OPTICAL FINE-NEEDLE ASPIRATION BIOPSY FOR MINI-INVASIVE SURGERY

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Abstract. The work describes the methodologyand technical implementation of optical biopsy system for focal and diffuse tumors diagnosticsduring fine needle aspiration biopsyin abdominal organs. Preliminary measurements results seem promising for further development of this approach.

Introduction. Currently, development of new diagnostic criteria for focal and diffuse tumors verification is one of the relevant problems of abdominal surgery. In particular, it is important in case of liver cancer, which is one of the most common and lethal cancers[1]. Early and accurate diagnosis is necessary for the timely determination of treatment tactics, which improves the prognosis of patient survival. A procedure called fine needle puncture and aspiration biopsy (FNAB) is considered as the "gold standard" of morphological preoperative diagnosis of tumors. This method includes taking cells from several areas of interest using a fine needle for further cytological studyingof the obtained material. The main advantage of FNAB is safety as a puncture using a fine needle allows for obtaining material without significant violation of tissue integrity. However, FNAB involves the preparation of a sample followed by study under a microscope, which takes several days; therefore,it does not provide real-time information.

One of the rapidly developing approaches, which can compensate this downside of classical FNAB technique, is calledoptical biopsy. This termincludes the application of spectroscopic and imaging methods for non-invasive acquisition of information about biological tissues state*in vivo* in real time. These methods estimate such parameters as metabolic processes rate, chromophores content, oxygenation and blood perfusion.

At present, the method of fluorescence spectroscopy (FS) is widely used for studies of tissue and cellular metabolism. The application of FS foroncological researchis based on differences occurring in fluorescence of healthy and malignant tissues. Another method calleddiffuse reflectance spectroscopy (DRS) provides information about the lightabsorbed in biological tissues. This method allows performing a differential diagnosis of malignant and benign tumors, as tumor tissues undergo significant morphological changes.The combination of two aforementioned methods providesmore complete information about tissues state, which can help inthe diagnosis of pathological changes. However,the application of optical methods during FNAB is associated with technical and methodological problems today.

Previous studies have shown the possibilities and further prospects ofmultimodal approach; at the same time, several limitations were revealed, mainly causedbythe anatomical features of areas of interest [2]. Therefore, transfer of this technology to a more compact format of traditional FNAB provides direct access to tumor tissues and allows information to strengthen the cytological and morphological data of traditional biopsy.

Thus, the aim of the work wasdevelopingthe technical implementation and testing the possibilities of multimodal optical biopsy setup during FNABfor the diagnosis of focal and diffuse neoplasms of the liver.

Materials and Methods. A fiber-optic system implementing FS and DRS methods was specially developed for the research. The FS channel includes 365 nm and 450 nm light sources used for NADH and FAD fluorescenceexcitation. The accumulation of these coenzymes in mitochondria during the Krebs cycle reflects metabolic activity. The content of NADH and FAD affects the fluorescence intensity; therefore, this parameter reflects metabolic processes and can be used for vitality evaluation[3]. DRS channel source is HL-2000-FHSA Tungsten Halogen Light Source («Ocean Optics», USA) with a wavelength range of 360–2400 nm. Receiving channel includes optical filters to attenuate backscattered radiation in fluorescence measurements. The light collected from biological tissue is analyzed by CCD FLAME Spectrometer («Ocean Optics», USA) in the range of 350–1000 nm. Further processing, as well as system control, is carried out using custom developed software in Matlab program environment.

Both channels are combined into a fiber optic probe with an outer diameter of 1 mm making it compatible with a standard 17.5G fine needle. The probe includes 10 optical fibers. 9 fibers (100 μm) includes 3 fibers for each radiation source. They are located around the central (200 μm) fiber, which collects and transmits light to the spectrometer. The number and orientation of optical fibers provide equal bright illumination of the diagnostic volume and high signal-to-noise ratio. The probe has a bevel angle of 20 degrees to ensure reliable contact with the tissues.

Preliminary measurements were conducted at the department of interventional radiology of Orel Regional Clinical Hospital (Orel, Russia). The work involved 4 patients aged 62±11.The studywas approved by the Ethics committee of Orel State University (record of the meeting №14 of 24.01.2019) and was carried out in accordance with the 2013 Declaration of Helsinki by the World Medical Association.The patients signed informed consent indicating their voluntary willingness to participate in the study.

The methodology assumes the use of designed setup during the standard FNAB procedure under the ultrasound control aimed at verification of liver neoplasm type. The surgeon inserts a fiber optic probe through the outer 17.5G needle into the tumor. The operator turns on one of the monochromatic light sources and puts the corresponding optical filter. The radiation from the tissue is transmitted by the fibers through the optical filter to the spectrometer. Processing and visualization are performed using a personal computer. After the measurement, the operator turns on the other source of monochromatic radiation and puts another filter, or turns on halogen light source and removes the filter to perform diffuse reflectance measurement. Measurements can be repeated as many times as necessary. After the measurements, the surgeon obtains a tissue sample from the same area for cytological analysis.

Comparison of two types of biopsies allows one to check the presence of pathological changes in tissues and to obtain data for a more accurate interpretation of the results of optical measurements. A database of fluorescence and diffuse reflection spectra in combination with traditional biopsy results will be used to develop new diagnostic criteria.

Results and Discussion. The measurements were performed in intact liver tissue and supposed tumor tissue. The results of conventional FNAB confirmed the presence of a malignant tumor in 2 out of 4 cases, which are shown next. The parameters analyzed were diffuse reflectance intensities and maximums of fluorescence intensity after normalization by backscattered radiation. Reflectance measurements clearly show the pronounced reflectance peak in region 450–500 nm. The absence of peaks of oxyhemoglobin absorption on the tumor spectra indicates tissue ischemia. Similar results can be seen for fluorescence intensity under 365 and 450 nm excitation. However, fluorescence intensity under 365 nm is higher in intact tissue for case 1 and in tumor tissue for case 2. It can be caused by the differences in cancer cells type, phase development of carcinogenesis and other factors, which require more detailed future research.

Conclusion. FNAB is a valuable diagnostic method necessary to confirm the diagnosis for further treatment. However, the need for morphological preoperative diagnosis of tumors in real time determines the relevance of optical biopsy. Simultaneous registration of fluorescence and diffuse reflectance provides information on metabolic processes and morphological structure of tissues. The proposed methodologywill be used to develop diagnostic criteria for an automatic classifier of neoplasms. This approach will make the diagnosis in minimally invasive abdominal surgery faster and more accessible and increase its accuracy and reliability.

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