Original Article

Optical Spectroscopy: A Promising Diagnostic Tool for Breast Lesions

Pathology Section

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ABSTRACT

Background: With the rising trend of breast cancer, there is a need to develop a diagnostic procedure which can solve the problem of differentiating the benign and malignant lumps and of detecting breast cancer at an early stage. Intrinsic fluorescence is an important step in this regards. Auto-fluorescence from biological tissues involves evaluating the biomolecular environment through optical spectroscopy.

Aim of study: To study the role of optical spectroscopy in differentiating the benign from the malignant breast lesions by detecting the change in the intrinsic fluorescence, especially of Flavin adenine dinucleotide (FAD) and porphyrins.

Method: A study of 37 patients with breast lumps was done, out of which 14 had benign and 23 had malignant lesions. After excision for the intrinsic fluorescence study, fresh samples were taken and excited by using an optical spectrophotometer.

Results: This study showed that intrinsic fluorescence is a good method of investigation with a sensitivity of 68% and a specificity of 73%.

Conclusion: Optical spectroscopy has a bright future in differentiating the benign and malignant lesions of the breast. Furthermore, it can detect cancer at an early stage.

Key Words: Intrinsic fluorescence, Flavin adenine dinucleotide, Porphyrins, Breast lesions

INTRODUCTION

Having a breast lump is an enigma for a female at any age. Cancer of the breast stands out as one of the most ominous of all the cancers in females. Also, despite the vast knowledge of the disease, its incidence has never shown a declining trend. It is also one of the most treatable forms of cancer, if it can be diagnosed at its early stage and so the best way to curtail the effects of the disease and to improve the survival is to diagnose it as early as possible. According to the National Cancer Institute, up to 10% of all the breast cancers, roughly 20,000 cases per year in the United States, fail to be discovered by X-ray mammography. Also, X-ray mammography uses ionizing radiation and it requires uncomfortable breast compression. It also suffers from a significant number of false positives that often lead to unnecessary biopsy, since biopsy is generally required to determine malignancy in most of the women with an abnormal mammogram. All the three techniques, X-ray mammography, ultrasound, and magnetic resonance imaging provide high spatial resolution, but comparatively little information about the molecular-level changes in the breast tissue [1-3]. On the other hand, if we could differentiate whether the breast lump is benign or malignant by a single investigation which is not only non-invasive / minimally invasive, then it would definitely become the investigation of choice. It is in this setting of the diagnostic dilemma of the diseases of the breast that fluorescence studies hold promise. The morphological and chemical changes that occur when a tissue proliferates in an exaggerated normal or abnormal fashion cause the fluorophores inside the tissue to fluorescence differently as compared to their normal environment. Fluorescence spectroscopy can differentiate the biochemical and morphological changes of the normal and diseased tissues. In our study, the fluorophores which were studied were FAD and porphyrins, because they have been proven to best correlate with the number of dividing cells and the metabolic activity of the cell. When they are excited at 436 nm, they show peak fluorescence at 530 nm and 630 nm respectively.

METHODS

A study on 37 cases was done in a period of one year from January 2007 to January 2008. Of these 37, 14 benign and 23 malignant samples (histologically proven) were considered for fluorescence analysis.

INTRINSIC FLUORESCENCE STUDIES

Fresh tissue samples were obtained immediately after surgery. The suspected tumour tissue was selected, it was separated from the normal tissue and it was sent for laser spectroscopic studies. Simultaneously, the surrounding normal tissue was also taken for fluorescence analysis. The tissue which was selected for study was taken into the chunk and thoroughly cleaned and washed with normal saline to remove blood and slimy material. Then, it was placed in normal saline. During the experiments, the tissue was kept at room temperature and it was kept moist with isotonic saline. The tissue was placed on a quartz plate of size 3 cm x 1 cm x 2 cm. The fluorescence spectra of the tissues were recorded by using a Fluorolog-3 spectrofluorometer (Jobin Yvon, USA). The samples were excited with an Ozone free Xenon lamp of 450-W power, which delivered light from 240 nm to 850 nm for the sample excitation by using an emission spectrometer and a photo multiplier tube (PMT, Model R928) and for the simultaneous recording of the elastic scattering spectra. The output of the detector was connected to a computer for data acquisition and analysis.

The samples were excited by using vertically polarized light. The parallel (VV) and perpendicular (VH) components of the fluorescence

were collected in the reflection geometry. In the same geometry, the parallel and perpendicular components of the scattered light were also collected. In this research, we have studied the cross-polarized (VH) fluorescence spectra of the sample and have compared it with the intrinsic fluorescence of the sample. The fluorescence power per unit area which escaped from the tissue was related to the distribution of the excitation radiation within the tissue.

For histopathological examination, the corresponding tissue was taken and it was placed in 10% buffered formalin and was processed for making paraffin blocks for routine histological diagnosis by the haematoxylin and eosin method.

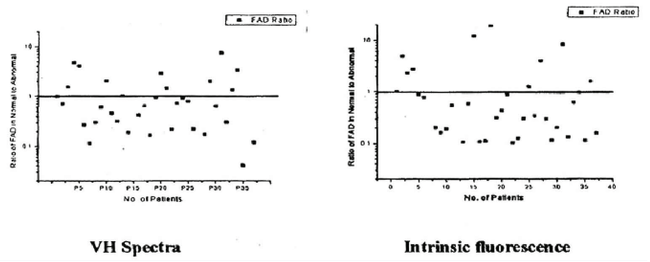
RESULTS

Breast tissue consists of fibrous and fatty tissues. The fluorescence spectra were recorded in parallel (VV) and perpendicular (VH) and the simultaneous scattering was also recorded. Considering the fact that in the emission spectra, the maximum intensity at 530 nm is due to FAD and that at 630 nm is due to porphyrin we calculated the area under 20 nm bandwidth at each of these wavelengths i.e. 530 ± 10 and 630 ± 10. This was done for the tumourous tissue and for its normal counterpart also. Thus, we could plot the ratio of FAD in abnormal tissues (benign and malignant) to that in normal tissues and also similarly for porphyrin; for each patient. The comparison between VH and intrinsic fluorescence could be done if we made a scatter plot of the ratio of the area under the 20mm bandwidth of FAD and porphyrin in normal tissues to that in cancerous tissues for both VH and intrinsic fluorescence [Table/Fig-1 and 2]. A ratio which was less than one would show that the concentrations of FAD and porphyrin were more in the cancerous (benign or malignant) tissues as compared to those the normal tissues.

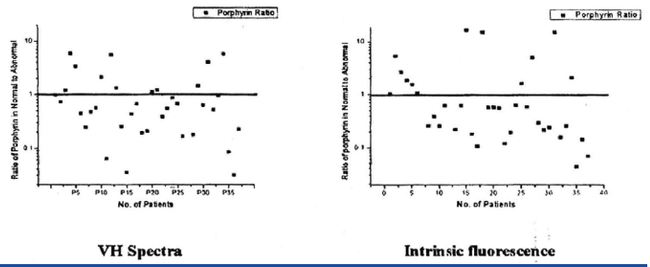
Thus, the intrinsic fluorescence was much accurate as compared to the VH fluorescence when we took all the benign and malignant tissue samples together [Table/Fig-1 and 2]. Though this held true if we considered the malignant tissues only [Table/Fig-5 and 6], there was an insignificant difference between the VH and intrinsic fluorescence spectra when only the benign tissue [Table/Fig-3 and 4] samples are considered. We classified the lumps as benign or malignant histologically. Then, we compared the results of different spectra in the normal as well as in abnormal tissues [Table/Fig-7] and compared the results of the benign and malignant lesions [Table/Fig-8].

DISCUSSION

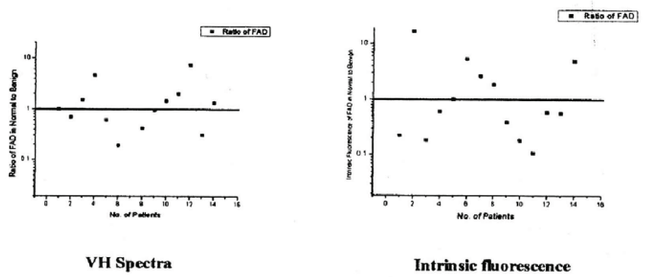
Auto fluorescence from biological tissues has been under investigation for many years. This involves the evaluation of the biomolecular environment through optical spectroscopy. This study was aimed at comparing an entirely new technique optical spectroscopy in differentiating the abnormal from the normal breast tissue by detecting the chemical changes that occurred at the molecular level. There are a large number of biomolecules in tissues that emit light (auto fluoresce) under photo-excitation. The most dominant tissue fluorophores are tryptophan, collagen, elastin, NADH, flavoproteins, and porphyrins. Each fluorophore has a distinct



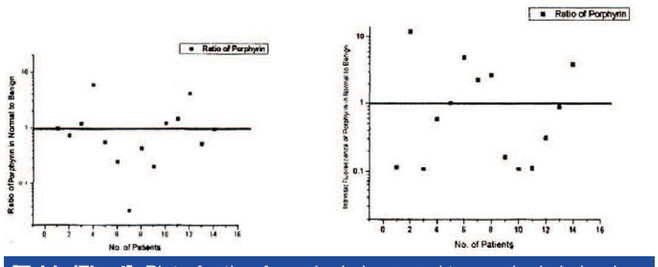
[Table/Fig-1]: Plot of ratio of FAD in normal to FAD in abnormal (benign and malignant) for VH and intrinsic fluorescence.



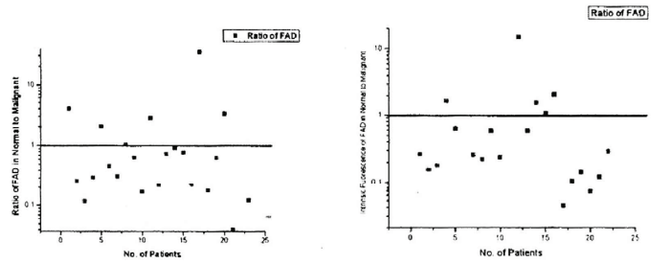
[Table/Fig-2]: Plot of ratio of porphyrin in normal to porphyrin in abnormal (benign and malignant) for VH and intrinsic fluorescence



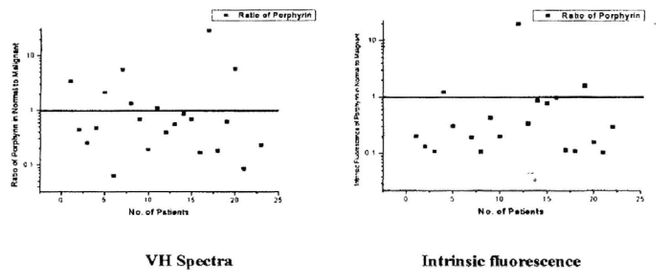
[Table/Fig-3]: Plot of ratio of FAD in normal to FAD in benign breast tissue for VH and intrinsic fluorescence



[Table/Fig-4]: Plot of ratio of porphyrin in normal to porphyrin in benign breast tissue for VH and intrinsic fluorescence



[Table/Fig-5]: Plot of ratio of FAD in normal to FAD in malignant breast tissue for VH and intrinsic fluorescence



[Table/Fig-6]: Plot of ratio of porphyrin in normal to porphyrin in malignant breast tissue for VH and intrinsic fluorescence

absorption and emission spectrum. Similarly, for the application of optical spectroscopy for the characterization of organic or inorganic materials, auto-fluorescence spectroscopy can be used to quantify the relative distribution of the different fluorophores in the tissue components for diagnostic purposes. Pioneering work by Alfano et al. highlighted the potential of autofluorescence spectroscopy for cancer detection [4]. Gupta et al. reported in an in vitro study which involved 63 patients and aimed to evaluate autofluorescence spectroscopy under excitation in the near-UV region (nitrogen laser) [5], that significant changes were observed in the spectrally integrated auto-fluorescence intensity from the normal, benign, and cancerous breast tissues. The intensity ratios of the cancerous tissues to the benign tumour and normal tissues were found to be 3.2 and 2.8, respectively. A discrimination parameter based on the spectrally integrated intensity alone provided a sensitivity and specificity of up to 99.6%. A similarly high sensitivity was reported by Hage et al. who used laser-induced auto-fluorescence spectroscopy under 548 nm excitation [6]. The experiments in the study by Palmer et al. included the characterization of tissues by using autofluorescence spectroscopy under multiple excitation wavelengths in the ultraviolet-visible range. They were successful in discriminating the malignant and non-malignant tissues, with a sensitivity and specificity of 70% and 92%, respectively [7]. The analysis of the results suggested that the important fluorophores for breast cancer diagnosis, most likely were tryptophane, NAD(P)H, and flavoproteins. Polarized fluorescence spectral profiles and anisotropy showed a definite distinction among the malignant, benign and normal human breast tissues in a study which was conducted by Laxmi et al in 2001 [8]. Majumdar et al, in 1999, discriminated human breast malignant lesions by using nitrogen excited auto-fluorescence spectra, which showed a sensitivity of 85.5% and a specificity of 87% [9]. Svensson et al, in 2005, showed intra and inter subjects as well as contra lateral variations of optical and physiological properties in breast tissues, as measured by using four wavelength time resolved spectroscopy [10]. Recently, an idea for the diagnosis of breast cancer by the non-invasive probing of calcification by using transmission Raman spectroscopy was given by Matousek et al in 2007 [11]. Krishna et al, in 2008, reviewed the Raman spectroscopic approach for metabolic fingerprinting in breast cancer detection [12]. Our study also showed comparable results.

We can differentiate benign and malignant tissues on the basis of the ratio of the normal and abnormal tissues, which if less than one, denotes tumourous conditions. Our results showed that the intrinsic fluorescence study was a good investigative method with a sensitivity of 68 % for FAD and 73% for porphyrin, though it was still in a very early stage of development. This research needs a longer study period and analysis with more number of samples for it to give better results with a high diagnostic accuracy.

Name of the spectra	Ratio less than 1 (abnormal)	Ratio more than 1 (normal)	Sensitivity
VH_{FAD}	24	13	65%
$VH_{PORPHYRIN}$	25	12	68%
IF_{FAD}	25	12	68%
$IF_{PORPHYRIN}$	27	10	73%

[Table/Fig-7]: Comparative study of results obtained by different spectra (all the sample)

In all the cases, it was possible to diagnose whether a tumour was actually present or not (by checking for a significant change in the area under the curve at 530 ± 10 nm and 630 ± 10 nm for the tumour tissue as compared to the normal tissue). The sensitivity for the malignant lesions was more than that for the benign lesions . Intrinsic fluorescence spectra is a better screening tool as compared to the VH spectra. Porphyrin is a better biochemical parameter in the intrinsic fluorescence spectra, whereas FAD is a better biochemical parameter in the VH spectra for abnormal (especially malignant) lesions. Also, porphyrin is a better biochemical parameter in the VH spectra for benign lesions. As compared to histopathology, this technique had an overall diagnostic accuracy of around 83% in the intrinsic spectra in detecting porphyrin accumulation. This is a relatively good value for a new investigative method. The study is an aid for the development of a probe which can be used to detect early malignant changes in vivo. Overall, optical spectroscopy, mainly the intrinsic fluorescence analysis for FAD and porphyrin, has shown encouraging results It has a bright future in diagnostics to differentiate the benign and malignant legions of the breast. Furthermore, it can detect cancer an an early stage, and can thus aid in better patient management.

The histological examination of breast tissues is currently being performed by selecting one of the well-developed invasive breast biopsy techniques (i.e., excisional biopsy, axillary node dissection, sentinel node dissection, or fine needle aspiration), depending on the location, size, palpability, and the characteristics of the abnormality. Breast excisions remain one of the most common surgical operations for diagnosing and treating breast cancer. In the current setting, while frozen section analysis is available, there are technical limitations in cutting certain types of tissues and as a result, an immediate histological analysis is not possible or practical. Therefore, developing a technology that can offer the detection and delineation of the tumour margins in real time may be very useful for a surgeon during a diagnostic or therapeutic procedure. The progress to date in using various optical spectroscopy methods for the classification of breast tissues arguably provides a solid foundation for the development of spectroscopy- based instrumentation for real time pathological assessment. Most of the approaches use single point measurement techniques that interrogate a small volume of tissue at each measurement. As mentioned earlier, the potential role of this technology in addressing clinical needs which are related to breast cancer detection and treatment may be used for intraoperative tissue characterization in real time or via designing thin fiberoptic needles to reach the suspected location within the breast for the evaluation of a suspected lesion [13-15].

Histologically	Benign	Benign		Malignant	Malignant	
Name of the spectra	Ratio less than 1 (Abnormal)	Ratio more than 1	Sensitivity	Ratio less than 1 (Abnormal)	Ratio more than 1	Sensitivity
VH_{FAD}	07	07	50%	17	06	73.9%
$VH_{PORPHYRIN}$	09	05	57.14%	16	07	69.56%
IF_{FAD}	08	06	57.1%	17	06	73.9%
$IF_{PORPHYRIN}$	08	06	57.1%	19	04	82.6%

[Table/Fig-8]: Comparative study of results obtained by different spectra (benign and malignant separately)

REFERENCES

[1] Carney PA, Kasales CJ, Tosteson ANA, Weiss JE, Goodrich ME, Poplack SP, et al. Likelihood of additional work-up among women who undergo routine screening mammography: the impact of age, breast density, and hormone therapy use. *Prev Med* 2004;39 (1):48–55.

[2] Hindle WH, Davis LWD, Wright D. Clinical value of mammography for symptomatic women who are aged 35 years and younger. *Am J Obstet Gynecol* 1999;180(6 Pt 1):1484–90.

[3] Hata T, Takahashi H, Watanabe K, Takahashi M, Taguchi K, Itoh T, et al. Magnetic resonance imaging for the preoperative evaluation of breast cancer: a comparative study between mammography and ultrasonography. *J Am Coll Surg* 2004;198 (2):190–97.

[4] Alfano RR, Tang GC, Pradhan A, Lam W, Choy DSJ, Opher E. Fluorescence spectra from cancerous and normal human breast and lung tissues. *IEEE J Quantum Electron* 1987; 23: 1806-11.

[5] Gupta P, Majumder S, Uppal A. Breast cancer diagnosis by using N2 laser excited autofluorescence spectroscopy. *Lasers Surg Med* 1997;21 (5):417–22.

[6] Hage R, Galhanone P, Zangaro R, Rodrigues K, Pacheco M, Martin A, et al. Using laser-induced fluorescence spectroscopy for the differentiation of normal and neoplastic human breast tissues. *Lasers Med Sci* 2003;18 (3):171–6.

[7] Palmer G, Zhu C, Breslin T, Xu F, Gilchrist K, Ramanujam N. Comparison of multiexcitation fluorescence and diffuse reflectancy spectroscopy for the diagnosis of breast cancer. *IEEE Trans Biomed Eng* 2003;50 (11):1233–42.

[8] Laxmi BV, Panda RN, Nair MS, Rastogi A, Mittal DF, Agarwal A, et al. Distinguishing normal benign and malignant human breast tissues by using visible polarized fluorescence. *Laser in the Life Sciences* 2001; 9:229-43.

[9] Majumder S K, Gupta P K, Jain B, Uppal A. UV excited autofluorescence spectroscopy of human breast tissue for discriminating the cancerous tissues from benign tumours and normal tissues. *Lasers Life Sci* 1999; 8: 249-64.

[10] Svensson T, Swarling J, Taroni P, Torricelli A, Pia Lindblom, Ingvar C, et al. Characterization of normal breast tissue heterogenecity by using time resolved near infrared spectroscopy. *Phys. Med Biol*. 2005; 50: 2559-71.

[11] Matousek P, Stone N. Prospects for the diagnosis of breast cancer by the noninvasive probing of calcifications by using transmission Raman spectroscopy, *J Biomed Opt* 2007; 12(2): 024008.

[12] Krishna CM, Kurien J, Mathew S, Rao L, Maheedhar K, Kumar KK, et al. Raman spectroscopy of breast tissues, Expert. *Rev Mol Diagn*. 2008; 8(2): 149-66.

[13] Bigio I, Bown S, Briggs G, Kelley C, Lakhani S, Pickard D, et al. Diagnosis of breast cancer by using elastic-scattering spectroscopy: preliminary clinical results. *J Biomed Opt* 2000;5 (2):221–8.

[14] Andrews R, Mah R, Da Silva L. The NASA smart probe project for real-time multiple-microsensor tissue recognition. *SPIE Proceedings* 2004;5326:92–7.

[15] Zhu C, Labawy C, Burnside E, Harter J, Ramanujam N. Development of an optical sensor for the spectroscopic detection of breast cancer during needle biopsy. *Lasers Surg Med* 2005; 6(6 15 Suppl. 17).

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