Original Article

Applicability of Optical Reflectance Spectroscopy for Detection of Precancerous Lesions in Uterine Cervix *in Vivo*

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Abstract

Background: We assessed the performance of single fiber reflectance spectroscopy (SFRS) in discriminating cervical squamous intraepithelial lesions (SIL) from non-SILs and the probable influence of environmental factors on its performance.

Methods: SFRS was used to measure the reflected light from cervical tissue of 157 patients undergoing standard colposcopy. Seven parameters extracted from the spectra in addition to two biographic parameters were used to compare the biopsy-confirmed SILs with non-SILs. The tissue classification capability was reported by receiver operating characteristic (ROC) curves. In addition, the effect of five interfering parameters, including the probe, clinician, hospital, menopausal status and age of the patient on spectroscopic parameters were investigated by the Kruskal-wallis test.

Results: The average vessel diameter and beta-carotene concentration were found to be the parameters contributing to tissue discrimination. SFRS could differentiate between SILs and non-SILs with sensitivity, specificity and area under the ROC curve of 63% ± 6%, 68% ± 6% and 0.69 ± 0.04, respectively. None of the five environmental parameters interfered with the discriminator spectroscopic parameters.

Conclusions: SFRS was found as a noninvasive, fast, compact, cost-effective, independent, and acceptably accurate system to help the clinician to reduce the number of unnecessary biopsies during the colposcopy procedure.

Keywords: Biomedical optics, cervical intraepithelial neoplasia (CIN), detection of pre-cancer, single fiber reflectance spectroscopy (SFRS), squamous intraepithelial lesion (SIL)

Cite this article as: Hariri Tabrizi S, Farzaneh F, Aghamiri SMR. Applicability of optical reflectance spectroscopy for detection of precancerous lesions in uterine cervix *in vivo*. *Arch Iran Med*. 2014; **17(9):** 602 – 607.

Introduction

Cervical cancer is one of the most common cancers among women around the world, especially in developing countries. Current screening and diagnostic protocols, Papanicolaou test (Pap smear) followed by colposcopy, have some limitations including low sensitivity[1] and poor specificity of colposcopy which often leads to unnecessary biopsies.[2] Therefore, there is increasing interest in alternative or complementary methods which may diagnose the undetectable lesions and reduce the number of unnecessary biopsies. One of these approaches is light spectroscopy.[3]

Since the late 20th century, many researchers have investigated the use of optical spectroscopy as an adjunct to colposcopy in order to reduce screening and surveillance costs and to improve the detection of squamous intraepithelial lesions (SILs).[3] Reflectance spectroscopy is a method that allows noninvasive determination of the scattering and absorption properties of a turbid medium such as tissue. This information can be used to describe aspects of tissue physiology (e.g. vascular oxygen saturation, blood volume fraction) and morphology (e.g. tissue, cell, organelle size and density). One such method is single fiber reflectance spectroscopy (SFRS) which benefits from small probe size and simple device design.[4] This technique has the potential to assist the clinician in tissue sampling during standard biopsy procedures and has been applied to different tissues like lymph nodes.[5]

The applicability of SFRS in detection of squamous intraepithelial lesions (cervical intraepithelial neoplasia grade one (CIN I) and worse) has been previously examined in a pilot study.[6] The present study has covered a larger population to assure an acceptable performance of the SFRS system in detection of neoplastic lesions in cervical tissue. A quantitative relationship between the significant optical parameters and the health status of the tissue was developed. In addition, five environmental parameters that can interfere with the diagnostic capability of the system were fully studied.

Material and Methods

Single fiber reflectance spectroscopy (SFRS) system

The system setup and the model used to analyze the reflected light from cervical tissue have been described previously.[6] In summary, visible light illuminates the cervical epithelium through a contact optical fiber of 0.8 mm or 1 mm in diameter. The reflected light from the tissue is collected using the same probe. A non-linear model which includes seven unknown parameters fits the SFRS spectra.[7,8] These parameters constitute the spectroscopic parameters which were used in differentiation between SILs and non-SILs. The spectroscopic parameters consist of Mie scattering amplitude (Mie-amp), Mie scattering slope (Mie-sl), Rayleigh scattering amplitude (Ray-amp), blood volume fraction (Bl-vol), blood oxygen saturation (StO_2), average vessel diameter (Ves-

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Accepted for publication: 20 June 2014

diam), and beta-carotene concentration (B-car).

In vivo measurement

The sample size calculation assumed obtaining sensitivity and specificity of 84% and 76%, respectively[9] with a precision of 14%. The prevalence of CIN I and worse was found to be 19%.[6] As a result, 143 samples were found to be sufficient to fulfill the sensitivity and specificity values with 95% confidence interval (CI) of (70% – 98%) and (62% – 90%), respectively. Assuming a loss of 10%, a sample size of 159 was calculated.

All non-pregnant patients who were referred for colposcopy following an abnormal Pap smear, post-coital bleeding (PCB) and/or chronic cervicitis resistant to treatment were invited to participate in the research. Before the experiment, each patient filled in a demographic questionnaire and signed an informed consent (with the approval from ethical committee of Shahid Beheshti University of Medical Sciences). The clinical *in vivo* study was conducted at Emam Hossein Medical and Educational Center and Taleghani General Hospital in Tehran.

The SFRS spectra were taken during standard colposcopy procedure by gynecologic oncologists. Before application of the probe, it was disinfected by 4% deconex® 53 PLUS (borer Co.) solution for at least half an hour. After application of 3% acetic acid to the cervix, the optical probe was placed in contact with the suspicious as well as colposcopically normal sites and SFRS spectra were acquired. In order to reduce the noise, five measurements were made for each probe position which took less than one second. Afterwards, the average of the five spectra was fitted by the model described previously.[6] For ethical considerations, only the colposcopically abnormal appearing sites were biopsied. Special attention was given to obtain the biopsy from the same location as that of the measurement. Cervical tissue biopsies were fixed in formalin and submitted for histological examination. The tissue was classified into seven categories: normal, inflammation, HPV-associated change, CIN I, CIN II and CIN III. The latter three cases are integrated as squamous intraepithelial lesion (SIL).

Data analysis

Tissue classification was performed based on the biopsy-confirmed spectra to discriminate between SIL and non-SIL. The classification was done using seven spectroscopic parameters and two biographic parameters including menopausal status (Mens) and age (Age) of the patient using the logistic regression method. The algorithm was validated using five-fold cross validation (5FCV) method and represented by receiver operating characteristic (ROC) curves. The optimal sensitivity and specificity were also provided. These parameters are defined for a point on the ROC curve with the shortest distance from the point of perfect separation (100% sensitivity and 100% specificity). Furthermore, to find the relevant parameters in tissue diagnosis, stepwise logistic regression was used. $P < 0.05$ was chosen as the criterion for including a parameter in the model while $P > 0.1$ was used for exclusion of that parameter from the model.

Additionally, the possible influence of environmental factors on the spectroscopic parameters was studied. These interfering parameters included the measuring probe, the clinician who performed the measurement, the study center, and biographic parameters including the patient's menopausal status and age. Kruskal-wallis test was applied to the spectroscopic parameters extracted from each measurement associated with different groups of the five interfering parameters. When the median value of a spectroscopic parameter differed among different groups of an interfering parameter ($P < 0.05$), it implied impressionability of the corresponding spectroscopic parameter by that interfering parameter.

Results

Tissue classification

During ten months of experiment in two clinical sites, 225 SFRS spectra from 157 patients were acquired. The criteria to exclude datasets were:

1. unsatisfactory biopsy specimen for histopathologic evaluation (n = 30),
2. unacceptable spectra fitting (n = 16),
3. interference of the colposcope light (n = 4),
4. tissue bleeding (n = 4),
5. unavailability of the pathology result (n = 2),
6. adenocarcinoma diagnosis (n = 2).

Therefore, 167 spectra from 113 patients (37 ± 8 years old) were used in data analysis. Table 1 summarizes the number of sites and patients measured for each pathology group.

Table 2 shows the mean and standard deviation (SD) of the spectroscopic as well as biographic parameters in the SIL and non-SIL groups. The *P*-values show the significance of difference between the mean values in the two groups using Wilcoxon rank sum test. Through stepwise logistic regression, Ves-diam and B-car parameters were found to significantly contribute to the tissue classification model and decrease the root mean square error. It is consistent with the *P*-values presented in Table 2. Also, it was found that the average age of the patients affected by SIL is less than the non-SIL group.

Applying the 5FCV algorithm to the dataset to discriminate SILs from non-SILs resulted in the area under the ROC curve (AUC), sensitivity and specificity values of 0.69 ± 0.04, 63% ± 6% and 68% ± 6%, respectively. It is important to note that the values after the "±" signs specify the 95% CI (≈2 SDs) for the mean values. The corresponding ROC curve is shown in Figure 1.

Effect of the interfering parameters

Probe

Four different probe settings were used in this study. They included one probe with an optical fiber with a diameter of 1000 µm and three probes with a diameter of 800 µm labeled as 800, 800(1) and 800(2). Kruskal-wallis test was used to determine if the seven spectroscopic parameters differed among the measurements done with each probe. It was found that Mie-amp, Bl-vol, Ves-diam and B-car parameters were significantly different among the four utilized probes (Table 3). Through a two-by-two comparison, the main difference was observed between measurements performed by 800(1) and 800(2) probes. While the 800(1) probe showed a significantly higher Mie-amp and B-car than 800(2), this relation was reversed for Ves-diam and Bl-vol.

As stated before, only Ves-diam and B-car were found as diagnostically important parameters. The former increases with progression of neoplasia whereas the latter decreases (Table 2). Hence, the dependence of these two parameters on the utilized probe was the result of the fraction of SIL cases measured by each probe. More than 17% of the measurements with 800(2) probe were diagnosed as SIL while it was less than 2% for 800(1) probe. To verify this, the analysis was repeated while the measurements with pathology results of SIL were excluded from the dataset. Implementation of the Kruskal-wallis test on the new dataset demonstrated that only the Ray-amp parameter was different among the four probe settings ($P = 0.0478$).

Clinician

Ten physicians including two gynecologic oncologists, one gynecologic oncology fellow and seven gynecology trainees contributed to the measurement procedure. Each had different experience and skill in the colposcopy procedure. The probe pressure they applied on the cervical tissue might have an impact on the SFRS signal. Therefore, any difference in the spectroscopic parameters due to the clinician's skill was investigated. None of the spectroscopic parameters showed any dependence on the clinician who performed the measurement ($P > 0.1$).

Study center

The measurements were conducted in two clinical locations. Although one of the clinicians who contributed to the measurements was common between the two centers, environmental conditions might have an effect on the spectra. Among the seven spectroscopic parameters, only StO_2 was found different among measurements performed in the two centers. The median value for StO_2 for measurements in Taleghani hospital (n = 22) was higher than Emam Hossein hospital (n = 145) ($P = 0.0055$).

Patients' age and menopausal status

In order to investigate the effect of biographic situation on the reflected light from cervix, the age and menopausal status of the patients were taken into account. The patients were divided into two groups of under 40 years (n = 117) and equal to or over 40 years (n = 50). As for the menopausal status, the patients were divided to three groups of pre, peri and post-menopausal status. They included the premenopausal patients aged below 40 years (n = 128), premenopausal patients aged 40 years and above with normal menstruation (n = 21), and postmenopausal patients (n = 18), respectively.

Using Kruskal-Wallis test, only the Mie-amp parameter was found as a significantly different parameter among the three groups of menopausal status. Multiple comparison test showed that the pre-menopausal group had lower Mie scattering amplitude than the post-menopausal group ($P = 0.0007$).The influence of age on the spectroscopic parameters was similar to that of the menopausal status as long as they were dependent parameters. Again, Mie-amp of the younger patients was lower than the older ones ($P = 0.0077$).

Discussion

System accuracy

Using the 5FCV algorithm, the 95% CI of the sensitivity and specificity were found to be (57% – 69%) and (62% – 74%), respectively. Although the achieved specificity overlapped with the desired range used in the sample size calculation procedure, the obtained sensitivity was about 13% less than the expected range. In comparison with the similar studies, however, the sensitivity of the SFRS system was near the results obtained by Chang, et al.[10] and Mirabal, et al.[11] and its specificity was in line with the studies by Werner, et al.[12] and Mirkovic, et al.[13] The resulting AUC was in the range of studies addressing the use of optical spectroscopy in cervical tissue.[3] Therefore, the performance achieved by the SFRS system was comparable with the similar studies. Nevertheless, higher specificity implied better potential of the device in decreasing the number of unnecessary biopsies than detecting suspicious lesions.

As for comparison with colposcopy, inconsistent performances were found in the works done by Mitchell, et al.[14] and Alvarez, et al.[15] The performance of the SFRS system was between two ends of the ROC curve defined by the two authors. Better comparison between the performance of SFRS and colposcopy can be made by using the results of this investigation to detect the SILs using the SFRS system. Then, the histopathologic results of the biopsies taken based on diagnosis of both methods can be compared fairly. It is the task of the phase III trial which is in progress.

Spectroscopic parameters

The spectroscopic parameters used in this study had physical and biological interpretations. Among seven spectroscopic parameters, only two parameters contributed to tissue diagnosis. Increase in vessel diameter with neoplasia progression (Table 2) is in accordance with an immunohistochemical study that showed the proportionality of vascular volume with cervical precancerous grades.[16] Contribution of Ves-diam to the diagnostic model has been shown in our pilot study, as well.[6] Other optical spectroscopy studies showed the similar effect explicitly[17] or implicitly through observing higher absorption in the neoplastic cervical epithelium.[18–20]

It was found that beta-carotene concentration decreases with neoplasia progression (Table 2). In a chromatography study of 105 cervicovaginal epithelial cells, it was observed that beta-carotene levels were significantly lower in women with CIN and cervical cancer than the control group ($P < 0.0001$).[21] Therefore, SFRS could find this effect optically. To the best of our knowledge, contribution of beta-carotene to optical spectroscopy of the cervical tissue was not previously shown in the similar studies. Mirkovic, et al. were the only group to mention the presence of beta-carotene in cervical tissue but they could not find any remarkable difference between its level in the healthy and neoplastic groups.[13]

Biographic parameters

Many researchers have shown significant relation between the biographic characteristics of patients and the optical spectra acquired from their cervical tissue.[9,22–24] For example, Chang, et al. concluded that age and menopausal status must be taken into consideration when developing diagnostic tools based on various optical parameters.[25]

In order to address the probable relevance of the diagnostic performance with the biographic parameters of age and menopausal status, two approaches were used: 1) inclusion of them as diagnostic parameters in the logistic regression model and 2) studying them as interfering parameters. When stepwise logistic regression was applied to the nine spectroscopic and biographic parameters, none of the biographic parameters was found diagnostically important. On the other hand, neither Ves-diam nor B-car, diagnostic parameters, was found different in various groups of age and menopausal status. This independence is valuable especially when one knows other researchers' effort like Mourant, et al. [22] to remove this dependence using numerical methods.

Table 1. Characteristics of the measured sites and patients

Pathology	Number of sites	Number of patients[a]
Normal	31	22
Inflammation (mild – severe)	110	72
HPV effect	9	7
CIN I	11	7
CIN II	4	4
CIN III	2	1
Total	**167**	**113**

[a]The patients with more than one biopsy were assigned to the worst diagnostic group based on pathology result.

Table 2. Mean values for the 9 spectroscopic and biographic parameters in the SIL and non-SIL groups (threshold = CIN I). The *P*-value of the Wilcoxon rank sum test shows the significance of the difference between their mean values

Spectroscopic / biographic parameters	Mean ± SD		*P*-value
	< CIN I (n = 150)	>= CIN I (n = 17)	
Mie-amp (-)	0.44 ± 0.18	0.38 ± 0.12	0.1746
Mie-sl (-)	-1.2 ± 0.4	-1.0 ± 0.4	0.2411
Ray-amp (-)	0.0067 ± 0.0095	0.0079 ± 0.0078	0.2721
Bl-vol (-)	0.0033 ± 0.0037	0.0028 ± 0.0017	0.4733
StO_2 (%)	75 ± 14	74 ± 14	0.8344
Ves-diam (mm)	**0.009 ± 0.009**[a]	**0.017 ± 0.014**	**0.0088**
B-car (μM)	**6.0 ± 4.5**	**4.2 ± 4.7**	**0.0227**
Age (years)	**37 ± 9**	**33 ± 8**	**0.0347**
Mens (-)[b]	1.6 ± 0.7	1.8 ± 0.7	0.2959

[a]Bold values in the table represent the statistically significant different averages ($P < 0.05$); [b] 0, 1 and 2 values were assigned to the three menopausal states of pre, peri and post-menopausal, respectively.

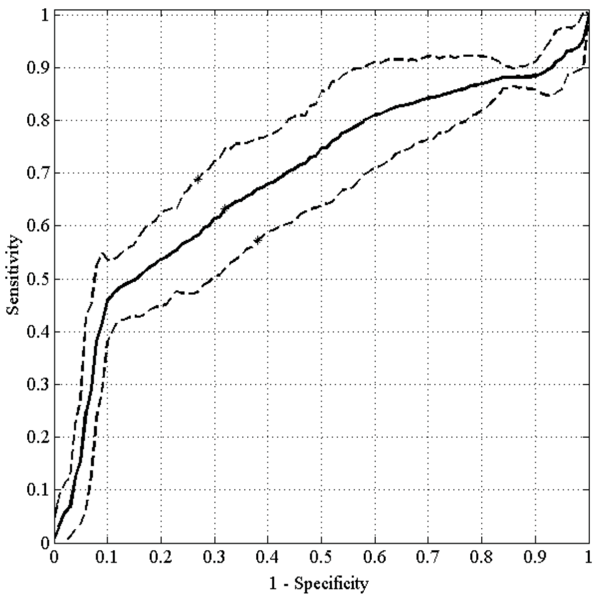

Figure 1. ROC curve obtained by 5FCV method for SIL versus non-SIL discrimination with the corresponding 95% CI (broken lines). Average vessel diameter and beta-carotene concentration were used in this tissue classification. The asterisks show the optimal sensitivity and specificity with their corresponding 95% CI.

Interfering parameters

Dependence of the intensity and shape of the cervical fluorescence spectra on the system generation, user, measuring probe and spectrophotometer has been recognized in previous studies.[26,27] Mourant, et al. tried to eliminate the dependence of the reflectance parameters to the four clinicians and two probes involved in their study using numerical methods.[17,22] However, optical signal calibration is supposed to remove any dependence on external factors other than the sample measured.

In this study, three parameters, in addition to the biographic ones, were studied to find their probable interference with the diagnostic capability. Neither the clinician who performed the

Table 3. The mean and standard deviation of the spectroscopic parameters which were significantly different among the four utilized probes. *P*-values were reported by Kruskal-wallis test

Spectroscopic parameters	800 (n = 48)	800(1) (n = 59)	800(2) (n = 46)	1000 (n = 14)	*P*-value
Mie-amp (-)	0.40 ± 0.16	0.49 ± 0.19	0.40 ± 0.15	0.41 ± 0.18	0.0290
Bl-vol (-)	0.0027 ± 0.0032	0.0028 ± 0.0027	0.0038 ± 0.0046	0.0044 ± 0.0030	0.0393
Ves-diam (mm)	0.0082 ± 0.0076	0.0084 ± 0.0097	0.013 ± 0.011	0.012 ± 0.012	0.0449
B-car (*μ*M)	5.9 ± 4.5	6.9 ± 5.1	4.2 ± 3.4	5.9 ± 3.9	0.0235

colposcopy and measurements nor the center in which the study was performed showed any effect on the diagnostically important parameters. Even though there were differences between the Ves-diam and B-car values in the measurements done using different probes, it was due to the higher fraction of neoplastic tissues measured by one probe compared to another. It occurred in spite of random assignment of the probes to each patient.

Independence of the diagnostic parameters on the four probes, ten clinicians and two clinical locations in this study shows the success of SFRS system in judging the tissue based on its characteristics, not the environmental conditions.

System simplicity

Most of the optical systems, such as Medispectra[15] and FastEEM,[23] which utilize fluorescence or its combination with reflectance spectroscopy in cervical tissue diagnosis, are huge and complicated. The SFRS system is based on reflectance spectroscopy of unfiltered white light. The system comprises of only one lamp, one spectrophotometer and one optical fiber in the probe. Compared with a point probe and compact device,[28] the SFRS system is more than an order of magnitude lighter and has at least one half dimensions. The simplicity of the device suggests advantages of easier and cheaper service and maintenance procedures. Also, lighter device is more transportable and capable of being used in remote places which may suffer from poor infrastructure for the cervical screening program.

Acknowledgments

We cordially acknowledge the contribution of the gynecologists, trainees and staff of the gynecological departments of Emam Hossein and Taleghani hospitals. Also, special thanks go to Prof. H.J.C.M Sterenborg and Dr. A. Amelink at Erasmus Medical Center for their technical support. Ms. S. Saghamanesh deserves gratitude for her kind contribution in revision of the manuscript. We would also like to thank all the patients who participated in this study.

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