AUTOFLUORESCENCE OF SKIN CANCER - TOOL FOR INITIAL DIAGNOSIS AND MONITORING OF THERAPY

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Many years of investigations and significant improvement of the spectral detection technologies still do not introduce on the market easy-to-use system for cutaneous autofluorescence cancer detection and differentiation. One could ask himself, why such system, which in principle does not seems to be very complicated technically, still does not exist. Of course, there are some attempts to introduce such diagnostic systems into standard clinical practice, such as fiber-based fluorimeter - SkinScan system (JobinYvon, France), where fluorescence of endogenous aminoacids is used for cutaneous lesions' investigations [1], or more recently developed DYADERM system (Biocam GmbH, Germany), which is applied for photodynamic diagnosis with exogenous photosensitizers [2]. However, up to our days there is no such universal clinical apparatus, based on autofluorescence detection of skin surface, which could be used as a general tool for early cancer detection and diferentiation. The reasons for such instrument absence in the field of clinical equipment based on the autofluorescence detection of skin cancer are very complex.

Problems for development of such reliable universal diagnostic fluorescence system for skin cancer detection are related to the great variety of benign and malignant forms of cutaneous pathologies, for example basal cell carcinoma lesions have more than 15 sub-types, squamous cell carcinoma lesions, have about 10 different subtypes, and all of them have variety of benign and dysplastic forms, as well as they are different, including by their fluorescence properties, on different stages on the lesion growth. Positive is the fact that due to these changes, depending on the lesion growth, we could use light-induced autofluorescence spectroscopy (LIAFS) for evaluation of the lesion stage, negative point is that we will need to compare this exact situation with great variety of other possibilities, such as lesion kind, stage of growth, and even patient skin general conditions, such as influence of medicines, ages, cutaneous phototype, typical work conditions (office/outside), etc.

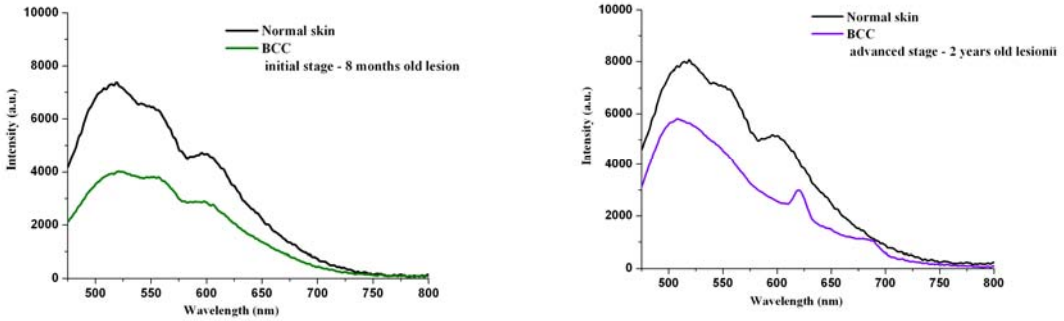
However, that variety of spectral information, which we could obtain from different skin pathologies and its diversity, does not mean that we could not use LIAFS, as a tool for early cancer detection. Moreover, exactly due to high sensitivity of fluorescence, LIAFS could be applied, as a very precise tool for initial diagnosis, for planning, and monitoring of therapeutic procedures. In the current report we will present several practical applications of LIAFS system as an initial diagnostic tool, as well as a tool for therapeutic monitoring and a decision tool for treatment planning.

This investigation is a part of a clinical trial for introduction of spectral diagnostic system for skin cancer detection in the daily practice of the dermatological department of University Hospital "Queen Jiovanna" [3], as well as for monitoring of electrochemotherapy procedures in the frames of National Oncological Center. Autofluorescence spectroscopy is applied to several different classes of malignant non-melanoma cutaneous lesions. Initially, they were classified visually and dermatoscopically. Second step was detection of lesion' and surrounding normal skin autofluorescence using different excitation wavelengths, namely 365, 385, and 405 nm. In the end for every lesion histological examination is used as a "gold standard" for all our investigations. The spectra and dermatoscopic evaluations were obtained from more than 400 patients up to now. Spectral properties of variety of benign cutaneous lesions are also evaluated for development of more precise discrimination algorithms for diagnosis of cancer lesions. The origins of diagnostically significant spectral features are evaluated and differentiation schemes are developed.

Typically the patients observed have one cutaneous tumour which needed to be diagnosed. Major trends in the fluorescence signals obtained are related to changes in the fluorescence intensity, as well as appearance of secondary fluorescent maxima, depending from the tumour subtype, and observation of increased absorption from the major skin pigments – melanin and hemoglobin, related to the tissue conditions. Basal cell carcinoma (BCC) lesions have decreased fluorescence intensity than surrounding normal skin, squamous cell carcinoma (SCC) in opposite, has revealed fluorescence intensity usually comparable and higher than that of normal skin tissues. Compounds, which fluoresce are collagen type I – at 400-405 nm; its cross-links – at 460-490 nm; elastin – with maxima at 400-420, 460 nm; elastin cross-links – about 500 nm; NADH – at 440-470 nm; keratin – at 430-460, and around 500-520 nm, and flavins.

Influence of the hemoglobin and melanin pigments is well pronounced in the received in vivo fluorescence spectra related to relative decrease of the short-wavelength vs. long-wavelength intensity, as well as appearance of minima at 420, 543 and 575 nm respectively. This relation to the absorbers' kind in the tissue under investigation is diagnostically usable spectral feature, related to the tissue condition and has significant evaluation weight, when one try to differentiate pigmented BCC and malignant melanoma, for example.

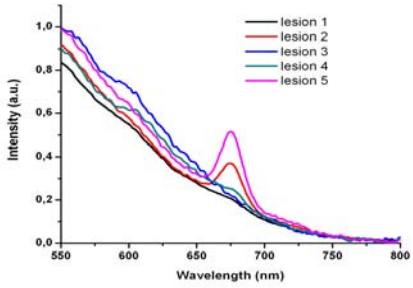
In the cases of advanced BCC lesions, a red fluorescence, related to endogenous porphyrins accumulation is also observed. This feature is useful and has been applied in our clinical practice for development of treatment planning for patients, having multiple BCC lesions, which clinical condition does not allow simultaneously treatment of all pathologies. We planned our treatment according received fluorescence data for the lesions, which correlate with the stage of growth and severity of tumour itself.



(a) (b)

Fig. 1. Fluorescence spectra of normal skin and two BCC lesions of one patient, second lesion has appeared about eight months before the light-induced fluorescence measurements carried out (a) and the first about two years before the observation (b)

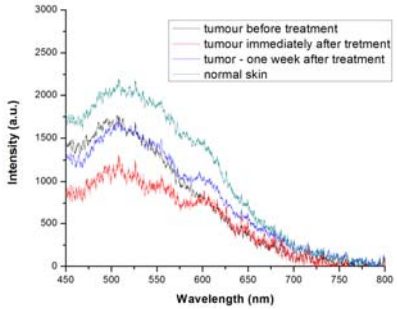
Convenient fact is that when we compare spectra of multiple lesions from same kind in one patient, we do not need to develop compensation procedures, related to inter-patient differences. Based on fluorescence spectra results initially more advanced lesion was treated, and after patient recover – initial tumour was also treated. The benefits for treatment planning, using fluorescence data from the lesions are very obvious when patient has multiple lesions – five, six or more.



Such porphyrin-like signals in advanced stages of the BCC lesions allow to develop mixed treatment plan for one 69 years old patient. Based on the fluorescence spectra obtained, lesions #2 and #5, which were on the most advanced stage, where surgically removed and chemotherapy was applied as well. Lesion #4 – intermediate stage was treated using chemo- and radiotherapy, and lesions #1 and #3 – initial stage – where treated using local chemotherapy with Aldara 3 months later.

Fig. 3. Red part of the fluorescence signal of BCC lesions on one patient, having multiple BCC tumours on different stage of growth.

Autofluorescence detection is applied for monitoring of electrochemotherapy of tumours as well. The therapeutic procedure itself - electrochemotherapy (ECT) combines chemotherapy and electroporation to increase locally cytostatic drug delivery in the cancer cells. The electroporator is battery supplied, associated with isolated ECG signals amplifier, QRS detection and synchronization circuits. The injection of local anaesthetic (1% lidocain) and cytostatic drug (bleomycin) in very small concentration direct to the tumor lesion, which is followed by application of electrical pulses. Drug delivery conditions (electric field intensity) and dose of cytostatic drug are personal for every single case. To monitor the effects of application of the electrochemotherapy fluorescence spectra are taken from the lesion and surrounding healthy skin, prior to, immediately after treatment and at the control check-ups. Patients are followed up at the first week after treatment, the first month and third month.



On the fig.4. are presented results from such therapeutic monitoring, using 365 nm excitation of BCC lesion and normal skin. BCC tumour has lower intensity than normal tissue. It is clearly observed immediate reaction after therapeutic procedure application – appearance of specific minima at 543 and 575 nm, related to increased hemoglobin absorption. One week later the fluorescence intensity of the lesion area is higher and approach to the "normal skin" spectral shape, which is indication for successful treatment of the tumour.

Fig.4. Fluorescence spectra of normal skin, BCC lesion before and immediately after ECT procedure, as well as one-week follow up of the lesion.

Clinical trial is currently under implementation and with broadening of the database with fluorescence spectra of major skin benign and malignant pathologies we expect to receive objective tool for cancer detection and treatment monitoring.

Acknowledgements: This work is supported by the National Science Fund of Bulgarian Ministry of Education, Youth and Science under grants MU-03-46/2011 "Development and introduction of optical biopsy for early diagnosis of malignant tumours", DO-02-112/2008 "National Center of Biomedical Photonics" and DO-02-178 "Cancer cells and solid tumours treatment using appropriate electric pulses selection"

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