Multi-spectral diffuse reflectance imaging for detection of cervical lesions: a pilot study

V. G. Prabitha, S. Suchetha, J.L. Jayanthi, P. Rema, K. V. Baiju, Nita Sukumar, Anita Mathews, Paul Sebastian, N. Subhash

Abstract - Early detection of cervical cancer is a growing concern worldwide. Current screening techniques based on Pap smear and colposcopy burden the health care system with excessive costs, unnecessary anxiety, discomfort and the patient has to wait for a few days for biopsy results. This paper reports a non-invasive and real-time diagnostic tool based on diffuse reflectance (DR) imaging for screening of cervical cancers. The DR spectra of white light consist of dips at 545 and 575 nm due to oxygenated hemoglobin absorption and the previous studies on oral cancer patients using a multi-spectral DR imaging system has shown that reflectance image ratio R545/R575 could effectively discriminate healthy tissue from premalignant and malignant lesions. In the present study DR images were recorded from 41 patients and the image ratio R545/R575 was determined from the monochrome images recorded at 545 and 575 nm and the same was pseudo-color-mapped to examine variations in the ratio value across the lesion. The DR image ratio value from the biopsied site was compared with histopathology results and the specificity and sensitivity of the imaging technique was determined. It was observed that low-grade cervical intraepithelial neoplasia (low grade CIN) could be discriminated from normal tissues with a sensitivity of 87.5% and specificity of 78.3% whereas high-grade cervical intraepithelial neoplasia (high grade CIN) could be discriminated from normal with 100% sensitivity and specificity. In comparison, low-grade CIN was discriminated from high-grade CIN with a sensitivity of 88.9% and specificity of 87.5%. The results of the study show the potential of DR image ratio in non-invasive and real-time screening cervical precancers and in the identification of the most malignant site in the cervix for biopsy.

Index Terms— Cervical intraepithelial neoplasia, image intensity ratio, multi diffuse reflectance imaging system, oxygenated hemoglobin absorption,

I. INTRODUCTION

Cervical cancer is one of the leading causes of death in women worldwide, which can be prevented with effective screening techniques for early detection of precancerous lesions. Lack of resources and expensive infrastructure has led to a high death rate from cervical cancer in developing countries [1]. Conventional techniques for early detection of neoplasia using pap smear and colposcopy have significant limitations. Colposcopic image interpretation relies mainly on the expertise of personnel [2], [3]. Multiple visits, low specificity of visual examination and discomfort during biopsy prevent women from regular screening [4]. Visual inspection with acetic acid (VIA) has been explored as an alternative to pap smear screening and colposcopy in many developing countries [5]-[7]. Even though VIA is inexpensive and requires minimal infrastructure, it is also based on visual interpretation, which is subjective. Thus, there is a need to develop an effective and affordable see-to-treat screening strategy for cervical cancer.

Advances in solid state technologies and image processing techniques with automated image diagnosis algorithms have led to development of high quality optical imaging systems at relatively low cost. Several studies have established the potential of different diagnostic image analysis tools to discriminate neoplastic cervical tissues from normal [8]-[10]. The reliability of cervical cancer diagnostic results of digital colposcopy compared with conventional binocular colposcopy is significant [11].

Spectroscopy is a non-invasive method capable of measuring structural and molecular changes of cervical tissues during neoplastic progression. Spectroscopic imaging based on tissue fluorescence has shown its potential for the detection of cervical malignancies [12], [13]. A multimodal hyper-spectral imaging device that collects and analyses both fluorescence and reflectance spectra from the cervix in vivo was able to detect CIN with improved accuracies than a simultaneously obtained Pap smear [14]. Gustafsson et al [15] introduced a hyper-spectral imaging spectrograph to measure the fluorescence and reflectance of cervical tissues in vivo by generating a hyper-spectral image cube. Agrawal et al [16] developed an algorithm to discriminate non-dysplastic tissues from
dysplastic cervical tissue using multimodal multi-spectral imaging (MMI) device based on fluorescence and reflectance. These studies have shown that spectroscopic imaging can become an adjunct to conventional colposcopy and possibly replace the traditional diagnostic techniques in the near future.

CT and MRI scanning are commonly used for cervical cancer imaging. In particular, CT scan directed biopsy is believed to be useful for obtaining histological confirmation of recurrence. There are concerns, however, that these techniques may result in false positives due to the inability to distinguish between tumor masses and masses of necrotic or scar tissue, and false negatives due to the inability to identify small tumors. In comparison, optical coherence tomography (OCT) is a non-invasive technique capable of providing images of tissue structures at a cellular level to diagnose cervical lesions without taking a biopsy [17], [18]. Confocal optical imaging can detect superficial lesions and therefore seems suitable for early detection of precancerous lesions [19]. Positron emission tomography (PET) with 18F fluorodeoxyglucose (FDG) is proposed as an alternative to CT and MRI to confirm cervical cancer. PET is commonly used with the biological tracer FDG, which allows evaluation of glucose metabolism.

Since all these imaging techniques are more complex and expensive to implement, development of a cost-effective screening strategy that retains the advantages of spectroscopic imaging is essential for widespread cervical screening in the third world countries. The Multi-spectral Diffuse Reflectance Imaging System (MDRIS) that was developed in our laboratory for oral cancer screening makes use of the advantages compactness, safety and cost as compared to laser based systems [20]. Even though the point monitoring systems that use fiber optic probes [21], [22] are simple and efficient, the probe tip when kept in contact with cervix during measurement exerts a mild pressure that leads to bleeding and obscuration of diagnostic information from the recorded DR spectra. Therefore, use of a noncontact imaging camera would be more suited as it can collect the diffusely reflected light from the irradiated tissue in the preferred oxygenated hemoglobin absorption bands at 543 and 577 nm using suitable filters. Using MDRIS diffusely reflected white light from tissues up to a depth of 1-2 mm can be obtained and this device was found to be very effective in locating potentially malignant areas of oral lesions from the R545/R575 image ratio with the help of a suitable algorithm developed for tissue differentiation [23].

The clinical study aims to extend the multi-spectral DR imaging technique for screening of cervical lesions and develop an objective diagnostic algorithm for classification of different grades of cervical lesions from the DR image ratio R545/R575. Towards this, cervical tissues were grouped as benign or normal, low-grade cervical intraepithelial neoplasia (CIN) and high-grade CIN. The DR image ratio algorithm developed for cervical tissue discrimination was subjected to a blind test to determine the diagnostic accuracies of measurement and the results are presented.

II. MATERIALS AND METHODS

A. Study Population

This clinical trial was conducted at Regional Cancer Centre (RCC), Thiruvananthapuram after obtaining the ethical approval of the Human Ethic Committee of RCC (No. HEC No-28/ 2011). The study was afterwards registered in the Clinical Trial Registry of India (REF/ 2013/ 10/ 005904). Study population consisted of 41 patients (average age: 40 ± 12 years) with different grades of cervical lesions, such as benign or normal, low grade cervical intraepithelial neoplasia (low grade CIN) and high grade cervical intraepithelial neoplasia (high grade CIN). After explaining the details of the clinical trial, written informed consent was obtained from all participants before initiating any study related measurement. The inclusion criteria included patients with positive pap smear reports, those referred for colposcopy examination and patients with clinically suspecting lesions in the uterine cervix. Patients with transmittable diseases and those unwilling to participate were excluded from the study.

B. Multi-spectral diffuse reflectance imaging system

The schematic of the multispectral diffuse reflectance imaging system (MDRIS) shown in Fig.1 consists of an EMCCD camera (Model: LUCA-R, DL-604M-OEM, Andor Technology, UK) with 1004 (H) x1002 (V) pixels, a Nikon AF 35-70 zoom camera lens and a liquid crystal tunable filter (LCTF) of 7 nm bandwidth (Model: VIS-07-20-STD, CRI Inc, USA) that can be tuned electronically to any wavelength in the 400-720 nm range.
A tungsten halogen lamp (3V, 12W) was used for white light illumination of the cervix during imaging. The LCTF was controlled using proprietary software of CRI Inc and the monochrome DR images and computation of the image ratios were carried out using the Solutions for Imaging and Spectroscopy (SOLIS) program (Andor Technology, UK, Ver. 1.0).

![Image of multi-spectral diffuse reflectance imaging system for cervical cancer detection.]

**C. Data acquisition**

The patient is asked to lie down on the examination table in lithotomy position with her legs spread out wide and supported under the knee by obstetrical stirrups. A strap is attached to each padded stirrup to hold patient's legs securely in place. After ascertaining patient’s comfort, a lubricating gel is applied inside the vagina and the Cusco vaginal speculum is inserted by the surgeon with its handle pointing downwards so that cervix can be seen properly seen with white light illumination from the tungsten halogen lamp positioned at an angle to the axis of imaging. The cervix of the patient is wiped gently with saline-soaked cotton swab to remove excess mucous. The imaging camera was mounted on a tripod at a distance of 50 cm from the cervix, and focused/zoomed to get a clear view the cervix on the laptop. Care was also taken to minimize specular reflection from the cervix by adjusting the position of the illumination lamp. After setting the appropriate image acquisition parameters (typical exposure time of 0.01s) background subtracted DR images were recorded sequentially at 545 and 575 nm by sequentially tuning the LCTF to these wavelengths.

Minute shifts in pixel position between sequentially recorded images were corrected using Math Lab program and the image ratio (R545/R575) was computed arithmetically. Increase in pixel intensity of the monochrome image ratio signifies higher levels of malignancy. The ratio image was further pseudo colored mapped (PCM) to facilitate easy identification the most malignant site in the lesion for biopsy. Tissue biopsy was taken from the identified site and sent for histopathological examination.

**D. Data processing**

Although the histopathology results of biopsy classifies the cervical lesions into seven separate categories, owing to the limited number of patients enrolled in this pilot study, we collapsed the categories into three, viz., normal, low-grade cervical intraepithelial neoplasia (CIN) and high-grade CIN, as shown in Table I.

<table>
<thead>
<tr>
<th>HPRS</th>
<th>No of Sites</th>
<th>Study Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>normal</td>
</tr>
<tr>
<td>Squamous metaplasia</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Koilocytic atypia</td>
<td>7</td>
<td>Low grade CIN</td>
</tr>
<tr>
<td>Cin1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Cin2</td>
<td>1</td>
<td>High grade CIN</td>
</tr>
<tr>
<td>Cin3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>CIS</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Grouping of different cervical lesions
Squamous metaplasia and benign tissues of the cervix were grouped under the normal category. Low-grade CIN comprising of tissue abnormalities consistent with koilocytic atypia and CIN 1 were grouped together, while high-grade CIN comprising of CIN 2, CIN 3 and Carcinoma in-situ (CIS) were grouped separately [24]. The histopathology results of biopsy showed that there were 23 cases of normal, 9 cases of low-grade CIN and 9 cases of high-grade CIN. The image intensity ratio (R545/R575) was calculated to distinguish between healthy and diseased sites from the PCM ratio image intensity values. For each identified site, 50 region of interest (ROI), 10 pixel lengths and 10 pixel heights each, were selected in the ratio image and the mean of these intensity values were estimated as the ratio value for that site. A scatter plot diagram using these values was plotted for classifying tissues as healthy, low-grade CIN and high-grade CIN. The weighted mean of the averages of successive groups are the cut-off value for discriminating these pairs.

### III. RESULTS

Figs. 2 shows the images recorded from the cervix of two patients diagnosed with low-grade CIN (left) and high-grade CIN (right). Whereas Figs. 2 a were recorded with a Nikon camera, Figs 2 b show monochrome DR images acquired at 545 nm and Figs 2 c are those recorded at 575 nm with the EMCCD camera. DR intensity changes associated with low-grade and high-grade CIN can be clearly distinguished from the PCM image ratios (R545/R575) shown in Figs. 2 d. The most malignant area identified by MDRIS is enclosed inside the yellow rectangle. The green and blue regions in the PCM image ratio denote normal tissue and as the ratio values increase the representative color changes to red, yellow and white. R545/R575 image ratio values were noted from the median pixel intensity values of the identified lesion regions in Fig. 2d.

![Fig 2: Images for a typical low-grade (left) and high-grade (right) cervical intraepithelial neoplasia; a) digital color photo, b) monochrome image at 545nm, c) monochrome image at 575nm, d) zoomed ratio image showing biopsy site in a rectangular box. The color palette shows the range of median pixel intensity values for tissue discrimination.](image)

The mean pixel value of the R545/R575 image ratio for normal (23), low-grade CIN (9) and high-grade CIN (9) were calculated and are 1.42 ± .62, 2.41 ± 0.71 and 4.05±.68, respectively, showing 70% and 185% variances for low- and high-grade CIN with respect to normal values. A scatter plot diagram of DR ratio R545/R575 values for different sites is shown in Fig. 3. The cut off values, drawn at weighted average for distinguishing low-grade and high-grade from normal are 1.912 and 3.23, respectively. Out of the 23 negative cases, 5 were misclassified as low-grade CIN, whereas out of 9 low-grade CIN cases, only one case was misclassified as normal and one as high-grade CIN. Also, one out of the 9 high-grade lesions was misclassified as low-grade CIN. The sensitivity and specificity for distinguishing low-grade CIN from normal are 87.5% and 78.3% respectively and those for distinguishing high-grade CIN from low-grade CIN are 88.9% and 87.5%, respectively. The positive predictive
value (PPV) and negative predictive value (NPV) estimated for each pair is shown along with the sensitivity and specificity values in Table II.

![Image](image_url)

**Fig 3:** R545/R575 ratio value scatter plot for different groups of cervical lesions. The continuous line represents the cut-off value for each pair.

**Table II: Diagnostic accuracies for different lesion pairs**

<table>
<thead>
<tr>
<th>Lesion pairs</th>
<th>Sensitivity (Se)</th>
<th>Specificity (Sp)</th>
<th>Positive predictive value (PPV)</th>
<th>Negative predictive value (NPV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal-Low grade CIN</td>
<td>87.5</td>
<td>78.3</td>
<td>58.3</td>
<td>94.7</td>
</tr>
<tr>
<td>Low grade CIN-High grade CIN</td>
<td>88.9</td>
<td>87.5</td>
<td>88.9</td>
<td>87.5</td>
</tr>
<tr>
<td>Normal-High grade CIN</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

CIN – Cervical intraepithelial neoplasia; Sensitivity (Se) = True positive (TP) / TP + False negative (FN); Specificity (Sp) = True negative (TN) / TN + False positive (FP); Positive predictive value (PPV) = TP / TP + FP; Negative predictive value (NPV) = TN / TN + FN.

**IV. DISCUSSION**

Morphological and biochemical properties of cervical tissue alter in accordance with neoplastic transformation. Dysplastic regions tend to have increased metabolism, vascularity and larger blood flow than normal tissues, and the decrease in DR intensity can be attributed to the changes in heme synthesis. The reduced activity of the ferrochelatase enzyme that catalyzes the insertion of ferrous iron into protoporphyrin to form heme results in lower hemoglobin production and corresponding lower absorption at 545 and 575 nm in the oxygenated hemoglobin spectra [25]. As the grade of malignancy increases, an overall reduction in DR intensity in the corresponding spectra can be observed. Mallia et al [26] in a clinical study on patients with oral cavity cancer has reported that the DR ratio R545/R575 increases with increasing malignancy and that the spectral ratio R545/R575 could be a useful indicator to discriminate different stages of oral cancer with relatively high sensitivity and specificity.

Compared to fiber optic point spectroscopy, multispectral imaging has the advantage of providing a real-time assessment of the entire lesion. Since colposcopy is time consuming and labour intensive, patients prefer fast, see and treat methods. Retaining these advantages, MDRIS provides information on tissue oxygenation changes characterizing all parts of the cervix at 545 and 575 nm, which helps locate regions of malignancy from the R545/R575 image ratio [23]. Increase in the value of the image ratio R545/R575 is visualized by PCM with relative ease. The multispectral image ratio derived from monochrome images recorded at 545 and 575 nm has shown maximum contrast between normal and abnormal lesions. The ratio value further enables differentiation between neoplastic and non-neoplastic lesions and helps to identify the most malignant site in a lesion for biopsy site.
The histopathology results obtained for the lesions identified by MDRIS underwent 3-tiered classification as normal, low-grade CIN and high-grade CIN. It is important to distinguish high-grade CIN from low-grade because high-grade lesions were considered to be true precursors of invasive cancer and need treatment. If left untreated, high-grade CIN leads to invasive carcinoma. Most low-grade CIN regress to normal within relatively short periods or do not progress to high-grade lesions and need only follow up.

In this study, the overall sensitivity and specificity obtained by MDRIS for distinguishing different cervical lesion pairs are significant. Even though the sample size is less; it was possible to distinguish between low-grade and high-grade CIN with 88.9% sensitivity and 87.5% specificity, whereas diagnostic accuracy achieved for differentiating high-grade CIN from normal is 100%. In order to improve the efficiency of population-based screening for cervical cancer, screening tests with a high diagnostic performance is crucial and MDRIS could save women from an uncomfortable, scarring, expensive biopsy in future.

The specificity and PPV for detecting low-grade CIN from normal were 78.26% and 58.3%, respectively. This comparatively low specificity is due to few false positives, which may reduce expenses towards further unnecessary investigations. The positive predictive value tells us how often the disease is present, when the test is positive. The problem of discriminating inflammation from low-grade CIN may be responsible for the false positive results. Quite often, it is spectroscopically difficult to discriminate inflammatory conditions from low-grade CIN. Since, the proposed algorithm has high sensitivity and NPV, it could reduce a large number of false negative results.

In a similar study using MDRIS for oral cancer detection, Jayanthi et al reported a sensitivity of 93% and specificity of 98% for discriminating healthy/normal oral lesions from pre-malignant and malignant lesions [22]. In another clinical study involving 106- normal, 20- pre-malignant and 29- malignant sites, Manju et al reports a sensitivity and specificity of 76% and 80%, respectively for discriminating premalignant oral lesions from malignant [23]. In another pilot study, digital reflectance images of the cervix acquired from 29 women showed that an automated image analysis algorithm could identify the presence and spatial extent of high-grade precancers with 79% sensitivity and 88% specificity when compared with histopathological analysis [14]. Burke et al reported a sensitivity and specificity of 93% and 94%, respectively for discriminating CIN against benign changes including normal, inflammation and metaplasia using fluorescence spectroscopy with 337-nm excitation [27]. Life Spex Inc. has developed a system to image fluorescence from cervical epithelium at multiple excitation-emission wavelength pairs. The results of the study conducted in over 100 patients show that the device discriminates precancerous cervical lesion from normal tissue with a sensitivity of 98% and specificity of 95.4% [28]. Ferris and Litaker obtained a sensitivity of 97% and a specificity of 70% for hyper-spectral wide-field imaging as compared to colposcopy-directed biopsy or loop electrosurgical excision [3].

Although LCTF has the inherent property of transmitting light of a single polarization, it was not able to completely filter out specular reflection from the tissues illuminated with white light as we could not use linearly polarized white light to illuminate the tissue. Nonetheless, the bright areas caused by light reflection from the moisture present on cervical tissue surface could possibly have misled some biopsy sites in this study, thereby lowering the diagnostic accuracies reported. Further studies are planned to improve the diagnostic accuracies and to test the MDRIS on a larger population with a view to refine this modality for non-invasive detection of cervical malignancies during its early stages.

V. CONCLUSION

Quantitative detection of cervical intraepithelial lesions of the cervix without the need for tissue removal is of great clinical significance. The present pilot study carried out with MDRIS establishes the potential of DR imaging at the oxygenated hemoglobin absorption peaks along with the utilization of the DR image ratio R545/R575 for screening and identification of the most malignant site in the cervix for pathology. It is hope that this see-and-treat method after further detailed studies could lessen health care costs and reduce the number of patients lost during follow up with conventional screening techniques for cervical cancer. Effects of cervical inflammation, menopausal status are not included in this study and need further investigation.
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