

Multimodal hyperspectroscopy screening in women at risk of cervical cancer: Results of a pilot study in a developing country

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ABSTRACT

Background: Screening and treatment of pre-cancerous lesions is important for prevention of cervical cancer. Currently, most available screening tests for cervical cancer are limited by low sensitivity, prohibitive costs, logistics and technical concerns. This study evaluates the role of multimodal hyperspectroscopy (MHS) as a cost-effective, sensitive and user-friendly point-of-care machine for early detection in women at risk of pre-cancer lesions.

Materials and Methods: Multimodal hyperspectroscopy of the cervix using the LuViva® Advanced Cervical Scan was performed first in a 1-minute procedure among 100 previously screened for cervical cancer using either visual inspection after application of acetic acid (VIA) or cytology within the last 120 days. This was then followed by obtaining human papilloma virus (HPV) samples and biopsies from women for histology.

Results: Of the 22 women with abnormal Pap tests of at least low-grade squamous intraepithelial lesion, 3 had CIN2+, 6 had CIN1, 4 were free of dysplasia at histopathology while 9 had cervicitis. All 3 of the CIN2+ recorded high likelihood of CIN2+ by MHS. However, HPV was negative for all 3 women. The machine classified 1 of 1 CIN1s and 7 of the 13 women without dysplasia or cervicitis as low or moderate risk for CIN2+ (40% specificity); of the 37 women who were VIA+, 81% were classified as high risk, and 66% of 37 women with normal Pap tests and biopsy were either at moderate or low risk.

Conclusions: The findings from this pilot study show that MHS reduced the percentage of unnecessary colposcopy and biopsy by 37.5%. It was also able to differentiate between VIA+ and Pap negative women suggesting its potential of being a point-of-care primary and objective screening test.

Key words: Cervix; hyperspectroscopy; pre-cancer; screening; sensitive.

Introduction

Cervical cancer remains one of the leading causes of cancer-related mortality among women in developing countries with about 266, 000 of such mortalities per year.^[1] Like most cancers, cervical cancer is easily treated if diagnosed early, and the uterine cervix is one of the few cancer-afflicted organs that can be accessed and visualized in a noninvasive manner. If detected in its pre-cancerous state, treatment can lead to almost a 100% survival rate at 5 years.^[2]


The Pap test, invented in the 1940s by Greek physician Georges Papanicalou, is the most common method for screening, especially in the developed countries of the world. Various meta-analyses have shown the detection rate or sensitivity of the Pap test to be highly variable, ranging 20–52% with a corresponding specificity of 90–95%.^[3] Findings after a

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Pap test can include atypical squamous cells of undetermined significance (ASC-US) and low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL) or invasive cancer. Although these findings provoke millions of follow-up Pap tests, colposcopies and biopsies at a cost in excess of \$6 billion annually, only about 5% of ASC-US and 10% of LSIL Paps actually reflect an underlying cervical intraepithelial lesion 3 (CIN3), an immediate cancer precursor.^[4,5] In addition, for various reasons, many women with abnormal Pap tests are lost to follow-up before receiving a definitive diagnosis or appropriate intervention. Even with colposcopy, diagnosis is imperfect, with reported sensitivities ranging 53–85% for high-grade dysplasia^[6-8] and reported specificities of 48% and 52% based on any dysplastic lesion detected. Therefore, a significant number of women have colposcopies and biopsies for benign conditions. Of the screening Pap tests conducted, approximately 8–9% result in ASC-US readings and approximately 5% are LSIL,^[9] resulting in over 6 million potential ASC-US/LSIL triage tests. An additional 2.24% are diagnosed as HSIL. Human papilloma virus (HPV) DNA testing is currently approved by the FDA for use in detecting the presence of the cancer-causing, high-risk HPV types. The American Society of Colposcopists and Cervical Pathologists (ASCCP) 2006 Consensus Guidelines indicate that ASC-US cytology findings can be followed either with HPV testing or repeat cytology. Women with LSIL cytology are referred to colposcopy if over the age of 20, but the new guidelines indicate that 12-month follow up is appropriate for women aged 20 and under. HPV testing is not indicated for use in young women due to its very high false positive rate and correspondingly low positive predictive value (10% in its intended use population according to the ALTS trial) or in women of any age with LSIL or HSIL as 84% of LSIL and nearly all of HSIL is HPV DNA positive. In addition, the Bethesda 2001 (and 2008 revisions) cervical cytology guidelines^[10] categorize ASCs as “undetermined significance (ASC-US)” or “cannot exclude HSIL (ASC-H),” the latter category can also refer directly to colposcopy.

The implication of these are the associated high expenses, both human and financial, to the patients, health care providers and to the society in general. Many women with benign cervical disease experience anxiety and confusion over ambiguous test results in addition to lost productivity and expenses to undergo invasive procedures. These procedures are not without risk, including possible morbidity due to over-referral. At the same time, excessive demand for repeat and/or invasive diagnostic procedures not only increases the cost of health care but also delays access to definitive diagnosis and treatment for some women who truly are at significant risk for developing cervical cancer. This problem has been exacerbated by the recently documented increase

in false positive results produced by the increased use of thin layer cytology and HPV testing.^[11]

There remains, therefore, a significant need for a point-of-care test to improve the screening and diagnosis as well as management of cervical disease, especially for improving sensitivity and lowering the false positive rate. Such point-of-care tests include devices based on the principle of fluorescence and reflectance spectroscopy. Fluorescence and reflectance spectroscopy have been shown to be valuable in cancer diagnosis by several investigators. An early review has outlined the various spectroscopic methods and challenges.^[12] However, much of the work done in this area has been on excised tissue. Several biophysical changes occur rapidly within tissue following excision. For example, changes in blood oxygenation and perfusion in the tissue vasculature cause changes in the reflectance readings and the change in the redox state of NADH, a key tissue fluorophore that affects fluorescence measurements made using 340–350 nm excitation which, in our view, may have limited utility as criteria for *in-vivo* diagnostics.

Several groups are investigating the potential of spectroscopic techniques to provide a quantitative measure of neoplasia without tissue removal. The main advantage of such spectroscopic techniques relies on their being able to detect and quantitatively assess changes in the cellular milieu and tissue architecture associated with the progression of disease. Several *in-vivo* studies appear in the literature showing the performance of either fluorescence or reflectance spectroscopy in discriminating between normal tissue and different epithelial cancer grades. These include cervical,^[13-16] colon,^[17-20] gastrointestinal tract,^[21] skin^[22] and lung^[23] cancers. These studies use point measurements of areas that are either suspect or normal based on heuristic determinations. For cervical cancer and its precursors, sensitivities in the range of 80–90% and specificities in the range of 70–80% have been reported for discriminating between LSIL and HSIL. Only a few investigators have used multiple spectroscopic modes to improve their diagnoses, and in these cases, improvement over individual modes has been documented.^[24] By using multimodal spectroscopy in an imaging system, a unique, cost-effective approach that has the potential to reduce morbidity from over treatment due to unnecessary referrals and significantly improve patient care while simultaneously reducing the enormous costs currently associated with cervical disease management was developed. One of such devices is LuViva® (Guided Therapeutics, Norcross, GA USA), a multimodal hyperspectroscopic (MHS) device.^[25] The device non-invasively collects and analyses fluorescence and reflectance spectra from the cervix without contrast agents such as acetic acid. Light from the arc lamp is band passed,

filtered to limit exposure of the cervix to three distinct regions centered at 340 nm, 400 nm and 460 nm, which excites fluorophores associated with neoplastic processes. The resultant fluorescent spectral output of the cervical tissue is imaged onto a charge coupled device (CCD) camera and stored for processing and analysis. In addition to the fluorescence and reflectance spectroscopy channel, the LuViva also contains a separate colposcopy quality imaging channel. The purpose of this imaging channel is two-fold: (1) to allow real-time imaging of the cervix for centration guidance while the cervical guide is being placed inside the vagina and (2) to allow images to be taken for documenting the placement of the device and appropriate centering of the cervix within the 1-inch diameter measurement area.^[26]

We, therefore, set to evaluate this MHS (LuViva®) for the early detection of cervical cancer in a population of women at risk of cervical cancer.

Patients and Methods

Study population

The participants in this study were 100 women previously screened for cervical cancer using either VIA or conventional cytology, irrespective of the result, within the last 120 days prior to the day of enrollment. To be considered for enrollment in the study, a participant had to be 21 years old or above, able to give informed consent, had a negative pregnancy test or with documentation of acceptable birth control and willing to undergo LuViva®, HPV testing, colposcopy and biopsy of the cervix on the day of the study following previous positive VIA test, normal cytology test, LSIL or HSIL from cytology screening. A potential participant was not considered for enrollment in the study when she had any pregnancy, menstruating, previous macroscopic cervical disease or prior hysterectomy. Moreover, conditions relating to the cervix that would render the test difficult to perform, including but not limited to excessive blood or mucus that cannot be removed, congenitally or acquired abnormal cervix or inability to tolerate speculum or LuViva® single use cervical guide, made a woman ineligible for enrollment.

Study procedure

Each participant had, after consenting to the study, placement of the LuViva® system. A video image from the device was used to guide placement of the hollow cervical guide for centering of the cervix in the visual field, capture locations of lesions for comparison with the spectroscopic image and as a quality check after spectroscopic measurements for detecting whether excessive movement of the cervix occurred or if there was excessive mucous or blood. Hyperspectroscopy of the cervix using the LuViva® Advanced Cervical Scan was performed in

a 1-minute procedure. After the LuViva® test, a sample for Hybrid Capture 2 HPV testing, using a cytobrush was collected for all patients followed by colposcopy and biopsy from the ectocervix after application of 5% acetic acid from the observed abnormal area and from the quadrants if no obvious abnormality was observed. If a lesion was not seen with acetic acid, Lugol's solution was applied. Thus, the gold standard will be negative VIA and negative HPV for women diagnosed as normal and most severe histopathology result for women with disease. A key component of effective screening studies designed to evaluate new detection modalities is verification of the gold standard comparison by which estimates of sensitivity and specificity are generated. Prior to beginning this pivotal trial, the site study team were trained on 5 patients. Data from these training cases were not included in the clinical trial data set.

Results

The participants' baseline characteristics analysis showed that the mean age was 44.2 (SD = ±8.2) years. Most women (84%) were multiparous with a modal parity of 3. The mean age at first sexual exposure was 17.1 (SD = ±3.5) years, and the mean age at first delivery was 26.4 (SD = ±2.6) years (Table not shown).

In this pilot study, 26 of the 100 participants were referred to colposcopy based on an abnormal Pap result, 37 based on positive VIA test and 37 with normal Pap smear results to serve as control.

Table 1 shows the cytology and VIA results as a function of histopathology. Of the 37 patients with negative results from cytology, histopathology could identify 5 (13.5%) as normal, 4 (11.4%) as CIN I; 2 (5.4%) as CIN 2 and 2 as CIN 3 (1 with and without cervicitis each). There were 24 (64.9%) cases with various degree of cervicitis without dysplastic changes. Histopathology confirmed 1 of the ASC-US as CIN 3 and the other one as normal while the 2 atypical glandular cells (AGC) were confirmed non-dysplastic (one each with and without cervicitis) by histopathology. Four of the 16 LSIL were confirmed normal, 6 with cervicitis, 5 as CIN 1 (3 without and 2 with cervicitis, respectively), one case of CIN 2 with cervicitis and no case of CIN 3 with histopathology while 3 of the HSIL were confirmed with cervicitis with 1 each confirmed as CIN 2 and CIN3, respectively, with histopathology. For the 37 participants referred based on positive VIA, 11 (29.7%) were confirmed normal by histopathology, 17 (45.9%) with cervicitis, 5 as CIN 1 (one without and 4 with cervicitis, respectively), 4 (10.8%) as CIN2 (one without and 3 with cervicitis, respectively) and none as CIN3. From this study, the overall sensitivity of cytology in identifying dysplastic changes of the cervix was 60% and specificity of 64.4% while for VIA it was 68.4% and 69.1%, respectively.

Table 1: Cytology and VIA+ results as a function of histopathology

Cytology	Histopathology									
	Normal	Cervicitis	CIN I	CIN I+ cervicitis	CIN II	CIN II+ cervicitis	CIN III	CIN III+ cervicitis	Total	
Negative	5	24	4	0	2	0	1	1	37	
ASCUS	1	0	0	0	0	0	1	0	2	
AGC	1	1	0	0	0	0	0	0	2	
LSIL	4	6	3	2	0	1	0	0	16	
ASC-H	0	0	0	0	0	0	0	0	0	
HSIL	0	3	1	0	1	0	1	0	6	
VIA+	11	17	1	4	1	3	0	0	37	
Total	22	51	9	6	4	4	3	1	100	
	22	51		15		8		4		

Table 2: HPV results as a function of histopathology

HPV results	Histology			
	Normal	CIN 1	CIN 2	CIN 3
HPV+	5	2	1	3
HPV-	64	15	5	3
HPV (no result)	2	0	0	0
Total	71	17	6	6

An important finding from this study is the high rate of cervicitis in the patients at 62% (62/100). While the cervicitis, in various degree of severity, was the only histopathological finding in 51 of the 62 cases and with no dysplastic changes, it was a co-existing finding in 6 of the 15 cases of CIN 1, 4 of the 8 cases of CIN 2 and 1 of the 4 cases of CIN 3.

In all 14 histopathology confirmed cases of CIN1 of which 5 (35.7%) were recorded RED (high likelihood of CIN2+) by MHS, 8 cases of CIN 2 of which 7 (87.5%) were recorded RED and 4 cases of CIN3 all (100%) of which were recorded as RED. Using CIN2+, the sensitivity of MHS at RED threshold was 91.7% (11/12). Of the 51 cases diagnosed with cervicitis only, histopathologically 10 (19.6%) were recorded as GREEN (low likelihood of CIN2+) and 7 (13.7%) were recorded as YELLOW by MHS whereas the remaining thirty-four (66.7%) were recorded as RED by MHS.

Overall, 11.2% (11/98) of all women in the study were positive for high-risk HPV [Table 2]. This included 5 of the 69 with normal histopathology results, 2 of 17 CIN1, 1 of 6 CIN2 and 3 of the 6 with CIN3.

In all the sensitivity of MHS in identifying any dysplastic lesion of the cervix was 92.3% whereas the specificity was 37.5%.

Discussion

Characteristics of the participants

This pilot study evaluated the ability of LuViva^(R), an MHS device, to correctly identify women at risk for cervical cancer in mixed population of previously screened women. A study

using the same device in our environment was among women without prior cervical cancer screening history.^[27]

Cytology and VIA+ results as a function of histopathology

Sixty-five of the 100 women in the study were referred to colposcopy based on either an abnormal Pap result or a positive VIA. Visual inspection with application of acetic acid could identify 7 CIN1, 5 CIN2 and only 1 CIN3, which is the goal of screening. Referral of patients with Pap smear results at the LSIL threshold identified 5 of 10 CIN1 as positive, missed 1 CIN2 and identified 3 of 5 CIN3 as positive (sensitivity of 60%). At histopathology, 3% of the VIA positive cases and 12% of the LSIL cases from cytology were diagnosed as CIN 3.

Cervical inflammation has been hypothesised to be capable of influencing the carcinogenic process of the cervix driven by HPV.^[28] The cervicitis, especially the infective types, can be risk factors for the persistence of HPV^[29] and the chronically induced dysplastic lesions. These and the high rate of cervicitis of 62% in this study might suggest the need for further studies to ascertain the role of genital microbial co-infection with HPV in the aetiopathogenesis of cervical pre-cancer and cancer lesions.

Positive HPV results were lower among these patients than expected based on the population of referred women who were previously screened. In all, only 11.2% of 98 women with HPV results in the study were positive for high-risk HPV (hrHPV). This included 5 of the 69 women with normal histopathology and available HPV results 2 of the 17 CIN 1 (11.8), only 1 of 6 with CIN2 (16.7%) and 3 of 6 with CIN3 (50.0%). The reasons for this are not completely clear but could include pre-, intra-, or post-analytical errors and/or degradation due to environmental conditions. Another possible explanation might be the average age of women in the study which was 45 years. Studies have shown that HPV infection rates can be much lower in women above the age of 40 and that the sensitivity of HPV is also lower in this

Table 3: Multimodal Hyperspectroscopy Triage Performance

a. Green/Yellow threshold			b. Yellow/Red threshold		
Sensitivity (CIN 2+)	Sensitivity (CIN 1)	Specificity (no dysplasia)	Sensitivity (CIN 2+)	Sensitivity (CIN 1)	Specificity (no dysplasia)
100% (3/3)	0% (0/5)	33% (5/15)	100% (3/3)	20% (1/5)	40% (6/15)

age group.^[30] In support of this, we found that HPV false negatives occurred more in older Nigerian women (median age = 51.5 years) compared with women having true positive HPV results (median age = 43.0 years), although sample sizes are small.

Multimodal hyperspectroscopy triage performance

Of the 100 women enrolled in the study, 20 qualified as Luviva® screening for possible triage based on referral Pap results of ASC-US/AGUS ($n = 4$) or LSIL ($n = 16$). An additional 6 patients with HSIL Pap smear were tested, however, HSIL is contra-indicated for Luviva® due to the high likelihood of significant cervical disease. Of the 24 women with abnormal Pap smear of at least LSIL, 3 were found to have CIN2+ at histology. All these 3 recorded RED (high likelihood of CIN2+) by Luviva® giving 100% sensitivity. It is of interest that HPV was negative for all 3 of these patients. Review of the remaining 21 women without CIN2+ showed that 5 were found to have CIN1, of which LuViva® recorded four as RED (high likelihood of CIN2+) and one as YELLOW (moderate likelihood of CIN2+). Fifteen of the referred patients were found to have cervicitis ($n = 9$) or normal histology ($n = 6$), 5 of whom were recorded as GREEN (low likelihood of CIN2+) and 2 were recorded as YELLOW (moderate likelihood of CIN2+) by Luviva®. The remaining 8 were recorded as RED (high likelihood of CIN2+) by Luviva®. Therefore, the sensitivity and specificity of the triage patients were as follows. At the Green/Yellow threshold, the Luviva® cervical scan sensitivity was 100% for cervical lesions with likelihood of CIN2+ while the specificity was 33%. The sensitivity remained 100% at Yellow/Red threshold with slight improvement of the specificity to 40% [Table 3].

Screening performance of Luviva® cervical scan device

Because the prevalence of CIN2 and especially CIN3 was very small in the population of women with normal Pap results we compared the Luviva® results for women screened positive by visual inspection with acetic acid (VIA+; $n = 37$) with those with Negative Pap results ($n = 37$). Using the Luviva® algorithm developed for triage of HPV+ (but Pap negative) referred patients, 23 of the 35 women (66%) with negative Pap results produced a LuViva result in the GREEN (low likelihood of CIN2+) or YELLOW (moderate likelihood of CIN2+) zone, whereas only 7 of the 36 VIA+ women did (19%). Thus, LuViva could differentiate VIA positive women from Pap negative women at a sensitivity of 81% and a specificity of

66%. Additional work with larger populations is needed to re-calibrate LuViva®'s algorithms to identify CIN3+ cases within screened populations.

Strength and limitations

The main strength of our study is its inclusion of both screened negative women and women referred as VIA and cytology positive, thus giving the examiners a wide range of participants with normal to pathological cervical status and a reduction of the risk of selection bias. Other strengths are that all the biopsies were analysed in a single-site laboratory and the included women were all examined in a single centre. Moreover, screening with the hyperspectroscopy device was performed by the same person, thus reducing the problem of intra-observer variations. The main weakness of our study is the small sample size making the result less generalizable. However, as a pilot, especially in a developing country, it is a template for a larger study.

Conclusion

This study showed that MHS when used in clinical setting has 92.3% sensitivity of identifying pre-cancer lesions of the cervix. It also reduced the percentage of unnecessary colposcopy and biopsy by 37.5% without any false negative result. These findings were consistent with other studies conducted in North America.^[25,26] It has the added advantage to differentiate between VIA+ and Pap negative women. The implication of all these is that the machine has the potential of being a point-of-care primary and objective screening test for pre-cancer lesions of the cervix.

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Conflicts of interest

There are no conflicts of interest.

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