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Diagnosis of pelvic lymph node metastasis in prostate cancer using single optical fiber probe

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ABSTRACT

Elastic light single-scattering spectroscopy system (ELSSS) is a biomedical tool which is used for detection of cancerous tissues ex-vivo. ELSSS spectra depend primarily on the size of scatterers in the tissue and are not directly related to changes in the absorption which are caused by variations of the biological macromolecules. In the present study, we aimed to detect metastasis in the pelvic lymph node by using combination of Principal Components Analysis (PCA) and Linear Discriminant Analysis (LDA). Single-scattering spectra in the 450–750 nm wavelength regions were obtained from the total of 83 reactive lymph node and 12 metastatic lymph node samples from 10 prostatic cancer patients. The ELSSS spectral data were compared against the "gold standard" histopathology results. Data analyses were done via using PCA, followed by LDA. Receiver Operating Characteristic (ROC) curve analysis was employed for differentiating performance. The classification based on discriminant score provided sensitivity of 100% and specificity of 96.4%, in differentiating non-metastatic (reactive) from metastatic pelvic lymph nodes, with a Positive Predictive Value (PPV) of 0.8, a Negative Predictive Value (NPV) of 0.99 and the area under the ROC curve (AUC) of 0.99, respectively. In this study, it was shown that ELSSS system can accurately distinguish reactive and metastatic pelvic lymph nodes of prostate cancer with high PPV and NPV. It can be concluded that diagnostic accuracy of ELSSS system allows detecting metastatic tissues during operation.

1. Introduction

Prostate cancer is the second-leading cause of cancer death in men. Cancer cells are commonly spread (or metastasis) to the other areas of the body via lymphatic channels or bloodstream. For this reason, the pelvic lymph nodes close to the prostate may be removed to control for spreading. Pelvic Lymph Node Dissection (PLND) is used for detection of metastasis in prostate cancer during Radical Prostatectomy (RP). PLND is invasive and can be associated with specific complications and several serious side-effects, a build-up of fluid in the lymph node area (seroma) and in the affected leg (lymphoedema), nerve and blood vessels injury, infection, pain, swelling and bruising, which negatively affects the patient's quality of life. The lymph node drainage of the prostate primarily drains to the obturator and the internal iliac channels. There is also a posterior lymphatic connection with the external iliac, presacral, and the paraaortic lymph nodes [1].

Frozen Section (FS) analysis is not routinely performed unless the nodes are grossly suspicious. Histological examination of FS's is the gold standard for appraising the presence of metastasis in pelvic lymph nodes in patients during intraoperative diagnosis. FS analysis in lymph nodes allows the pathologist to analyze the samples during operation but results may take 20–30 min because of the difficulties of the technique and the experienced pathologist skills. Size and shape of the cell nucleus and nucleus-cytoplasm ratio are important morphological criteria for pathologist that these structures are often abnormal in cancer cells.

Minimally invasive or real-time fiber optic pelvic lymph node diagnosis involvement would ensure considerable utility to patients who undergoing RP. ELSSS system is used for detection of pelvic lymph node metastasis in men which sensitive to the epithelial morphology and nuclear size [2–4]. During elastic

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scattering there is a negligible energy transfer and there is only spatial distribution of light changes in the tissue. ELSSS system has been used previously as a diagnostic technique [2–8] moreover; there has been a great deal of investigation in cancer detection by using other spectroscopic techniques [9–13]. Significant portion of light is scattered from the nucleus which is the major internal scatterer in the cell and the difference in the index of refraction between the cytoplasm and the cell membrane causes light scattering [12,13]. Backscattered light from cell is obtained as spectral data and dependent to the wavelength. Recently developed nanoparticles are used as molecular imaging agents for early detection and diagnosis of cancer. For example, superparamagnetic iron oxide nanoparticles are used for detection of pelvic lymph node metastasis in genitourinary cancers and technetium-99 m sulfur colloid nanoparticles are used for mapping of sentinel lymph in invasive breast cancer. Multimodal nanoparticles can be designed to image of cells in deeper tissue by imaging techniques such as Near-Infrared Fluorescence, Surface-Enhanced Raman Scattering and photoacoustic imaging [14].

Multivariate statistical analysis was used to construct diagnostic algorithms capable of discriminating between metastatic and reactive pelvic lymph nodes. ELSSS results are objective and do not need interpretation by pathologist and decision support for urologist.

In the present study, we aimed to detect metastasis in lymph node of prostate cancer by using ELSSS technique without time consuming and pathologist experience in FS room. The acquired spectrum were evaluated in combination with PCA and LDA to create a technological basis for decision support during operation.

2. Materials and methods

2.1. The study protocol

The clinical study was conducted at Akdeniz University Hospital with the approval of the institutional ethical committee. The preliminary histopathological diagnoses of patients were prostate malign neoplasm with a high prostate-specific antigen level and a high Gleason score after prostate biopsy. 10 patients who underwent RP at Akdeniz University Urology Department were selected for the study and the spectral measurements were performed on total of 95 lymph nodes by the collaboration of pathology. During RP operation pelvic lymph nodes were excised from the obturator, external and internal iliac regions and materials were transported to the histopathology in buffered formaldehyde (10%) for routine pathologic examination within 10 min. The classical pathological method begins with palpation and visual inspection of the node for defining its borders and removing all the fat from the surface using scalpel and fingers. Lymph nodes with most of the fat removed were serially sectioned at 3–5 mm gaps in the longitudinal or transverse plane. These sections and small nodes which were not sliced (<5 mm) were placed on a black plastic sheet to prevent back-reflection during spectral data acquisition. At least 16 ELSSS measurements were collected per node (depending on the size) randomly and perpendicularly placing tip of the optical fiber probe on the lymph node cut surfaces within 3 to 5 min. This step was performed very carefully in order not to change the optical properties of the nodes mechanically. Then all nodes were coded and stored in containers that let the formalin fixation to solidify them and prevent the denaturation of proteins within the cells. Thin (5 μm) sections of the nodes were cut using a microtome and stained with Hematoxylin and Eosin. Then pathologists review the slides under the microscope. These procedures took about 7 days to get final pathology report of the nodes. ELSSS spectrum was obtained in the wavelength range of 200–1100 nm, and each spectrum was corrected in the wavelength range of 450–750 nm. ELSSS spectrum and pathology results were compared to find a correlation. In the present study, pathologic examination was used as a standard reference for tissue diagnosis and spectral shape is used as a diagnostic parameter that is related to scattering of the light from the cells and cellular organelles. Of the total 95 lymph node specimens 83 were classified histologically as reactive and 12 as metastatic.

2.2. Elastic light single-scattering spectroscopy and optical measurements

The ELSSS system contains a single-fiber optical probe (100 μm diameter and with a numerical aperture of 0.22) which is used for both delivery and detection of light on the tissue by touching, a tungsten halogen white-light source (illumination power of 0.1 mW), a spectrometer (USB2000 with OOIBase32TM Platinum Spectrometer Operating Software, Ocean Optics, Tampa, FL) which has optical resolution ~0.34 nm and a laptop computer to record the spectra as illustrated in Fig. 1. One proximal end of the fiber was connected to the tungsten halogen white-light source and the other one to the spectrometer. The light travels from the light source to the single-fiber optical probe through the fibers and reflects back from lymph node surface and goes to spectrometer to be analyzed. Short term (<2 s) and gently probe pressure effects were performed for collection and recording of spectrum in order to minimize the negative influence of the diagnostic performance and spectral measurements. The average acquisition time of each ELSSS spectrum was 200 ms. We kept the spectral intensity below a saturation value 40,000 and above 10,000 counts by modifying the software of the spectrometer and changing integration time automatically. If the maximum intensity of the tissue spectrum is higher than 40,000 or lesser than 10,000 counts, the software ignores it and takes another spectrum. Before data acquisition and analysis from lymph nodes, each spectrum is normalized to the integration time and three spectra were measured for system calibration. The first one was a background spectrum which was taken from pure water in a black container $R(\lambda)_{bg}$. The spectral distribution of the light source was measured by the second spectrum $R(\lambda)_c$ where the probe was placed nearly 1 mm above a white-light reflectance standard (Spectralon, Labsphere, Inc.) in water. Single fiber optical probe was tested by detecting singly-scattered photons from the turbid media which consists of %10

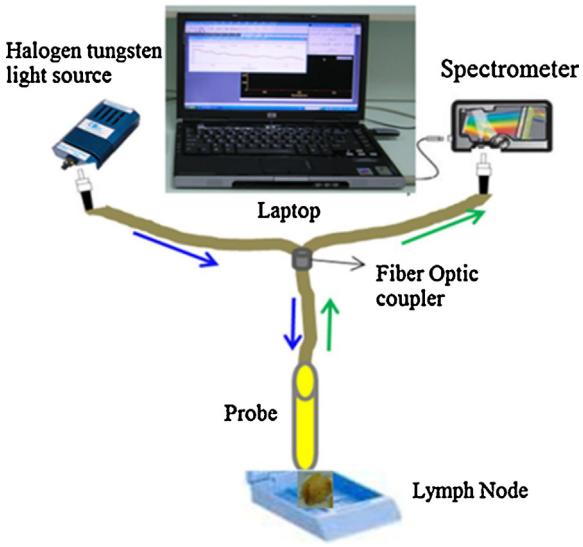

Fig. 1. The ELSSS system consists of a halogen tungsten light source, optical fibers, a coupler, a spectrometer, and a laptop computer. The light travels from the light source to the probe through the optical fibers and reflects back from the tissue surface to the spectrometer to be analyzed.