Research Article

The role of Multimodal Hyperspectroscopy for Detection of Cervical Neoplasia

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Abstract
Objective: to provide a prospective evaluation of the sensitivity and specificity of MHS as a new test for detection of high grade cervical dysplasia. **Methods:** 203 women previously screened with liquid based cytology were evaluated with multimodal hyperspectroscopy (MHS), colposcopy and biopsy samples taken for histopathology. **Results:** Sensitivity of MHS cervical scan for high grade intraepithelial neoplasia (CIN) 2+ was 86.6% and Specificity was 57.9%. **Conclusions:** MHS cervical scan is a noninvasive modality for detection of high grade cervical neoplasia with good efficacy.
Keywords: CIN, cervical cancer screening, multimodal hyperspectroscopy.

Introduction
Cervical cancer is the fourth most common female cancer worldwide.[1] Pap smear and colposcopy are widely-used methods for the detection of cervical cancer. 80% of all cervical cancer deaths are reported in developing countries, where these tests are not routinely practiced.[2] This emphasizes the importance of effective screening and early detection techniques. However, the existing screening techniques have been shown to suffer from high false results, which could be attributed to the subjective interpretations and may lead to more unnecessary referrals [3].

One of these approaches is light spectroscopy.[4] Spectral imaging appears to be a powerful approach which is starting to become applied to medicine [5] after it has been largely exploited in other areas, such as mineralogy, remote sensing, drugs screening and food qualification[6]. In fact, spectral imaging appears to be successful in distinguishing between tumor and normal tissues[7], and has been used to study skin lesions.[8] and breast cancer [9].

While cytology testing relies on morphological and staining patterns, biospectroscopy records the spectral information from tissues reflecting its biochemical composition at molecular levels, which occur before the changes in morphology are seen under the light microscope [10].

Reflectance spectroscopy allows determination of the scattering and absorption properties of a turbid medium such as tissue. It indicates the presence of structural changes within tissue (cell size, arrangement and organelle density, Neoangiogenesis).[11]

The fluorescence spectroscopy identifies metabolic changes associated with neoplasia. Intrinsic fluorophores can absorb light at different wavelengths and re-emit it, the most common fluorophores include collagen, elastin, tyrosine, nicotinamide Adenine Dinucleotide (NADH) and Flavin Adenine Dinucleotide (FAD).[12]
combining optical imaging techniques, referred to as multimodal imaging, allows for an improved diagnostic reliability due to the complementary nature of retrieved information[5].

Multimodal spectroscopy was implemented in a cost effective new device LuViva® that can be easily operated by trained medical personnel. It is supposed to have The advantage of early detection of cervical neoplasia [13].

Aim of the work:
To provide a prospective evaluation of the performance of MHS for detection of high grade cervical dysplasia

Methodology
This study included 203 women screened using

liquid based cytology. a participant considered eligible for the study if they were 21 years old or above, and willing to undergo MHS cervical scan, colposcopy and biopsy. Patients were excluded if they had any pregnancy, menstruating, prior hysterectomy, congenitally abnormal cervix, or excessive blood or mucus in the examination filed that cannot be removed.

Each participant had full history and clinical examination had been undertaken.
Cervical spectroscopy was performed using a noninvasive device (LuViva, Guided Therapeutics, Inc. Norcross, GA, USA). The system consisted of: base unit (light source, computer and monitor), handheld unit (optical systems), and the sight tube, (a hollow tube that is inserted into the vagina through a speculum).
The device collects and analyses fluorescence and reflectance spectra from the cervix without contrast agents. Light from the arc lamp is band passed, filtered to limit exposure of the cervix to three distinct color regions at wavelengths of 340nm, 400nm and 460nm, which excites fluorophores associated with neoplastic processes.

The resultant spectral output is imaged onto a charge coupled camera and stored for processing and analysis. In addition, the device contained a separate colposcopy quality imaging channel.

While patient in lithotomy position, speculum inserted, mucus or blood was removed by suction. the sight tube attached to the device to set the distance between the cervix and device while blocking ambient light. After calibrating the device, the tube was inserted into the vagina. This process was viewed on a monitor screen to ensure proper positioning and focus. Scan was performed in a 1 minute procedure.
The output results were color coded: - Green (Low risk): negative, further evaluation is not necessary - Orange (Moderate risk): other medical factors should be considered before further evaluation - Red (High risk): positive, further evaluation is necessary.

Then, a conventional colposcopy with 5% acetic acid was performed. endocervical curettage was performed for subjects that had LSIL or HSIL cytology. Then, biopsy from the ectocervix from abnormal areas or from the quadrants if no obvious abnormality was observed. Biopsy specimens were sent to histopathology.

Results

Study included 203 cases with mean age of 42.80±8.5 years and parity of 3.2±1.5. participants were subjected to MHS cervical scan based on different indications, 88 case (43.3%) were referred following result of abnormal cytology, 22 cases (10.84%) had history of contact bleeding, 26 cases (12.8%) were referred due to suspicious cervix, 3 cases were presented for follow up after LEEP biopsy for CIN 1, another 2 with mastectomy, one case known as HIV patient. And 61 cases (30%) were referred for routine screening. 11 cases had history of HPV infection, none of the included cases had history of HPV vaccination.
Cytology was normal in 46.7%, ASCUS was the most prevalent abnormality found in 22.6%, followed by ASCUH (8.9%) then LISL and HSIL (10.8% and 6.4%) and one case with AGC.

Colposcopy was negative in 60.1% of cases, 24 cases (11.82%) showed signs of chronic infection while CIN was detected in 32 cases (15.76%). Unsatisfactory colposcopy in 4 cases (1.97%), Acetowhite areas and abnormal vascularity were seen in 15 and 6 cases respectively.

For MHS: 108 cases were low risk (53.2%), 27 cases were with moderate risk (13.3%), and 65 cases were with high risk for cervical neoplasia (32%).

Test failed in 3 cases (1.48%) due to failed visualization of the cervix. 2 of them due to excessive blood, the device reported “poor contact”, the 3rd case failed due to abnormal light reflection from the threads of IUCD, the device reported “excessive light”. although, the test was performed in many cases with IUCD with no errors.

Normal histopathology was reported in 45.81% of cases, benign conditions included: inflammatory changes (17.73%), polyps (3.45 %), and metaplastic changes (11.33%). CIN 1 in 29 case (14.29%), and CIN2+ in 15 cases (7.39%). MHS was “high” in 46% of cases with abnormal cytology, while it was ‘low’ in 67.9% of cases with normal cytology.

Table 1: MHS correlated to cytology

MHS	Cytology						Total
	Negative	ASC-US	ASC-H	LSIL	HISIL	AGC	
Low	70	17	5	11	4	1	108
Moderate	12	8	2	2	3	0	27
High	19	21	10	9	6	0	65
Failed	2	0	1	0	0	0	3
Total	103	46	18	22	13	1	203

MHS reported high risk in 72.72% of abnormal colposcopic findings, it showed low risk in 70.83% of cases with normal colposcopy.

Table 2: MHS correlated to colposcopy

MHS	Colposcopy			Total
	Normal	Abnormal	Unsatisfactory	
Low	102	6	0	108
Moderate	17	8	2	27
High	23	40	2	65
Failed	2	1	0	3
Total	144	55	4	203

MHS was found 'high' in 30 cases out of 44 cases of dysplasia (75%). Adding the moderate risk MHS as a positive screening result, so 39 case were considered as positive out of 44 (88.6%).
In cases with severe dysplasia (CIN2+) MHS was positive in 13 case out of 15 (86.6)

Table 3: MHS correlated to histopathology

MHS	Histopathology				Total
	Benign	AGC	CIN1	CIN2+	
Low	104	1	2	1	108
Moderate	18	0	6	3	27
High	35	0	20	10	65
Failed	2	0	0	1	3
Total	159	1	28	15	203

Sensitivity of MHS for detection of 'any' dysplasia was 88.63%, and it had a specificity of 67.94%, with PP and NP values of 42.39% and 95.49% respectively
For high grade lesions (CIN 2+) MHS had a 86.66 % Sensitivity, 57.97% specificity, 14.13% PPV and 98.19% NPV. Excluding the failed cases increased the sensitivity to 92.85%, with 58.6% specificity, 14.44% PPV, and 99% NPV

Combining cytology and MHS results, the sensitivity raised to 100% for high grade lesions.
One case was examined post LEEP biopsy, reported as high risk with MHS despite being negative histopathology.

Using the kappa test, cervical spectroscopy showed 56.3% agreement with liquid-based cytology, and 74.5% with colposcopy, with high significance (p=0.001).

Discussion

This study included 203 women presented to the outpatient clinic either for primary (routine) screening or secondary screening or to be followed after treatment of cervical neoplasia.
We included this category of patients to our study for research reasons, as colposcopy and biopsy are parts of our evaluation.
In this study, The Sensitivity of MHS for detection of any degree of dysplasia was 88.63%, and its specificity was 67.94%. While,

For high grade lesions MHS cervical scan had a little lower Sensitivity (86.66 %), but much lower specificity (57.97%).
Several previous studies examined the performance of cervical spectroscopy using histopathology as a gold standard endpoint

The early pre-clinical trials, the largest was carried on 572 patients, The sensitivity was 95.1% for CIN2+ with a corresponding 55.2% specificity for benign lesions.[14]
A study of 113 women, compared results of cervical spectroscopy and HPV testing, concluded that Spectroscopic scanning of the cervix is equally sensitive (95%), and 2-fold more specific than HPV testing (66%, 27% respectively). Thus the use of cervical spectroscopy may reduce the number of false positive HPV test results.[15]

The largest phase 3 study was carried on 1850 women either presented for regular screening or referred for colposcopy, the sensitivity of MHS was 100% for detection of high grade lesions, 71% specificity. The device performance was best in the diagnostic rather than screening population [16]
A multi-centre study of 1,607 women with positive cervical screening test; compared the results of HPV testing, colposcopy and biopsy, with MHS. The Sensitivity of MHS for CIN2+ was 91.3%, the potential reduction in referrals to colposcopy and biopsy was 38.9% for women with benign histology and 30.3% for women with CIN1[17]. In a complimentary study by the same authors, 802 women were followed up for two years. MHS identified 89.6% of CIN2 + prior to their discovery during the follow-up period. They concluded that MHS as a triage would have reduced the need for further testing.

In our results MHS showed better performance in low grade lesions than with high grade lesions, This is in contrast to the results reported by Twiggs et al who found higher performance of MHS in higher grade lesions[17]. But in our study there was one case of CIN 2 which was missed due to test failure, which considered as false negative and it affected the test performance.
Combining cytology and MHS results for detection of high grade cervical lesions, The sensitivity raised to 100%. this is in agreement with results from the study by Werner et al., [15] however, Twiggs et al., combined both test results and found no increase in the sensitivity but the specificity increased by 30% in detection of neoplasia [17].

Also, Louwers et al., studied the colposcopic dynamic spectral imaging in 275 women, they reported sensitivity of 79% in detection of high grade lesions, and 77% specificity, while sensitivity of conventional colposcopy was only 55%, combining both test results gave higher sensitivity 88% but lower specificity 69% [18]

post launch trials reported variable results. in a pilot study by Adewole et al., [19] the sensitivity of MHS was 92.3%. MHS reduced the percentage of unnecessary colposcopy and biopsy by 37.5%.
Another report indicated that LuViva performed with a specificity of 87% in a screening population and it had potential as primary screening tool, especially in areas with no infrastructure for cervical cancer screening[20]. however, Cantor et al., reported that the device performed best in diagnostic population.[21]
In the current work, findings of MHS showed better agreement with those of colposcopy (74.5%) than with liquid-based cytology (56.3%).

In a recent study with similar methodology, good correlation between spectroscopy and both cytology and colposcopy was noticed (79.3% , 47.9% respectively) [13].
In our study, 3 cases were examined for follow up after LEEP cervical biopsy, one of them had false positive result by MHS. It was noticed that this case were scanned no more than 6 month after the procedure. This may be attributed to the distorted anatomy in the early post-operative period.

In the current work one case with AGC by cytology underwent fractional endometrial curettage and cervical cone biopsy, histopathology reported CGIN. while it was reported as low risk by MHS. It buts a question on the ability of the device to detect endocervical lesions. However, other reports recorded high sensitivity for intracervical lesions up to 100%. As that by Wade et al., they reported that the emerging light can penetrate and detect the deep epithelial, supepithelial or endocervical

lesions in contrast to cytology which only smears the superficial layers of cells.[22]

Conclusions

MHS cervical scan had good efficacy in detection of high grade cervical neoplasia. It may be used as a triage for women who has low grade cervical cytology. And it can be used as screening tool for routine screening.

References

1. World Health Organization (February 2014). "Fact sheet No. 297: Cancer". 2014-06-24. http://globocan.iarc.fr/Pages/fact_sheets_cancer.asx
2. Torre LA, Bray F, Siegel RL. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65:87.
3. Koliopoulos G, Nyaga VN, Santesso N. Cytology versus HPV testing for cervical cancer screening in the general population. Cochrane Database Syst Rev. 2017;8(8): 1-119.CD008587.
4. Gajjar K, Ahmadzai AA, Valasoulis G, Trevisan J. Histology Verification Demonstrates That Biospectroscopy Analysis of Cervical Cytology Identifies Underlying Disease More Accurately than Conventional Screening: Removing the Confounder of Discordance. PLoS ONE 2013; 9(1): e82416.
5. Vogler N, Heuke S, Bocklitz TW, Schmitt M. Multimodal Imaging Spectroscopy of Tissue. Annu Rev Anal Chem (Palo Alto Calif). 2015;8:359-87.
6. Guolan Lua, Baowei Fei. Medical hyperspectral imaging: a review. J Biomed Opt. 2014; 19(1): 1-23.
7. Riccardo Cicchi, Suresh Anand, Riccardo Fantechi, Flavio Giordano, Valerio Conti, Gabriella Nesi. Multimodal fiber-probe spectroscopy as a clinical tool for diagnosing and classifying biological tissues. Clinical and Preclinical Optical Diagnostics. SPIE 10411, (2017); doi.org/ 10. 1117/12.2283278
8. Moy, Austin, Feng, Xu, Markey, Mia, Reichenberg, Tunnell, James. Noninvasive skin cancer diagnosis using multimodal optical spectroscopy. Photonic Therapeutics and Diagnostics.2016;9689:1605-7422
9. Alchab L, Dupuis G, Balleyguier C, Mathieu MC, Farcy R. Towards an optical biopsy for the diagnosis of breast cancer in vivo by endogenous fluorescence spectroscopy. J Biophotonics.2010;3(5-6):373-84.
10. Kelly JG, Angelov PP, Trevisan J, Vlachopoulou A, Paraskevaidis. Robust classification of low-grade cervical cytology following analysis with ATRFTIR spectroscopy and subsequent application of self-learning classifier. Anal Bioanal Chem. 2010; 398: 2191–2201.
11. Tatiana Novikova. Optical techniques for cervical neoplasia detection. Beilstein J Nanotechnol. 2017; 8: 1844–1862.
12. Drezek R, Brookner C, Pavlova I, Boiko I, Malpica A. Autofluorescence microscopy of fresh cervical tissue sections reveals alterations in tissue biochemistry with dysplasia. Photochem Photobiol. 2001;73: 636–41
13. Trahmono, Lusiana, Indarti. The performance of multimodal hyperspectral spectroscopy in the detection of precan-cerous cervical lesions. J. Phys.: Conf. Ser. 2017; 884: 012152.
14. DeSantis T, Chakhtoura N, Twiggs L, Ferris D, Lashgari M, Flowers L. 2007 Spectroscopic imaging as a triage test for cervical disease: A prospective multicentre clinical trial. J. Low. Gen. Tract. Dis.11 18-24.
15. Werner L., Griffith F., Ashfaq R, Wilkinson E, Faupel M. Comparison of Human Papilloma Virus Testing and Spectroscopy Combined With Cervical Cytology for the Detection of High-grade Cervical Neoplasia. Journal of Lower Genital Tract Disease. 2007; 11(2):73-79
16. Cantor SB, Yamal JM, Guillaud M. Accuracy of optical spectroscopy for the detection of cervical intraepithelial neoplasia: Testing a device as an adjunct to colposcopy. Int J Cancer. 2011;128(5): 1151–1168
17. Twiggs L., Chakhtoura, N., Ferris G., Flowers C., Winter L., Sternfeld R., . Wilkinson, J. Multimodal hyperspectroscopy as a triage test for cervical neoplasia: Pivotal clinical trial results. Gynecologic Oncology. 2013; 130(1): 147-151.
18. Louwers J, Zaal A, Harmsel WT, Graziosi G, Spruijt J, Papagiannakis E, Meijer C,Kemenade FV, Verheijenb R. Dynamic spectral imaging colposcopy: higher sensitivity for detection of premalignant cervical lesions. BJOG 2011; 118:309–318

19. Adewole F Isaac, Awolude A Olutosin, Akinwunmi O Babatunde. Multimodal hyperspectroscopy screening in women at risk of cervical cancer: Results of a pilot study in a developing country. Trop J Obstet Gynaecol. 2017; 34 (2): 134-139
20. Omoya SO, Obimakinde AM, Fasubaa OB, Olomojobi OG, Alabi OO. Cervical screening with Luviva machine for early detection of cervical dysplasia: Experience from Ekiti state, Nigeria. Trop J Obstet Gynaecol 2014;31:82-9.
21. Cantor Scott, José-Miguel, Martial Guillaud, Dennis. Accuracy of optical spectroscopy for the detection of cervical intraepithelial neoplasia: Testing a device as an adjunct to colposcopy. International journal of cancer. 2011; 128. 1151-68.
22. Wade R, Spackman E, Corbett M, Walker S, Light K. Adjunctive colposcopy technologies for examination of the uterine cervix – DySIS, LuViva Advanced Cervical Scan and Niris Imaging System: a systematic review and economic evaluation. Health Technol Assess. 2013;17(8):1-240