, metaplasia cells, intermediate squamous cells, Parabasal, PMN inflammation cells, lymphocytes, histiocytes, there was mild dysplasia, koilocytosis, binuclear cells and thus was suspected to be due to HPV infection. Pap smear screening test was used for early detection of low stadium cervical cancer, an effective way to detect pre-cancer lesions.¹⁷

A positive Pap smear cytology in this study showed mild dysplasia (LSIL) class 3 with HPV infection was low, only 3 samples (6.4%). The results of this study were in contrast with Nindrea's results in high-risk females (commercial sex workers) in Palembang where 70% were diagnosed with pre-cancer lesions.²⁴ Leung study in commercial sex workers in Hong Kong showed that the results were almost similar to this study,²⁵ with LSIL 3.89% and there was 1.78% of HSIL or squamous cell carcinoma and 0.11% glandular lesion. Leung *et al.* results showed 6.23% ASCUS, ASC-H as much as 0.44%. Retnowati showed the same results for Pap smear with most results showing unspecific chronic inflammation influenced by hygiene and poor sanitation of genitals.¹⁷

The benefits of Pap smear screening is for low stadium cervical cancer detection of and effective to detect pre-cancerous lesions, so the mortality due to cancer can be decreased and increased in survival rate.²⁶ Screening with Pap smear has an 84.2% sensitivity and 62.1% specificity.²⁷

The weakness of Pap smear examination is the limitations of sensitivity and specificity that is determined by the subjectivity of the health practitioner and the ability to detect HPV infection but cannot differentiate HPV infection and high-risk or low-risk HPV.²³ Conventional Pap smear has a lower sensitivity than Liquid Based Cytology (LBC) because it is affected by debris, blood, mucus and how many cervical cells

that are not obtained during the examination.

Other than HPV infection, cervical cancer can also be caused by other factors like sexual intercourse with multiple partners (6 or more) and having sex with high-risk males, which are done by female commercial sex workers, the risk of a female to obtain cervical cancer increases 10 fold,⁶ this is proven by this study of which only 2 samples had normal Pap smear results and 40 samples showed inflammation and LSIL.

Smoking can also influence cervical cancer growth, cervical mucus in smoking females contain substances in cigarettes and this causes the cervix to lose optimal endurance so it can be carcinogenic and easily infected by viruses. Passive smokers also have the same risk and are three times easier to get cervical cancer than females who are never exposed to cigarettes.⁶

Age also has played a role in causing cervical cancer. Most females with cervical cancer were 40 years or older. It was proven by all the results of Pap smear with LSIL (mild dysplasia) in 3 samples, were all from females above 40 years old, 53 years old, 46 years old, and 48 years old. This finding was due to HPV needing 10 -20 years to transform into cervical cancer. The latent period (from CIN1 to in-situ carcinoma) varies, according to the immunity of the patient; 3 – 20 years (usually 5 – 10 years).⁷

The HC-II HPV detection method can predict whether a person can be infected with HPV before the virus makes changes in the cervix and cause cervical cancer, even though the number of HPV is minimal.³ Pap smear does not only detect low stadium cervical cancer but is also useful for detecting pre-cancerous lesions so the mortality due to disease will increase, while the survival rises.¹⁷

Statistical analysis results using the non-parametric Kruskal–Wallis had a $p=0.000$, because a $p < 0.05$ showed a difference of detecting HPV with the HC-II method and Pap smear. A Post hoc analysis to know which group has a difference used the Mann-Whitney test and results showed that there was a significant difference in the HC-II and Pap smear with a p of 0.000. $P < 0.05$ proven that HC-II was better for screening cervical cancer because it can detect high-risk HPV earlier before HPV changes the cervix into cervical cancer.

The sensitivity of HPV test (88-98%) was higher compared to Pap smear (51 – 86%), but the specificity of HPV (83 – 94%) was lower compared to Pap smear (92 – 99%). From 6.29% cases with positive HPV DNA,

only 3.9% had abnormal cytology. This finding was coherent with this study, where 6.4% Pap smear had dysplasia (LSIL) with HPV class 3 infection compared to the 68.1% of positive HC-II, where 29 samples had a negative Pap smears, but the HC-II results positive for high-risk HPV.

CONCLUSION AND SUGGESTION

Results for detecting high-risk HPV with the Hybrid Capture –II method has shown 32 positive sample results (68.1%). The Pap smear examination would dysplasia (CIN1) as much as three samples (6.4%) which cervical cell dysplasia was found that showed HPV infection. From the non-parametric Kruskal-Wallis test that was continued by posthoc Mann-Whitney analysis, showed that there was a significant difference for the HC-II examination with Pap smear examination ($p=0.000$) meaning that HC-II examination is better in using as a screening test for cervical cancer.

HC-II positive and negative examination must be done to compare it with the gold standard HPV test (PCR) and genotyping to know which HPV type infects commercial sex workers. The next study HC-II method can use a comparison of cervical biopsy or samples inserted into thin prep tube or sure path so the sample can be used directly for Liquid Based Cytology (LBC) examination, which results are better than conventional Pap smear and genotyping; there must also be a study on commercial sex workers with a positive HIV chromatography, so the risks for commercial sex workers with HIV to be infected with HPV and cervical cancer can be known.

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