Research Article

Papsmear Examination for Diagnosing Pre-Cancer Lesion in Invisible Squamo-Columnar Junction

Pemeriksaan Papsmear dan IVA untuk Diagnosis Lesi Prakanker pada Tampilan Sambungan Skuamo-Kolumnar Tidak Tampak

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Abstract

Objective: To know the concealed pre-cancer lesion in women with invisible squamo-columnar junction (SCJ) by Papsmear examination.

Method: This study was a descriptive cross-sectional design starting from August 2014 to March 2015 at several Public Health Cares in Jakarta. A total of 1,682 subjects were screened by Acetoacetate Visual Inspection (AVI) examination. After the data was collected, the process was continued by verification, editing, and coding. The descriptive analysis showed the percentage of SCJ in age distribution, the percentage of AVI examination based on SCJ, and the percentage of Papsmear examination in invisible SCJ according to negative AVI result.

Result: There were 1,484 (88.2%) women with the visible SCJ and 198 (11.8%) women with invisible SCJ. The percentage of invisible SCJ in the menopausal women group was 122 (61,6%); meanwhile, in the non-menopausal women group, it was 76 (38.4%). Almost half of the percentage from visible SCJ was found in menopausal women group 45.8% (103/225 women). The positive AVI result was 4 (7.1%) in the menopausal women group and 52 (92.9%) in non-menopausal women group. The result of Papsmear examination with invisible SCJ were 197 (100%) normal.

Conclusion: Almost half of visible SCJ was found in menopausal women group. Most of positive AVI result was found in the non-menopausal women group. All women with the invisible SCJ have a normal Papsmear result.

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Keywords: acetoacetate visual inspection, papsmear, pre-cancer lesion, squamo-columnar junction

Abstrak

***Tujuan**: Untuk mengetahui kelainan yang tersembunyi pada keadaan sambungan skuamo-kolumnar (SSK) tidak tampak melalui pemeriksaan Papsmear.*

***Metode**: Penelitian ini merupakan deskriptif potong lintang. Penelitian dilakukan pada periode Agustus 2014 sampai Maret 2015 di beberapa puskesmas di Jakarta. Sebanyak 1.682 subjek yang dilakukan pemeriksaan IVA (Inspeksi Visual dengan Asam asetat). Setelah data dikumpulkan, akan dilakukan verifikasi data, editing, dan proses pengkodean. Analisis data deskriptif berupa variabel kategori yaitu persentase letak SSK berdasarkan distribusi usia, persentase hasil pemeriksaan IVA berdasarkan SSK, dan persentase hasil pemeriksaan Papsmear pada SSK yang tidak tampak dari hasil pemeriksaan IVA negatif.*

***Hasil**: Perempuan dengan SSK yang tampak 1.484 (88,2%), yang tidak tampak 198 (11,8%). Sambungan Skuamo-Kolumnar (SSK) yang tidak tampak pada perempuan yang sudah menopause sebanyak 122 (61,6%), sedangkan pada perempuan yang belum menopause sebanyak 76 (38,4%). Hampir setengahnya proporsi SSK yang tampak didapatkan pada kelompok perempuan yang sudah menopause 45,78% (103/225 perempuan). Hasil pemeriksaan IVA positif didapatkan 4 (7,1%) pada kelompok perempuan menopause dan 52 (92,9%) pada kelompok perempuan yang belum menopause. Pada pemeriksaan Papsmear dengan SSK yang tidak tampak, persentase kelainan lesi prakanker yaitu sebesar 197 (100%) normal.*

***Kesimpulan**: Hampir setengahnya SSK yang tampak ditemukan pada kelompok perempuan menopause. Sebagian besar IVA positif ditemukan pada kelompok perempuan yang belum menopause. Seluruh perempuan dengan SSK yang tidak tampak memiliki hasil pemeriksaan Papsmear normal.*

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***Kata kunci**: IVA, lesi prakanker, papsmear, sambungan skuamo-kolumnar*

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INTRODUCTION

Cervical cancer is the second most common cancer that affects women in the world.[1-4] Apart from that, it is the most common causes of death among cancer, especially for women in developing countries.[4] Based on the recent world's estimation, there are 493,000 new cervical cancer cases occurred each year, whereas there are 409,400 (83%) cases happened in women in developing countries and only 84,400 (17%) cases in developed countries.[2] Unfortunately, more than 80% of the cases are diagnosed at advanced stage when the 5-year survival rate is less than 40%.[2]

The main cause of high incidence of cervical cancer in developing countries is due to the lack of effective screening programs to detect and manage the early stage of cervical cancer or pre-cancer lesions of the stage.[4] Of the various modality screening which have been studied, Papsmear has ability to decrease 70% of cervical cancer cases; however, this examination is difficult to implement in developing countries like Indonesia. It seems that visual inspection of the cervix is the best techniques to apply, especially in areas with limitation of health facilities resources.[2,4-7] This technique is known as the VIA test (Visual Inspection with Acetic acid application). It is defined as a technique of cervical direct observation after being applied with acetic acid without the use of any magnifying tools.

Visual Inspection of Acetic acid examination depends on the Squamo-Columnar Junction (SCJ) condition in the cervical region. In childbearing age women and pregnant women, SCJ is located in the ecto-cervical that it can be seen on direct examination. Whereas in postmenopausal women, SCJ is often located in the endo-cervical canal so it cannot be seen on direct observation. However, the percentage of visible SCJ in menopausal women is 64.28% and invisible SCJ is 11.66%.[8] This rate is high for the menopausal women. Another study conducted by Dhaubhadel, et al. prospectively and descriptively in women aged 20-50 years, the result showed negative VIA Papsmear test from all 46-50 year of group women.[2] The study did not describe the visibility of SCJ in the investigation. Therefore, it could make the false negative results. If the SCJ is not visible, it suggests doing the Pap smear test. Unfortunately, the problem is in the first-line health care facility, such as primary health centers with limited facilities, the invisible SCJ (in the second examination), particularly in menopausal women is justified without doing the VIA examination. Therefore, this study aims to determine the percentage of hidden invisible SCJ in abnormal cervical pre-cancer lesions through the Pap smear examination.

THEORITICAL OVERVIEW

Invasive cervical cancer is usually preceded by a long phase of pre-invasive lesions, which are microscopically seen as precursor lesions developing from atypical cells to the various level of cervical intraepithelial neoplasia (CIN) before progression to invasive carcinoma. The epidemiological studies have identified several risk factors that contribute to the development of the CIN and cervical cancer. The risk factors are Human Papilloma Viral (HPV) infection, sexual contact in early age, changing sexual partners, multi-parity, long-term of oral contraceptive use, smoking, low socioeconomic status, infection with Chlamydia trachomatis, micronutrient deficiency described by less intake of vegetables and fruits. The types of HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 are closely related to CIN and invasive cancer. Infection of one or more HPV types is suspected as the cause of cervical neoplasia. Infection of one or more of the oncogenic HPV types will contribute to the entrance of combined viral genome into the host cell genome; this formation will lead to cell neoplasia and turn into various level of CIN and finally, it will further develop into cervical cancer.[9-13]

Application of 5% acetic acid is believed to cause a reversible coagulation or precipitation of the cell protein. This application also causes swelling of the epithelial tissue and cell dehydration. The color of normal squamous epithelium is pink; while, the columnar epithelium will be in red color due to the reflection of light from the stroma in which its bottom is rich for blood vessels. If the epithelium contains a lot of protein cells, the acetic acid will coagulate this protein which will eliminate the color of the stroma. The result from this coagulation production is called as aceto-white area that can be seen with the naked eye and distinguished from the normal area which should be pink around it. Therefore, the effect of acetic acid depends on the amount of protein found in epithelial cells. Areas where the increased activity of the nucleus and DNA will change very clearly.[9,13]

When acetic acid is applied to normal squamous epithelium, the coagulation will occur in the superficial cell layer. Although the deeper part of the cell has more protein core, acetic acid cannot penetrate; thus, the result of precipitation is not sufficient enough to remove the color in the stroma. The CIN region and invasive cancer are an area where the protein coagulation will occur maximally because they contain of higher core protein and prevent light passing through the epithelium. As a result, the arrangement of blood vessels in the sub-epithelial disappears that the thick epithelium appears white. In the CIN, aceto-white area is limited to the transformation zone close to the SCJ; while, in cervical cancer, aceto-white area is seen around the cervix. Aceto-white area is not typical

for the CIN and early stage of cancer. This area can also be seen in other conditions due to the increase of core proteins, such as immature squamous metaplasia, healing or regenerating epithelium (associated with inflammation), leucoplakia (hyperkeratosis), and condyloma.[9,12,13] The symptoms of cervical cancer are vaginal bleeding, post-coital bleeding, vaginal discharge, lower abdominal pain, edema of lower extremity, obstructive uropathy, bowel obstruction, and also anemia.[12,14]

Aceto-white area in CIN and early stage of cancer are more whitish, thicker, and opaque as well as clear boundary; while, the aceto-white area in immature squamous metaplasia, the inflammation and regenerating epithelium will make less pale, thin, translucent, and ill defined. Aceto-white area caused by inflammation and epithelial healing process is usually spread to the cervix, not only in the transformation zone. Besides, this color will be disappeared within a minute. Leucoplakia and condyloma turn the color into grayish white after the application of acetic acid. The effect of acetic acid (aceto-white area) is slower in CIN lesions and early invasive cancer than immature squamous metaplasia and inflammation. This effect occurs after 3-5 minutes at CIN 2-3 and invasive cancer.[9,13]

METHODS

This cross-sectional design study is conducted in several primary health centers in Jakarta on the period of August 2014 to March 2015. The target population is all women who are married or have ever had sexual intercourse for VIA examination. We included women who did the VIA screening examination in primary health centers Jakarta in the period of August 2014 to March 2015. We excluded pregnant women and women with history of total hysterectomy. From the calculation formula, the total number of subjects needed in this study is 1,658 subjects. In this study, we performed VIA examination on 1,682 women.

All women that met the criteria of the study were labeled and subsequently did the Papsmear examination and the results were interpreted by expert pathologist. The result of Papsmear examination would be described with Bethesda system criteria in 2001. The data was analyzed where we conducted the descriptive statistics for categorical variables, namely the proportion of SCJ layout based on the age distribution, the proportion of the VIA examination results based on the location of the SCJ of the age distribution, and the percentage of the Papsmear examination results of the SCJ which was not visible from the IVA. All data were shown in frequency and percentage.

RESULTS

Of 1,682 subjects, only 9 (0.5%), 311 (18.5%), 638 (37.9), 492 (29.3), 232 (13.8%) women were less than 20, 20-29, 30-39, 40-49, and more than 50 years old; respectively.

Table 1. The Characteristics of the Subjects

Characteristics	n	%
Age		
< 20 yo	9	.5
20-29 yo	311	18.5
30-39 yo	638	37.9
40-49 yo	492	29.3
≥ 50 yo	232	13.8
Menopause state		
Not yet	1457	86.6
Menopause	225	13.4
Contraception state		
Not using contraception	354	21.0
Pill	127	7.6
Implant	43	2.6
Injection	603	35.9
IUD	470	27.9
MOW	27	1.6
Condom	58	3.4
SCJ		
Invisible SCJ	198	11.8
Visible SCJ	1484	88.2
Total	1682	100.0

In this study, according to the age group, we divided into group who were still menstruation (premenopausal state) and had stopped menstruation (menopausal state). There were 1,457 (86.6%) women who were still menstruation (premenopausal) and the others had stopped menstruation.

Table 1 showed that the majority of women (603 women (35.9%)) had ever used the injectable contraception. Meanwhile, the rate of permanent contraceptive acceptor (MOW) was only 27 (1.6%) women. There were 354 (21.0%) women had not ever used contraception. There were 1,484 (88.2%) women with visible SCJ and 198 (11.8%) women with invisible SCJ. The proportion of invi-

sible SCJ based on age group for more than 50, 40-49, 30-39, 20-29, less than 20 years old was 127 (64.1%), 35 (17.7%), 30 (15.2%), 6 (3.0%), and 0 (0.0%); consecutively.

Invisible SCJ proportion in women who had experienced menopause was 122 (61.6%) and 76 (38.4%) for premenopausal women. Almost half of visible SCJ obtained in the group of women who were menopausal (45.78% (103 women)).

Table 2 depicted the proportion of visible SCJ. For age group of 30-39, 40-49, 20-29, and less than 20 years old, there were 608 (41.0%), 457 (30.8%), 305 (20.6%), and 9 (0.6%); consecutively. The proportion of visible SCJ in menopausal women was 103 (6.9%) and 1,381 (93.1%) women in premenopausal state. However, in the premenopausal group, nearly half of 103 (45.77%) women had visible SCJ.

Table 2. The Proportion of Squamo-Columnar Junction (SCJ)

Characteristic	SCJ			
	Invisible SCJ	%	Visible SCJ	%
Age				
< 20 yo	0	0.0	9	0.6
20-29 yo	6	3.0	305	20.6
30-39 yo	30	15.2	608	41.0
40-49 yo	35	17.7	457	30.8
≥ 50 yo	127	64.1	105	7.1
Menopause State				
Not Yet	76	38.4	1381	93.1
Menopause	122	61.6	103	6.9

Table 3. Proportion of VIA Examination with Visible SCJ

Characteristic	VIA			
	Negative	%	Positive	%
Age				
< 20 yo	8	0.6	1	1.8
20-29 yo	291	20.4	14	25.0
30-39 yo	589	41.2	19	33.9
40-49 yo	439	30.7	18	32.1
≥ 50 yo	101	7.1	4	7.1
Menopause State				
Not Yet	1329	93.1	52	92.9
Menopause	99	6.9	4	7.1

In this study, the overall positive VIA test result was in 56 (3.98%) women. The proportion of positive VIA test result according to the age group were 19 (33.9%) for 30-39 years old, 18 (32.1%) for 40-49 years old, 14 (25.0%) for 20- 29 years old, 4 (7.1%) for more than 50 years old, and 1 (1.8%) for less than 20 years old. While, the proportion of positive VIA test result was obtained for 4 (7.1%) in menopausal women and 52 (92.9%) in menstruating women (Table 3).

In the Pap test with SCJ which was not visible, every test presented the normal result (100%) (Table 4). In this study, invisible SCJ was obtained in 198 subjects, but there was one subject that was not checked due to loss of data. However, this number still met the minimum of required sample (196 subjects).

DISCUSSION

This study is based on the data from the examination in several health centers in Jakarta. In this study, the total of 1,682 women were analyzed

Table 4. Proportion of Papsmear Test Result in Invisible SCJ

	ASCUS	%	LSIL	%	HSIL	%	Normal	%
Age								
20-29 yo	0	0.0	0	0.0	0	0.0	6	3.0
30-39 yo	0	0.0	0	0.0	0	0.0	27	13.7
40-49 yo	0	0.0	0	0.0	0	0.0	36	18.3
≥ 50 yo	0	0.0	0	0.0	0	0.0	128	65.0
Menopause stase								
Not Yet	0	0.0	0	0.0	0	0.0	74	37.6
Menopause	0	0.0	0	0.0	0	0.0	123	62.4

based on their characteristics of age, history of contraception, menopausal status, state of the SCJ, the results of the VIA examination and Papsmear. This study began between August 2014 and March 2015. Women with positive VIA test result were evaluated on subsequent visits. While the women had cervical abnormalities, such as cervicitis, it would be treated with topical antiseptics. If the doctor found other abnormalities, such as cervical polyps and suspicious cancer, the women would be sent to higher level of health care facility.

This study has strengths and limitations. The strengths of this study were the sample was taken by doctors and health workers who have had the training and experience from the Female Cancer Program (FCP) in identifying the SCJ, the abnormalities in the cervical region, the procedures for VIA and conventional smear of Pap-smear examination sampling. Additionally, in this study, one pathologist performed Papsmear examination. There were some flaws in this study among others, namely in the Pap-smear examination, sampling errors might occur that could affect the results.

In this study, there were 225 (13.37%) menopausal women. The proportion of invisible SCJ was among 198 (11.7%) women, whereas almost half of postmenopausal women had visible SCJ. In the study conducted by Nuranna, et al. in October 2007 until December 2010, it showed that of 3,791 postmenopausal women (16.49%), the proportion of invisible SCJ was on 2,680 (70.69%) women. More than half of the menopausal women had visible SCJ (2,437 (64.28%) women).[8]

In this study, in Pap-smear with invisible SCJ, all results showed normal (100%). Shwe, et al. conducted a study of cervical cytology in Myanmar from 2010 to 2011. Of 1,771 women screened, 762 women (43.0%) resulted in abnormal smear, 866 (48.9%) and 87 (4.9%) were diagnosed as inflammation and Atypical Squamous Cells of Undetermined Significance (ASCUS). There were 42 (2.3%) and 11 (0.6%) cases of Low Grade Squamous Intraepithelial Lesion (LSIL) and High Grade Squamous Intraepithelial Lesion (HSIL). The cases of squamous cell carcinoma (SCC) occurred on 3 (0.2%) women.[15] While the study conducted by Sengul, et al., there were 32,578 cases of Pap-smear examination performed and analyzed between January 2001 and April 2010. From the investigation which had carried out, the results showed that 1.18% of ASCUS; 0.39% of LSIL; 0.16% of HSIL; 0.07% of Atypical Glandular Cells of Undetermined Significance (AGUS); 0.02% of squamous cell carcinoma, and 0.006% of adenocarcinoma 0.006%.[16] Abnormal cytology is more common in cases with older age, low parity, and period of perimenopause.[16] However, this study did not have data on SCJ picture.

In this study, all results were 100% normal cervical cytology at the invisible SCJ. However, this did not completely rule out pre-cancer lesions abnormalities. Study by Pan, et al. explained that overall, the level of sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of cervical cytology for detecting cervical intraepithelial neoplasia (NIS 2+) were respectively 81.0 %, 95.4%, 38.3%, 99.3% and 94.9%.[17] A study held by Pak, et al. which aimed to compare the history of previous Papsmear test result in patients with cervical adenocarcinoma and squamous cell carcinoma of the cervix. In patients with cervical adenocarcinoma, the false negative results of Papsmear were very significant in the latest investigation. As already mentioned above, the normal Papsmear results did not guarantee not to develop the cervical cancer. In general, 157 patients (41.8%) carried out repeated screening within 2 years. Fifty-five (14.6%) carried out repeated screening within 2-5 years and 80 (22.3%) over 5 years. A total of 16.8% did not have documentation for Pap-smear examination results. The false negative results of Pap-smear for patients with cervical adenocarcinoma and squamous cell carcinoma were 9 (5.6%) and 2 (1.3%) patients.[18] Kirschner, et al. stated that the false negative of Pap-smear result was on 11 (9.8%) women.[19]

CONCLUSION

Almost half of invisible SCJ is found in the group of menopausal women and most visible SCJ is found in the group of women who have not menopausal yet. Positive VIA results are largely found in the group of women who have not menopausal yet. All the women with invisible SCJ have normal Papsmear examination results.

REFERENCES

1. Moore DH. Cervical Cancer. Clinical Expert Series, Am College Obstet Gynecol 2006; 107: 1152-3.
2. Dhaubhadel P, Vaidya A, Choudhary P. Early detection of precursors of cervical cancer with cervical cytology and vi-

sual inspection of cervix with acetic Acid. JNMA; n 2008; 47(170): 71-6.

3. Belinson JL, Pretorius RG, Zhang WH, et al. Cervical cancer screening by simple visual inspection after acetic acid. Obstet Gynecol 2001; 98(3): 441-4.
4. Ocviyanti D. Tes pap, tes HPV dan servikografi sebagai pemeriksaan triase untuk tes IVA positif: upaya tindak lanjut deteksi dini kanker serviks pada fasilitas kesehatan dengan sumber daya terbatas beserta analisis sederhana efektivitas biayanya. Indones J Obstet Gynecol 2007; 31(4): 202-3.
5. Blumenthal PD, Gaffikin L, Deganus S,et al. Cervical cancer prevention: safety, acceptability, and feasibility of a single-visit approach in Accra, Ghana. Am J Obstet Gynecol 2007; 196(4): 407.e1-8; discussion .e8-9.
6. Singh V, Sehgal A, Parashari A, et al. Early detection of cervical cancer through acetic acid application - an aided visual inspection. Sing Med J 2001; 42(8): 351.
7. Bharani B, Phatak S. Acetic acid visualization of the cervix an alternative to colposcopy in evaluation of cervix at risk. J Obstet Gynecol Ind 2005; 55: 530.
8. Nuranna L, Aziz MF, Irianti R, et al. The role of SCJ in VIA examination: can VIA still be done in menopause women? In: Nuranna L, editor. Asia-Oceania Research Organisation on Genital Infections and Neoplasia (AOGIN) Interim Meeting. Discovery Kartika Plaza Hotel, Bali: AOGIN; 2011.
9. RI DK. Buku Acuan Pencegahan Kanker Leher Rahim dan Kanker Payudara. Jakarta: Direktorat Pengendalian Penyakit Tidak Menular. Direktorat Jendral PP dan PL; 2007.
10. Benard VB, Eheman CR, Lawson HW, et al. Cervical screening in the National Breast and Cervical Cancer Early Detection Program, 1995-2001. Obstet Gynecol 2004; 103(3): 564-71.
11. Chong Q. The Abnormal-looking Cervix. The Singapore Family Physician 2003; 29(2): 56-9.
12. Andrijono. Kanker Serviks. 3rd ed. Fakultas Kedokteran Universitas Indonesia: Divisi Onkologi Ginekologi, Departemen Obstetri dan Ginekologi RSUPN-CM; 2010.
13. Sankaranarayanan R. A practical manual on visual screening for cervical neoplasia. World Health Organization 2003. (accessed)'.
14. Berek JS. Intraepithelial Disease of The Cervix, Vagina, and Vulva. In: Berek JS, ed. Berek & Novak's Gynecology. Philadelphia: Lippincott Williams & Wilkins; 2007: 570.
15. Shwe M, Harano T, Okada S, et al. Prevalence of high risk human papillomavirus (HR-HPV) infection among women with normal and abnormal cervical cytology in Myanmar. Acta Medica Okayama 2014; 68(2): 79-87.
16. Sengul D, Altinay S, Oksuz H. Population-based cervical screening outcomes in Turkey over a period of approximately nine and a half years with emphasis on results for women aged 30-34. Asian Pacific J Cancer Prev 2014; 15: 2069-74.
17. Pan Q, Hu S, Zhang X. Pooled analysis of performance of liquid based cytology in population-based cervical cancer screening studies in China. Cancer Cytopathol 2013; 121(9): 473-82.
18. Pak SC, Martens M, Bekkers R, et al. Pap smear screening history of women with squamous cell carcinoma and adenocarcinoma of the cervix. Au New Zealand J Obstet Gynaecol 2007; 47(6): 504-7.
19. Kirschner B, Poll S, Rygaard C, et al. Screening history in women with cervical cancer in a Danish population-based screening program. Gynecol Oncol 2011; 120(1): 68-72.