

Performance of the R-way Colposcopic Evaluation System in Cervical Cancer Screening

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Abstract

Aim: To investigate the diagnostic value of the R-way colposcopic evaluation system (R-way system) in cervical cancer screening.

Materials and Methods: Between August 2013 and August 2014, a total of 1059 cases referred for colposcopy at Peking University First Hospital were studied using both the R-way system and the conventional colposcopic method. Our study evaluated and compared the diagnostic ability of the two methods in detecting high-grade lesions and cervical cancer (hereinafter called CIN2+). Evaluating indicators including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), Youden index and the area under the curve (AUC) of the receiver operating characteristic (ROC) were calculated.

Results: The R-way system had a slightly lower specificity (94.52%) than conventional colposcopic method (95.99%) for CIN2+ detection ($p = 0.181$). However, the sensitivity (77.81%) was significantly higher than conventional colposcopic method (46.62%) ($\chi^2 = 64.351$, $p < 0.001$). Meanwhile, the AUC of the ROC for CIN2+ detection using R-way system (0.839) is larger than that with conventional colposcopic method (0.731) ($Z = 4.348$, $p < 0.001$). If preliminary results had been drawn from cervical exfoliated cytology before colposcopy referral, the combination of the R-way system with cytology could increase the sensitivity to 93.89% for CIN2+ detection (excluding ASCUS\LSIL), which is confirmed by multiple punch biopsy or ECC.

Conclusion: The diagnostic value of the R-way evaluation system is higher than that of the conventional colposcopic evaluation system in cervical cancer screening. Moreover, taking the ease of use and standardized quality control management into account, the R-way system is highly preferable.

Keywords: R-way colposcopic evaluation system, conventional colposcopic examination, cervical cancer screening

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Cervical cancer is one of the most prevalent cancers, with the fourth highest mortality among female cancers worldwide. In less developed countries, cervical cancer is the second most prevalent cancer, with the second highest mortality in women among all other cancers. In developed countries, effective cervical cancer screening has led to morbidity and mortality dropping significantly. Now cervical cancer is no longer one of the top ten cancers in developed countries.^{1,2}

In China, due to the large population and the lack of an effective cytology interpretation system, a population-based screening of high-risk human papillomavirus (HPV) test is more suited for first-line screening.³ Population screening is the secondary means of prevention and treatment of cervical cancer. For China to improve the quality of population screening, a feasible strategy would be to raise the level of colposcopy techniques of gynecologists. For cervical cancer screening, it is important that gynecologists practise a more standardized colposcopic evaluation system, especially for those in primary hospitals. R-way (R: Red; w: white; a: abnormal vascular features; y: yellow) is a standardized

colposcopic evaluation system based on standard colposcopy procedure. By combining the sequentially appeared features ("R", "w", "a", "y"), a preliminary diagnostic result will be drawn along with a suggestion of biopsy regions, if required. R-way system is built to help gynecologists easily locate CIN2+ lesions, thus providing technical assurance for decreasing the morbidity and mortality of cervical cancer in China.

Materials & Methods

Clinical Materials

Between 28 August 2013 and 28 August 2014, a total number of 1059 cases referred for colposcopy at Peking University First Hospital were studied using both the R-way system and the conventional colposcopic method. Excluded criteria were: 1) age of 25 years or less, 2) a history of hysterectomy, 3) a history of pelvic radiation therapy. Colposcopy was performed on non-pregnant women with a diagnosis of abnormal cervical exfoliated cytology, positive high-risk type HPV16/18 or suspicious for cervical lesions. A biopsy was conducted if either method suggested pathology. For all cases studied, the

histopathological diagnostic result of the cervix was included. The age of all studied cases was between 25–85 years old, with a mean age of 42.61 ± 10.13 years.

Research Methods

Conventional colposcopy

According to standard colposcopy procedure, first saline is applied to clean the surface of the cervix, followed by preliminary inspection of the cervical surface. Thereafter, 5% diluted acetic acid solution is applied to wipe the cervix for 50 seconds. If acetowhite epithelium occurs after application of acetic acid, images are captured at time points of 60, 90 and 120 seconds, the changes of the whitening effect during this period are observed and any abnormal vascular features are checked such as punctuation, mosaics and atypical vessels. Finally, Lugol's staining (Schiller's test) is performed to inspect iodine uptake. From the information collected during this period, one of the four preliminary diagnostic results, including suspicious for invasion, high-grade lesions, low-grade lesions and normal colposcopic findings⁴ can be drawn. For the cases in which colposcopic examination does not indicate suspicious cervical cancer or a high-grade lesion but cervical cytology shows a positive diagnostic result of ASC-H, HSIL, SCC, AGC+ (including AGC-NOS and AGC-FN), AIS or Adca, a multipoint biopsy or ECC is conducted afterwards. Collected tissue is fixed with 10% formalin and examined in the pathology department.

R-way system

Supplemental instructions and materials are added into the conventional colposcopy method: First, saline is applied to clean the surface of the cervix followed by inspection of surface vessels under a colposcope. Current images are captured with and without green filter to evaluate the "red" feature. If "red" is obvious, the corresponding region is marked in a quadrantal diagram (Figure 1) on the computer. After that, 5% diluted acetic acid is applied to wipe the cervix for 50 seconds. The changes of the surface of the cervix are observed continuously and images are captured at 60, 90 and 120 seconds. The whitening effect is inspected from these 3 images to see whether the acetowhite epithelium fades or becomes dense and thick. If acetowhite epithelium persisted or incrassated, this feature is defined as "white" and the corresponding area marked in a quadrantal diagram (Figure 2). If punctuation, mosaics and atypical vessels (a) appear in acetowhite areas, the corresponding region is marked in a quadrantal diagram (Figure 3). Finally, Lugol's iodine solution is applied to the cervix to see the region with no iodine uptake. The region with no iodine uptake is marked with the "mustard yellow" (y) (Figure 4). Based on different combinations of the above marks, the computer associates and compares the images captured during colposcopic examination, performs analysis using the pathology-based R-way specialized database, then provides preliminary diagnosis (suspicious for invasion, high-grade lesions, low-grade lesions or normal colposcopic findings) and suggests biopsy regions for cases of suspicious for invasion or high-grade lesions. If the R-way system failed to recognize cases of suspicious for invasion or high-grade lesions, the R-way will automatically correlate these two situations for biopsy: 1) If bleeding occurs on the surface of the cervix (or ulceration),

bleeding surface biopsy or ulceration biopsy is suggested; ECC is recommended if bleeding is from cervical canals. 2) A biopsy of random regions in the four quarters or ECC is suggested, if the cervical cytology result is ASC-H, HSIL, SCC, AGC+ (AGC-NOS and AGC-FN included), AIS or Adca. Collected tissue is fixed with 10% formalin and examined in the pathology department.

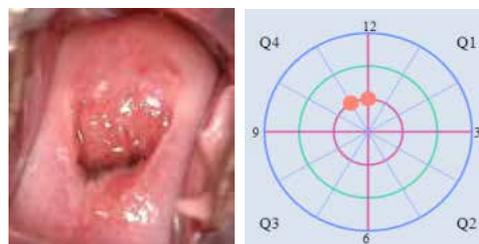


Figure 1: Red dots were marked for reddish domain.



Figure 2: White dots were marked for persistent or thickened acetowhite.

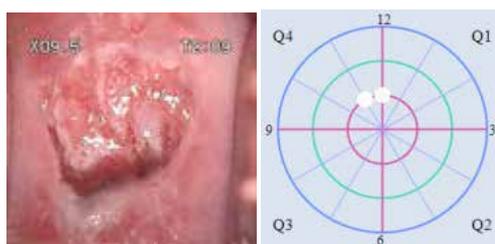


Figure 3: Dark red dots were marked for abnormal vascular features under green filter.

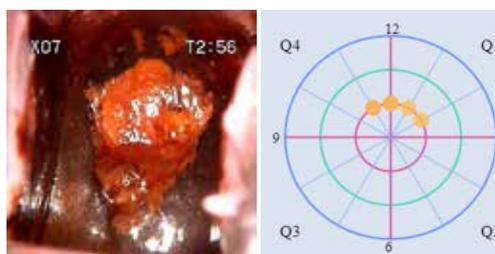


Figure 4: Yellow dots were marked for "mustard yellow" areas with no iodine uptake.

Preliminary colposcopy results

Preliminary results from colposcopy are classified into four groups including: suspicious for invasion, high-grade lesions, low-grade lesions and normal colposcopic findings. Preliminary

results of normal colposcopic findings and low-grade lesions are grouped as negative. Results of suspicious for invasion and high-grade lesions are classified as positive by using the conventional colposcopic method while active bleeding is also considered a positive group when using the R-way system. Among all cases, when suspicious for invasion or high-grade lesions are not recognized by colposcopy while cervical cytology shows a diagnostic result of ASC-H, HSIL, SCC, AGC+ (including AGC-NOS and AGC-FN), AIS or Adca, those cases are classified as positive enrolling in statistics analysis.

Pathology examination and results interpretation

Histopathology result is regarded as the gold standard in this study. According to the American Society for Colposcopy Cervical Pathology (ASCCP) guidelines published in 2013,⁵ pathological findings are classified into four levels, namely cervicitis, LSIL (CIN I), HSIL (CIN II-III) and invasive cancer. Pathological negative group refers to the low-grade lesions group, including cervicitis, LSIL (CIN I) and HPV relevant lesions. Pathological positive group refers to the high-grade lesions group including HSIL, adenocarcinoma in situ and invasive cervical cancer.

Statistics

All statistical analyses were conducted using SPSS17.0 software. The evaluation of the ability of the two methods to detect CIN2+ was performed by analyzing the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Youden index. The diagnostic ability of these two methods was assessed by comparing the AUC of the ROC. We respectively calculated chi-square test and Z test for comparisons between the evaluating indicators of the two methods. P values less than 0.05 were defined as significantly different.

Results

3Evaluating Indicators

Among 1059 cases enrolled in this study, 311 cases were diagnosed with cervical diseases by the gold standard, while the other 748 cases were confirmed normal. The R-way system showed a positive result of 242 cases (292 cases when combined with cytology) while the conventional method reported 145 positive cases (183 cases when combined with cytology). The sensitivity, PPV, NPV and Youden index of the R-way system (77.81%, 85.51%, 91.11%, 72.33%) were higher than those of the conventional method (46.62%, 82.86%, 81.22%, 42.61%) for CIN2+ detection. The specificity of the R-way system (94.52%) was slightly lower than that of the conventional colposcopic method (95.99%), and the difference ($p = 0.181$) was not statistically significant (Table 1).

AUC of the ROC

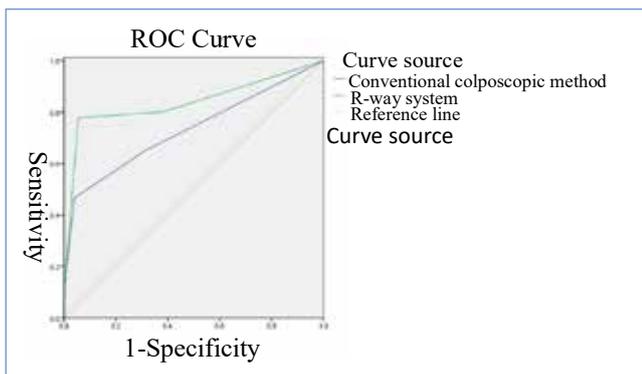
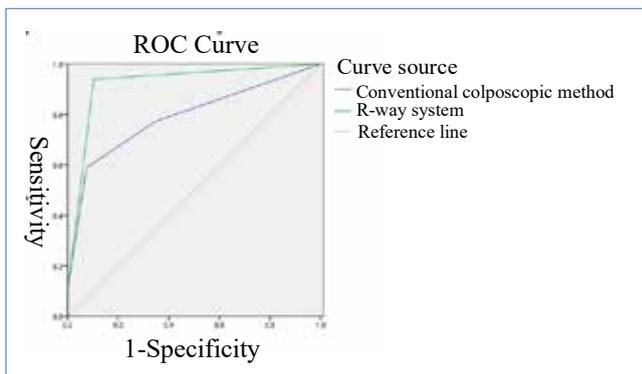
Receiver operating characteristics (ROC) curves were plotted to evaluate the sensitivity and specificity of conventional colposcopy and R-way system for CIN2+ detection. AUC above 0.5 was defined as clinically significant. From the results, two methods tested were clinically significant for CIN2+ detection ($p < 0.001$). The AUC of the R-way system was 0.839 (95% CI: 0.807–0.871), while the AUC of conventional colposcopic method was 0.731 (95% CI: 0.693–0.768). The AUC of the ROC assessed by two different method was significantly different ($p < 0.001$). Therefore, the R-way system performed better than the conventional colposcopic method for CIN2+ detection with regard to the AUC (Table 2, Figures 5 and 6).

Table 1: Results of two different methods for detection of CIN2 and higher lesions

Evaluating indicator	R-way system		Conventional colposcopy		χ^2	p
	%	95% CI	%	95% CI		
Not combined with cytology						
Sensitivity	77.81	72.87, 82.08	46.62	41.15, 52.18	64.351	< 0.001
Specificity	94.52	92.65, 95.93	95.99	94.33, 97.18	1.789	0.181
PPV	85.51	80.94, 89.14	82.86	76.58, 87.72	0.582	0.446
NPV	91.11	88.9, 92.91	81.22	78.51, 83.66	33.237	< 0.001
Youden index	72.33	-	42.61	-	-	-
Combined with cytology						
Sensitivity	93.89	90.66, 96.05	58.84	53.3, 64.17	105.836	< 0.001
Specificity	89.71	87.32, 91.68	92.51	90.4, 94.19	3.639	0.056
PPV	79.13	74.7, 82.97	76.57	70.8, 81.49	0.558	0.455
NPV	97.25	95.74, 98.23	84.39	81.75, 86.71	70.477	< 0.001
Youden index	83.60	-	51.36	-	-	-

Table 2: Comparison of the AUC of the ROC assessed by the R-way system and the conventional method

Method	R-way system	Conventional method	Z	P Value
Not combined with cytology				
AUC*	0.839	0.731	4.348	< 0.001
95% CI	0.807–0.871	0.693–0.768		
Combined with cytology				
AUC*	0.923	0.79	6.743	< 0.001
95% CI	0.904–0.942	0.756–0.823		

*Null hypothesis: AUC = 0.5, $p < 0.001$ **Figure 5:** Comparison of the AUC of the ROC assessed by R-way system and conventional method without combination of cytology**Figure 6:** Comparison of the AUC of the ROC assessed by R-way system or conventional method with combination of cytology

Discussion

Cervical cancer is one of the most common malignant tumors in women's genital tracts. Early diagnosis and treatment of cervical premalignant lesions is very important for the prognosis of cervical cancer. To effectively reduce the morbidity and mortality of cervical cancer, women at the right age are injected with HPV vaccine and cervical cancer screening campaigns are conducted in large scale. In China, HPV vaccine has not yet been approved by the China Food and Drug Administration (CFDA), hence cervical cancer screening remains an important way for cervical cancer prevention.⁶ In 2008, the Chinese government launched the 'Breast Cancer & Cervical Cancer' examination project for women in rural areas. In three years, over 10 million women were. In 2012, the coverage of the project increased to 10 million cases per year.

The primary method of cervical cancer screening is to apply Papanicolaou smear or VIA/VILI,⁷ and patients with positive diagnosis are then referred for colposcopy.⁸ As the second stage for cervical cancer screening, colposcopy goes as follows: observe the images of the cervical surface (which indirectly reflect the components of cervical tissues) to identify CIN2+ by finding whether abnormal cells and vessels are present on the surface of the cervix. This could improve biopsy accuracy and avoid excessive biopsy. Pathological biopsy under colposcope is the gold standard for determining whether treatment is needed or not. Therefore, the precise location of CIN2+ region is not only the assurance of a proper pathological diagnosis, but also a critical technical problem encountered in cervical cancer screening.

However, image interpretation is dominated by the subjective experience of the gynecologist during conventional colposcopy,⁸ with the sensitivity in detecting high-grade squamous intraepithelial lesions (HSIL) ranging from 48% to 87% in different reports.⁸⁻¹² Based on pathology, the R-way system identifies cases of high-grade lesions or suspicious for invasion by observing the staining effects of cervical epithelium after application of different solutions. Abnormal cervical tissues consist of abnormal newly-formed vessels and abnormal cells. If a large number of newly-formed vessels and abnormal cells are present on the surface of the cervix, terminal cervical cancer can be detected through visual inspection of changes such as cervical ulceration, necrosis, bleeding, irregular surface, or ectocervical dysplastic nodules in routine gynecological examinations.

The target group for cervical cancer screening is patients with HSIL. Therefore, early detection of HSIL is the primary task of colposcopy. HSIL is diagnosed with colposcopy rather than the naked eye when newly-formed vessels and abnormal cells are not sufficient for visual inspection. During colposcopy, by applying 5% dilute acetic acid solution and Lugol's solution (Schiller's test), images are captured by colposcope for further analysis. Those images are formed by the light which reflects from the stroma underlying the epithelial cells. By observation and analysis of image features such as the colour, shape, border and vessels, HSIL can be detected.

In normal cases, the cervical columnar epithelium is composed of a single layer of glandular cells and it appears reddish in colour because the thin single cell layer makes the underlying vasculature in the stroma easier to be seen. Due to multiple

factors, reserve cells under the columnar epithelium start to become squamous. At the beginning of this process, the underlying vasculature in the stroma also appears reddish in immature cervical transformation zones (TZ). When the cervix goes atrophic, differentiation of the intermediate and superficial cell layers becomes slower due to hormone level decreased, and usually only the bottom layer of squamous cells is visible. Therefore, the reddish feature appears again in atrophic cervical areas.

One typical feature of abnormal cells is the higher nuclear density compared with normal cells. 5% dilute acetic acid solution reacts with the nucleus protein of abnormal cells in the HSIL region, leading to a reduction of transmittance. While the light can reflect back from the stroma after passing through the epithelial cell layers of normal cervical tissues, forming the acetowhite pattern in contrast with the abnormal tissues. Special attention should be paid on another variant of HSIL, thin HSIL, with cell layers less than 10.¹³ Under these circumstances, transmittance of the tissue is only reduced slightly, together with no thick acetowhiting effects. In this case, the time length of whitening effects and vessels in acetowhite areas is critical in distinguishing abnormality from normality.

Appearance of abnormal cells is always accompanied with new vessel formation. During expansion, the newly-formed vessels are squeezed by abnormal cells and appear irregular in the acetowhite epithelium, and the commonly-seen abnormalities are coarse punctuation, coarse unsymmetrical mosaics and other atypical vessels.

Lugol's solution stains cells with abundant glycogen. Normally, in the cervix, there is little glycogen present in columnar epithelial cells, immature metaplastic cells and the intermediate and bottom layers of squamous epithelial cells, whereas glycogen is rich in original and newly formed mature squamous epithelium. Therefore, in 2011 IFCPC colposcopic nomenclature,¹⁴ Lugol's staining (Schiller's test) was categorized as a non-specific staining test, and it is recommended that Lugol's staining should be performed on the acetowhite epithelium during colposcopy.

Based on standardized time control of the examination procedure and observation of changes analyzed by the computer, the R-way system could automatically identify CIN2+. The R-way system shows a higher sensitivity (77.81%) than the conventional colposcopic method does (with a sensitivity of 46.62%) in identifying CIN2+. If the R-way system is further combined with cytology, the sensitivity could reach 93.89%. With the R-way system, the AUC of the ROC curve reaches 0.839 (95% CI: 0.807–0.871). All these indicate that the R-way system performs better than the conventional colposcopic method in identifying CIN2+ lesions.

All technology has its limitations, and colposcopy is no exception. The R-way system complies strictly with the pathological features of cervical lesions. The R-way system would suggest a biopsy from the cervical bleeding area or endocervical bleeding area for patients detected with the presence of abnormal newly-formed

vessels and abnormal cells under colposcope, especially for those with bleeding (or ulceration) seen on the cervical surface or endocervical canal after application of 5% diluted acetic acid solution. For patients with cytological report of ASC-H, HSIL, SCC, AGC+ (including AGC-NOS and AGC-FN), AIS or Adca, multipoint biopsy or ECC is recommended.

In America, professional colposcopists need to know the techniques of colposcopy and also have knowledge of relevant subjects of lower genital tract, including the pathophysiology, cytology, cytogenetics, preventive medicine, basic research, molecular biology, gynecological oncology and gynecological endocrinology. In low resource countries, it is difficult to elevate the levels of the primary gynecologist in a short period, therefore enhancing the accuracy of CIN2+ detection is the current approach. With the R-way system, colposcopy is better at detection of CIN2+. To complete a colposcopy, the colposcopist needs to know: how to use the relevant equipment, the standard colposcopy procedure, the identification of the colour features (red, white and yellow) after application of the three examination solutions, and how to use a computer. Another factor deals with the colposcopist's experience. For most colposcopists, especially the primary colposcopist in rural areas, the accuracy and consistency of preliminary results varied from case to case. During colposcopy, the operator identifies the colour features in each step and marks them in real-time if required via the R-way system. Then the computer associates and compares images captured, performs analysis using the pathology-based R-way specialized database, and a more objective result will be addressed in this manner. Through simple training, the primary colposcopist can quickly grasp the use of the R-way system. This easy-to-use system provides a basis for a more accurate diagnosis by diminishing subjective judgment, hence it is of great importance for enhancing the efficiency of cancer screening, especially in low-resource countries.

Conclusions

The R-way system performs better than the conventional colposcopic method in identifying high-grade cervical lesions. Through time control of the colposcopy procedure, the R-way system standardizes the time points for each image captured, identifies the colour changes (red, white and yellow) in each step and evaluates the presence of a transformation zone. For its ease of use and standardized management of colposcopy procedure, R-way system is highly recommended for cervical cancer screening.

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References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin.* 4 Feb 2015.
2. Sigel R, Ma JM, Zou ZH, et al. *Cancer Statistics.* 2014. *Cancer J Clin.* 2014;64(1):9-29.

3. Qiao YL, Sellors JW, Eder PS, et al. A new HPV-DNA test for cervical-cancer screening in developing regions: a cross-sectional study of clinical accuracy in rural China. *Lancet Oncol.* 2008; 9, 929-36.
4. Quaas J, Reich O, Küppers V. Explanation and Use of the Rio 2011 Colposcopy Nomenclature of the IFCPC (International Federation for Cervical Pathology and Colposcopy): Comments on the general colposcopic assessment of the uterine cervix: adequate/inadequate; squamocolumnar junction; transformation zone. *Geburtshilfe Frauenheilkd.* Dec 2014;74(12):1090-92.
5. Massad LS, Einstein MH, Huh WK, Katki HA, Kinney WK, Schiffman M, et al. ASCCP Consensus Guidelines Conference. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. *J Low Genit Tract Dis.* Apr 2013;17(5) Suppl 1:S1-S27.
6. Petry KU, Luyten A, Scherbring S. Accuracy of colposcopy management to detect CIN3 and invasive cancer in women with abnormal screening tests: results from a primary HPV screening project from 2006 to 2011 in Wolfsburg, Germany. *Gynecol Oncol.* Feb 2013;128(2):282-7.
7. Cristiani P, Costa S, Schincaglia P, Garutti P, de Bianchi PS, Naldoni C, et al. An online quality assurance program for colposcopy in a population-based cervical screening setting in Italy: results on colposcopic impression. *J Low Genit Tract Dis.* Oct 2014;18(4):309-13.
8. Thulaseedharan JV, Malila N, Esmay PO, Muwonge R, Hakama M, Sankaranarayanan R. Risk of invasive cancer among women visually screened and colposcopy triaged by trained nurses in rural south India. *Int J Gynaecol Obstet.* 31 Jan 2015.
9. Ghosh I, Mittal S, Banerjee D, Singh P, Dasgupta S, Chatterjee S, et al. Study of accuracy of colposcopy in VIA and HPV detection-based cervical cancer screening program. *Aust N Z J Obstet Gynaecol.* Dec 2014;54(6):570-5.
10. Tatiyachonwiphut M, Jaishuen A, Sangkarat S, Laiwejpithaya S, Wongtiraporn W, Inthasorn P, et al. Agreement between colposcopic diagnosis and cervical pathology: Siriraj hospital experience. *Asian Pac J Cancer Prev.* 2014;15(1):423-6.
11. Di Stefano L, Patacchiola F, Necozone S, Paolone G, Di Febbo G, Carta G. The correspondence between abnormal transformation zone grade 1 and grade 2 colposcopic parameters and histology. Clinical implications. *Eur J Gynaecol Oncol.* 2014;35(1):16-9.
12. Diedrich JT, Felix JC, Lonky NM. Contribution of Exocervical Biopsy, Endocervical Curettage, and Colposcopic Grading in Diagnosing High-Grade Cervical Intraepithelial Neoplasia. *J Low Genit Tract Dis.* 28 Jan 2014.
13. Darragh TM, Colgan TJ, Thomas Cox J, Heller DS, Henry MR, Luff RD, et al. Members of the LAST Project Work Groups. The Lower Anogenital Squamous Terminology Standardization project for HPV-associated lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. *Int J Gynecol Pathol.* Jan 2013;32(1):76-115.
14. Bornstein J, Bentley J, Bösze P, Girardi F, Haefner H, Menton M, et al. 2011 colposcopic terminology of the International Federation for Cervical Pathology and Colposcopy. *Obstet Gynecol.* Jul 2012;120(1):166-72.