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The performance of multimodal hyperspectral spectroscopy in the detection of precancerous cervical lesions

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Abstract. The aim of this study was to compare the performance of multimodal hyperspectral spectroscopy (MHS), which combines fluorescence and reflectance spectroscopy, with that of conventional laboratory-based screening tests, such as the Papanicolaou (Pap) smear test and human papilloma virus (HPV) DNA test, for detecting precancerous lesions of the cervix. The study utilized a cross-sectional design, and the kappa test was used in the analytical assessment. MHS scans were obtained from a sample of 70 consecutive patients, followed by sample collection for Pap and HPV DNA analysis and colposcopy referral, if indicated. Of the 70 patients evaluated, the results of cervical spectroscopy were normal in 38 (54.3%) patients, and they were abnormal in 32 (45.7%) patients. Based on the cytology results, 45 (64.3%) samples were normal, and 25 (35.7%) samples were abnormal. According to the results of the HPV DNA test, 47 (67.14%) samples were normal, and 17 (24.28%) samples were abnormal. Based on the results of the kappa test, the agreement between MHS and cytology was 0.793 ($p < 0.001$). The agreement between MHS and the HPV DNA test was 0.195 ($p = 0.086$), and the agreement between MHS and colposcopy was 0.479 ($p < 0.001$).

1. Introduction

Cervical cancer is the second most common cancer in women worldwide [1]. In Indonesia, it is the leading cause of death among gynecological cancers.1Most patients with cervical cancer present at an advanced stage. With earlier detection, the incidence of mortality due to cervical cancer has been shown to decrease drastically. There are a number of methods to detect precancerous cervical lesions. These include visual inspections with acetic acid, cytological screening using the Papanicolaou (Pap) smear test, HPV DNA tests, and colposcopies. In many countries, the Pap test is routinely used to screen for cervical precancer. Published results, including those of a meta-analysis, showed that the sensitivity and specificity of the Pap test were 50–90% and 80–90%, respectively, whereas the sensitivity and specificity of liquid-based cytology were both 76%.2,3 The same research reported that the sensitivity and specificity of colposcopy were 70% and 44%, respectively [2,3]. The more recent HPV DNA test resulted in sensitivity and specificity of 90% and 86.5%, respectively [2,4]. Although the Pap test has been used for decades, the incidence of false negatives with this test is relatively high (10–50%) [5]. Thus, other investigations are necessary to improve its accuracy.

The liquid-based cytology (e.g., ThinPrep) Pap test improves the detection of low-grade precancerous lesions. However, a clinical examination strategy for cervical cancer based on this test and follow-up colposcopies has a number of limitations, including high costs. Strategies based on new technologies need to be developed and tested to improve the detection of cervical cancer at an early

stage (i.e., precancerous lesions of the cervix)and at a minimal cost [6]. The aim of the present study was to compare the performance of multimodal hyperspectral spectroscopy (MHS), which combines fluorescence and reflectance spectroscopy, with that of conventional laboratory-based screening tests (e.g., Pap smear test and HPV DNA test) for detecting precancerous lesions of the cervix.

2. Materials and Methods

This study used an analytical cross-sectional design. The study group consisted of 73 consecutive patients with precancerous lesions who were referred to the Colposcopy Clinic of the Department of Obstetrics and Gynecology of Cipto Mangunkusumo General Hospital because of abnormal colposcopy or abnormal cytology results. Some of the patients had received treatment earlier for precancerous lesions. All the subjects underwent MHS and other tests currently performed as part of standard care. These included a Pap test, spectroscopy of the cervix (MHS) on the day of the study, a colposcopy, and a biopsy if needed. A biopsy of the cervix was not performed when the results of a colposcopy was normal. However, as per the standard of care, a biopsy was performed in cases of a high-grade squamous intraepithelial lesion (HSIL), according to the results of the Pap test. This study was approved by the Research Ethics Committee of the Faculty of Medicine, University of Indonesia, and all the participants signed informed consent forms prior to the examinations. The inclusion criteria were patients aged over 16 years, scheduled for a colposcopy examination, Pap test results in the last 120 days before enrollment, and willing to be the subject of research by granting informed consent. The exclusion criteria were pregnant patients, bleeding or menstruating during the examination, a history of radiotherapy of the genitourinary tract, and excessive cervical mucus or an excessive discharge that could not be removed during the examination.

MHS derives its name from the two kinds (fluorescence and reflectance) of spectroscopy that are used in the procedure. In MHS, using white light from a xenon lamp, multiple beams of light are shone in a prescribed pattern over the entire cervix. This causes the cells in the cervix to fluoresce and reflect light back to the device where it is measured and analyzed. The light used in MHS causes photons that vibrate to “walk straight” and produce electromagnetic radiation patterns, which run perpendicular to each other. These photons elicit chemical responses (fluorescence) and physical responses (reflectance) in cervical cells. The fluorescent range of wavelengths in MHS covers the spectrum of 300–500 nm [7,8]. The reflectance range is wider (350–900 nm) [7,8]. In addition to a xenon light source, the spectroscopy device features a monochromatic system, which is controlled by a computerized system and projects the wavelengths of the light above the cervix through an optical fiber [9,10]. The working principle of MHS is the transmission of monochromatic light through a media (solution) onto the cytoplasm of the cervix. Some of this light is absorbed, whereas the remainder is partially reflected and partially transmitted. The resulting energy from the monochromatic system is computerized, separated into its spectral components, and directed through a rotating mirror as a filter. The detector converts the light into an electrical signal, which is then converted into a readable digital format and shown on a monitor screen. This information is then converted to a unitary numerical output, which corresponds to a low (normal), moderate, or high risk (abnormal) of the likelihood of cervical precancer with Nutritional Impact Scale (NIS) of 2-3.

Cervical spectroscopy was performed using a noninvasive device (LuViva, Guided Therapeutics, Inc. Norcross, GA, USA). In the initial examination, if mucus or blood were present in the cervix, it was removed by suction, without acetic acid. A single-use, hollow black tube was attached to the spectroscopy device. The function of this tube was to set the optical distance between the cervix and device while blocking ambient light. Once calibrated, the tube was inserted through the speculum into the vagina, with the tip of the tube touching the annulus of the cervix. This process was viewed on a monitor screen to ensure proper positioning and focus. The entire process, including the MHS scan of the cervix, took approximately 4–5 min. After scanning, the results of the examination were shown on the device’s monitor screen. After the spectroscopic examination was completed, the tube was removed. A sample was then obtained for liquid-based cytology and HPV DNA testing, if indicated. In addition, a colposcopy with acetic acid and Lugol was performed [11].

3. Results and Discussion

3.1 Results

The study consisted of 73 patients. Three patients were excluded from the study because of menstruation or bleeding, and one subject could not be tested due to a device error warning. Table 1 presents the demographic data on the patients. The age range of the patients was 21–60 years, and the mean age was 44 years (95% confidence interval [CI] 41.76 to 46.24). Most of the patients (44.29%) were older than 40 years or 50 years (27.14%).Table 2 shows the age distribution of the patients according to the level of the lesions.

Table 1. Demographic data on the patients

Characteristics	Total (*n*, %) *n* = 70
Age (years)	
20–29	8 (11.43)
30–39	12 (17.14)
40–49	31 (44.29)
> 50	19 (27.14)
Range	21–60
Mean (SD)	44 (9.59) 95%CI 41.6 to 46.2
Education	
0–9 years	9 (12.86)
10–12 years	38 (54.28)
≥13 years	23 (32.86)
Range	6–18
Mean (SD)	12.4 (2.74) 95%CI 11.77 to13.03
Parity	
0	5 (7.14)
1	14 (20)
2–3	35 (50)
≥4	16 (22.86)
Range	0–6
Mean (SD)	2.4 (1.4) 95%CI 2.08 to 2.72

Table 2. Age distribution according to the degree of nutritional impact scale (NIS)

Level of the lesion	Mean age (years)	Age range (years)	95% CI
Non-NIS	46.74	22–59	44.51 to 48.97
NIS 1	41.21	21–56	9.04 to 43.38
NIS 2	49.2	35–60	47.12 to 51.28
NIS 3	41.82	26–59	39.55 to 44.09

Table 3 presents data on the characteristics of the patients according to risk factors for cervical cancer. These characteristics were obtained from each patient's history. None of the patients had more than two sexual partners, and the majority of the women 64/70 (91.43%) had just one sexual partner. A total of 57/70 women (81.43%) reported first sexual intercourse at age ≥21 years (range, 13–40). Most of the patients also did not report the use of oral contraceptives 62/70 (88.57%) or a history of smoking 67/70 (95.71%).

Table 3. Characteristics of the patients according to risk factors for cervical cancer

Characteristics	Total (*n*, %) *n* = 70
Age at time of first sexual intercourse	
<17 years	4 (5.71)
17–20 years	9 (12.86)
≥21 years	57 (81.43)
Range	13–40
Mean (SD)	23.9 (4.737)
Number of sexual partners	
1 partner	64 (91.43)
≥2 partners	6 (8.57)
Range	1–2
Mean (SD)	1.09 (0.282)
Smoker	
Does not smoke	67 (95.71)
Smoker (10–20 cigarettes per day)	3 (4.29)
Contraceptive pill	
No	62 (88.57)
<5 years	6 (8.57)
>5 years	2 (2.86)

Table 4. Results of liquid-based cytology and spectroscopy

Device	Liquid-based cytology				
Spectroscopy	Negative	ASC	LSIL	HSIL	Total
Normal	34	3	0	1	38
Abnormal	11	10	6	5	32
Total	45	13	6	6	70

*ASC including ASC-US, ASC-H, and AG-US;
ASC=Atypical Squamous Cells; ASC-US=Atypical Squamous Cells Underdetermined Significance; ASC-H= Atypical Squamous Cells-cannot exclude HSIL; AG-US= Atypical Glandular Cells of Undetermined Significance; LSIL=low-grade squamous intraepithelial lesion; HSIL=high-grade squamous intraepithelial lesion

Table 5. Comparison of spectroscopy, HPV DNA, and colposcopy findings

Device	HPV DNA			Colposcopy		
Spectroscopy	Normal	Abnormal	Total	Normal	Abnormal	Total
Normal	17	6	23	18	20	38
Abnormal	10	11	21	12	20	32
Total	27	17	44	30	40	70

Table 5 presents the findings of the comparison of spectroscopy, HPV DNA, and colposcopy. As shown in the table, the results of the HPV DNA test were normal in 27 patients (61.36%), and they were abnormal in 17 patients (38.64%). In 34/38 (89.47%) patients, the spectroscopy results were normal (low) in patients with negative cytology results, and they were abnormal (high or moderate) in patients with abnormal cytology results in 21/32 (65.62%) cases. In 17/23 (73.91%) cases, the spectroscopy results were normal in patients with a negative HPV DNA test, and they were normal in patients with negative colposcopy results in 18/38 (47.37%) cases. Of the 70 patients who were examined using the spectroscopy device, 26 did not undergo a HPV DNA test. Therefore, there was an insufficient number of HPV DNA test samples for inclusion in the analysis, and the results of this test

can only be discussed within a descriptive or anecdotal context. The agreement between cervical spectroscopy in detecting precancerous lesions, as assessed by the kappa test and liquid-based cytology and colposcopy. The results of cervical spectroscopy showed good agreement with those of liquid based cytology, with a kappa value of 0.793 ($p < 0.001$) and moderate agreement compared to colposcopy, with a kappa value of 0.479 ($p < 0.001$), based on the criteria of Altman.

3.2 Discussion

Several previous studies examined the performance of cervical spectroscopy in terms of its sensitivity and specificity using histopathology as a gold standard endpoint. However, no previous studies have examined cervical spectroscopy in a population of Indonesian women. To the best of our knowledge, this is also the first study to investigate the agreement between cervical spectroscopy and both liquid-based cytology and colposcopy in the detection of precancerous lesions using the well-accepted kappa test. The cervical spectroscopy device used in this study differentiates a normal from an abnormal cervical epithelium, and it is intended for use in primary or secondary screening. By design, it does not show the location of abnormalities. This device uses wavelengths of light that project onto the entire ectocervix and distal endocervix and penetrate completely the epithelium of the cervix. The device's use of fluorescence spectroscopy to assess biochemical changes, combined with reflectance spectroscopy to assess structural changes that occur in precancer, allows an accurate diagnosis to be made. Using cervical spectroscopy, the biochemical activity of the amino acids tyrosine, phenylalanine, and tryptophan and the metabolism of nicotinamide Adenine Dinucleotide (NADH) and Flavin Adenine Dinucleotide (FAD) in an undifferentiated epithelium (i.e., normal) can be detected and compared against reference values to detect disease progression.

Reflectance spectroscopy assesses morphological alterations associated with the progression of cancer, as detected by changes in the cell nucleus, cell size, cell cytoplasm, and cell structure. Neo-angiogenesis can affect spectroscopy because blood absorbs light within specific bands. In the present study, both biochemical and morphological changes were assessed by the MHS spectroscopy method. A previous study showed that this method improved the sensitivity in the detection of cervical precancers [11]. In terms of clinical efficacy, one study indicated that the sensitivity and specificity of cervical spectroscopy were 97% and 70%, respectively, and that its performance was similar to that of liquid-based cytology [12]. Another study reported that a combination of spectroscopy and the Pap test increased the specificity from 27.4% for the test Pap and HPV DNA compared to 65.5% for the Pap test and cervical spectroscopy [10]. The same study reported that the sensitivity of the two strategies was the same, with both the Pap and HPV DNA test and the Pap test and cervical spectroscopy having sensitivity of 95%. In this study, we did not calculate or compare the sensitivity and specificity of the various diagnostic tests because biopsies and subsequent histopathological results from our cohort of patients were not planned and therefore not available. This was because many of the patients had precancerous lesions of the cervix that had been treated previously and so there was no need for a biopsy. Instead, with the aim of determining the potential utility of cervical spectroscopy in the health care setting, the present study investigated whether the results of spectroscopy agreed with those of other tests. The results revealed that the findings of cervical spectroscopy showed good agreement with those of liquid-based cytology using the kappa test. This finding supports the use of cervical spectroscopy as a primary screening test. In addition, as shown by the results of the kappa test, cervical spectroscopy showed a moderate level of agreement with colposcopy findings. The results of the kappa test were highly significant. Thus, cervical spectroscopy can be used either as an adjunctive method for triage or as a primary screening tool, combined with other tests, in the early detection of precancerous cervical lesions.

The present study also considered the incidence of precancerous lesions of the cervix in relation to known risk factors for cervical cancer, although we did not systematically examine the relationship between the various risk factors. Other studies reported that the following factors were associated with an increased risk of cervical cancer: multiparous, a young age (<16 years) at the time of first intercourse, multiple sex partners, a history of smoking, and a history of taking the contraceptive pill.

As compared with existing tests, such as the Pap and HPV DNA, the use of spectroscopic devices in precancer cervical screening has similar advantages to those of the Internal Vapor Analyzer (IVA) test. For example, the results of both cervical spectroscopy and IVA are available immediately, and they do not require the infrastructure and resources of an anatomic pathology laboratory. However, cervical spectroscopy also has limitations. For example, it is not suitable for use with a menstruating patient. In addition, it cannot be used immediately after a biopsy or if there are excessive secretions that cannot be removed, as the latter can interfere with the transmission of light. Nevertheless, cervical spectroscopy is a noninvasive, fast, and convenient test, and it can be used as a primary screening tool in combination with other examinations as indicated in women with an increased risk of developing cervical cancer [13].

This study had a number of limitations. First, patient's health insurance does not cover them for certain procedures. Patients with precancerous lesions referred to a primary and secondary care center before being referred to the specialized tertiary hospital. As a result, many of the patients have advanced disease by the time they were referred to the hospital. Second, we were unable to compare the results of HPV DNA testing with those of cervical spectroscopy because HPV DNA testing was performed in only a subset of the patients. Thus, the HPV DNA results were summarized. Third, we did not include the IVA test as an additional comparative method for detecting cervical precancerous lesions because this study was performed in a tertiary hospital that typically does not perform the IVA test. Finally, three subjects could not be tested and were excluded, two because they were menstruating and one because of a device error.

4. Conclusion

Based on the kappa values, the results of MHS showed good agreement (0.61 to 0.80) with those of cytology, and they displayed moderate agreement (0.41 to 0.60) with those of colposcopies. The agreement of the findings of MHS with those of HPV DNA test was not statistically significant, primarily because few HPV tests in the cohort were positive, including those of patients with NIS 3. The results of this study support the use of MHS for primary screening because of its good agreement with the Pap test and colposcopy, both of which are used as the standard of care in most countries with established screening programs. MHS has the added advantages of immediate results and no requirement for a laboratory infrastructure.

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