Optical Biopsy of Breast Cancer Tissue

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Abstract—In this paper, we report results of Fluorescence Emission Spectra (FES) and Stokes Shift Spectra (SSS) of 19 cancer tissue of invasive ductal carcinoma of different grades in comparison with normal breast tissues (obtained away from tumor regions). We were able to get distinct differences in the spectral features of normal and malignant tissues in terms of the ratios of concentrations of biomolecules like tryptophan, collagen and NADH. The sensitivity and specificity were in the range of 75%. What was all the more important was the parallelism in the spectral features of normal and malignant breast tissue pieces of above set of subjects. The objective of our research is to evolve one such protocol and the first step is the spectral characterization of in vitro optical analyses of excised tumor tissues.

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1. INTRODUCTION

According to cancer report of 2008, breast cancer comprised 22.9% of all cancers in all over the world [1]. Clinical examination, mammography, needle or surgical biopsy is the most common steps of early detection and diagnosis and this has significantly reduced the morbidity and mortality. Surgical biopsies have long been the gold standard for determining whether growths are cancerous or benign. But at least three out of four biopsies following mammograms conclude that observed abnormalities are benign and that no intervention is needed. In case of surgical biopsy, stress on patients and additional cost of recuperation are serious concerns. In this context, optical biopsy based on optical coherence tomography (OCT), fluorescence diagnosis (FD) are expected to do paradigm shift in diagnosis. The OCT is optical analogue to ultrasound imaging, with resolution approaching that of histology. In breast tissues, regions of tumor, tumor margins, abnormal ducts, and foci of tumor cells can be identified based on increased scattering and morphological inhomogeneities [2–5]. Fluorescence diagnosis (FD) of cancer finds use in wide spectrum of medical disciplines such as urology, dermatology, gynecology and neurology. FD is based on the application of 5 δ ALA, a heme precursor which induces the endogenous accumulation of efficient photo sensitizer which can be easily identified by the characteristic fluorescence [6–11]. Apart from the above mentioned labeled fluorescence, Alfano et al. demonstrated the potency of laser spectroscopy for detection of breast cancer by native luminescence of tissues [12]. Subsequently such studies have been extended to prostate cancer tissue by Masilamani et al. [13].

Breast Cancer is a disease of humans and other mammals, originating from breast tissue most commonly from the inner lining of milk ducts or the lobules. Women, especially in the age groups of 40–50, are more affected, as compared to the rest. Cancers originating from ducts are assigned as mammary ductal carcinomas, and those originating from lobules are named as lobular carcinomas.

In the current study, we have selected a set of normal (N = 7), benign (N = 2), and malignant (ductal carcinoma N = 10) tissue samples. We have obtained fluorescence emission spectrum (FES), Stokes’ shift spectrum (SSS), and synchronous emission spectrum (SES) for all samples. Results are based on the relative intensity of bio fluorophores like tryptophan, tyrosine, collagen elastin, flavin, etc. This study provides significant discriminatory spectral features in the three sets so that it paves way for the screening of breast cancer.

2. MATERIALS AND METHOD

Excised breast tissue pieces were obtained by breast surgery from 19 patients of King Khalid University Hospital (KKUH) and King Faisal Specialist Hospital and research Center (KFSH & RC), Riyadh, Kingdom of Saudi Arabia. Before launching into research, the institutional review board ethical committee clearance
from KKUH and KFSH&RC and informed consents from the individual patients were obtained. The excised chips roughly in the size of $5 \times 2 \times 2 \text{mm}^3$, were kept immersed in saline water, stored in refrigerator and then carried through spectral analysis within 8 h. From each patient, a parallel set was taken for conventional histopathology. All the spectral diagnosis reported here are in comparison to the gold standard of histopathology.

Before taking spectra, each sample was washed five times in saline water, dried on blotting paper, minced, washed again to remove blood and loaded into a quartz cuvette.

Light from xenon lamp, of pre-selected wavelength, with a size of $2 \times 2 \text{mm}^2$ was allowed to fall on the tissue and excite native fluorescence, which was detected transverse to the incident light. The instrument used for the study was Perkin Elmer LS45 (Waltham, MA), which has the facility to take FES and SSS and SES in the range of 200–800 nm; the spectral resolution for excitation or emission spectra was 10 nm and scan speed was set at 1000 nm per minute.

In FES, we select one particular wavelength for excitation of the biofluorophores of the tissue and obtain the native fluorescence emission spectrum, by rotating the emission grating. In fluorescence excitation spectrum FXS, it is just the reverse. We select the peak of emission band and rotate the excitation grating to scan the excitation spectra (which is very similar to the absorption spectra) of a particular biofluorophores. In SSS, both grating are rotated synchronously, with an offset of 70 nm, to obtain the fluorescence excitation band of every biofluorophores in the range of 200–600 nm. We have tried other offsets, such as 30, 40, 50, and 80 nm, but found that the offset of 70 nm is the best. This is because, most of the fluorophores of our interest have stokes shift of 70 nm. For example, FXS for tryptophan is around 280 nm and FES of around 350 nm; FXS for elastin is around 327 nm and FES is around 390 nm. Since the resolution is excellent in SSS, the contrast between the normal and diseased samples are quite good and most of the results presented here are based on SSS. When the offset is 10 nm we obtain only synchronous emission spectra (SES) where individual bio molecules are excited and emission bands are obtained synchronously.