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ADVANTAGES OF LASER PHOTOACOUSTIC SPECTROSCOPY IN RADIOTHERAPY CHARACTERIZATION*

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Abstract. The present work describes an extremely sensitive apparatus based upon laser photoacoustic spectroscopy (LPAS) methods which can be used for the optical detection of breath ethylene concentration at patients treated by radiotherapy.
We analyzed the breath air from patients, subjected to radiation treatment with a high dose fraction, depending on the cancer type and we have found out that patients treated by external radiotherapy suffer a slight increase in the generation of oxidants; however, in accordance with the clinical practice, this increase is sufficient to assess the body response to the treatment: the smaller the increase, the higher the radio-resistivity developed by the patient.
With the relevant characteristics of high sensitivity and specificity, LPAS holds a great potential for medical diagnostics and future steps are to be taken in order to bring this method near the patient bad.

Key words: oxidative attack, cancer, radiotherapy, laser photoacoustic spectroscopy.

1. INTRODUCTION

Different agents and reactions such as ionizing radiation, UV radiation, autoxidation, and drugs induce damage and lesions to DNA. Up to 10000 oxidative damaging events upon the DNA of each cell in the human body are estimated to occur every day. Among reactive oxygen species, such as singlet oxygen, hydrogen peroxide, superoxide anion, hydroxyl radical, and hydroperoxyl radical, hydroxyl radical is believed to be the most damaging [1, 2]. Oxidative attack is able to disturb the oxidant-antioxidant balance in the human body in favor of the former,

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thereby influencing many pathological conditions. Such pathologies, including cancer, sepsis, osteoporosis, diabetes, multiple sclerosis, rheumatoid arthritis, and inflammatory bowel diseases are essentially associated with genetically programmed cell death, a phenomenon that plays an important role in normal development, morphogenesis, tissue remodeling, and immune regulation [3, 4].

Cancer as one of the most threatening human diseases is most commonly treated by radiotherapy and chemotherapy. The key problem associated with such cancer treatment is that radiotherapies are relatively non-specific. When considering ionizing radiations, a substantial part of the total interactions concerns water molecules, water being the major component of living tissue present in all biological systems. Consequently, water ions and radicals are mainly generated inside tissues as primary reactive species. Those reactive species interact with biomolecules, damage them (indirect effect of radiation) and may cause general systemic distribution with deleterious side effects on surrounding healthy cells [4].

Exhaled breath is very attractive as it offers a non-invasive, safe and straightforward observation of multiple biochemical processes occurring within the human body. A fast and affordable broad-spectrum health-screen run in-clinic is a common ambition held by many researchers in this area. Volatile substances are exhaled in the gaseous phase, while low-volatile and non-volatile substances (like lipids, proteins, cellular fragments, DNA, etc) are exhaled in sub-micron size droplets generated by turbulent motion of air due to airway reopening following airway closure. Exhaled air may also contain environmental contaminants that are not deposited in the airways during the respiratory cycle [5].

Oxidative attack causes a cascade of hormonal releases. The most well-known is the secretion of cortisol, but this is only one aspect of the body's response. Long term exposure to psychological stress has been reported to cause headaches, back pain and reduced cognitive function. Such observations arouse curiosity as to whether stress may be indentified in breath volatile organic compound profiles, and if so whether any such putative markers of stress might be potential confounding factors for diagnosis of disease by breath analysis [6].

This study, therefore, sought to engender a stress response in the exhaled breath of cancer patients, subjected to radiotherapy.

Experimental measurements were performed applying CO_2 laser photoacoustic spectroscopy (LPAS) technique at 9 patients with cancer treatment.

During the last years the LPAS method has been developed to a high degree of perfection and has emerged as a very powerful investigation method for monitoring and diagnostics, able of measuring trace gas concentrations at sub-parts per billion (ppb) level.

The LPAS technique that is developed in this research complies with following requirements: non-invasive and near real time monitoring, ensuring the advantages of health state assessment by monitoring the evolution of breath biomarkers in human organism, loading a simple equipment with high sensitivity and selectivity, ease for use in clinical practice.

2. THE PRINCIPLE OF EXPERIMENTAL METHOD

To measure the level of oxidative attack at patients with cancer, we evaluated the ethylene biomarker in the breath by using the LPAS method, because it is emerging as a standard technique for measuring extremely low absorptions independent of the path length and offer a degree of parameter control that cannot be attained by other methods.

The used experimental system is a photoacoustic (PA) spectrometer with a homebuilt, line-tunable and frequency stabilized CO_2 laser which emits cw radiation at wavelengths between 9 and 11 μm (Fig. 1).

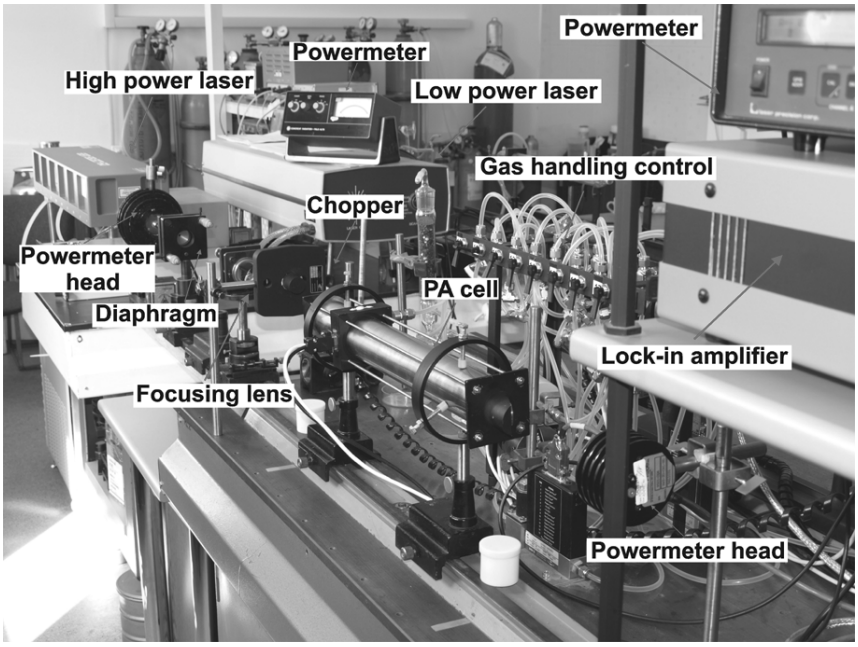

Fig. 1 – A view of the experimental LPAS installation.

This laser has a maximum power of 6.5 W on 10P(20) line and a tunability on 62 vibrational-rotational lines in all four spectral bands.

The light beam was modulated by a high quality, low vibration noise and variable speed mechanical chopper, operated at the appropriate resonant frequency of the cell (564 Hz) and then was focused by a ZnSe lens, and introduced in the PA spectrometer. The power of the laser beam (after passage through the PA spectrometer) is measured by a laser powermeter and its digital output is introduced in the data acquisition interface module together with the output from the lock-in amplifier. All experimental data are processed and stored by a computer [7–9].

In LPAS method, the resulting signal, processed by the phase sensitive detector, is directly proportional to the absorption coefficient and the laser power. The sensitivity of the technique is such that absorptions of $<10^{-7}$ cm^{-1} can be measured over path lengths of a few tens of centimeters.

Water vapors and carbon dioxide may influence the measurements and may impede the low concentration range detection. One way to overcome this problem is to partially remove these gases from the sample by absorbing them on a potassium hydroxide scrubber (KOH) inserted between the sampling cell and the PA spectrometer. The KOH scrubber must neither change the ethylene concentration level, nor introduce new interfering gases. We investigated the efficiency of the KOH scrubber using recipients with different volumes, and we found out that a quantity of minimum 120 cm^3 KOH pellets should be used for a sampling bag of 750 mL in order to keep the detection of ethylene traces free of CO_2 interference [10].

First of all, we analyzed the experimental values of ethylene absorption coefficients (detailed in [11, 12]), for all CO_2 laser wavelengths, unique for the laser frequency and ethylene molecules. These signatures are absolute entities and provide the specifics of instrument performance in terms of detection limit and interference rejection.

Using LPAS we proved that this technique is able to measure trace amounts of ethylene using CO_2 laser lines where ethylene [10P (14)] absorption coefficients have the maximum values.

3. RESULTS AND DISCUSSION

To understand the role of stress damage, it is important to focus on principal mechanisms that are responsible for cellular functions under oxidative stress conditions. Ethylene from the human breath is an indicator of stress damage in ionizing radiation damage of human skin and can be directly correlated to physiological events in the organism of the patients. So, we analyzed the breath ethylene from 9 subjects (females and males with age between 31 and 78 years old) with cancer, treated by X-ray (at Coltea Clinical Hospital, Bucharest), after tumor surgery. The breath probes were collected using multi-patient sampling bags (750 mL) before the X-ray external beam treatment, immediately after the treatment and at 15 minutes after the X-ray external beam treatment.

After the breath air is collected the sample gas is able to be transferred at any time into the PA spectrometer and analyzed by the LPAS technique. Firstly we evacuated thoroughly the previous gas mixture from all the handling system, and then flushed the system with pure nitrogen (it is a pure clean non expanding inert gas) at atmospheric pressure (1024–1034 mbar) for few minutes. After a third vacuum cleaning, the gas samples were transferred in the PA spectrometer and then evaluated.

The exhaled gas sample was transferred to the PA spectrometer (with a total volume ≈ 1000 mL) at a controlled flow rate of 300 sccm (standard cubic centimeters per minute) in about 2 minutes, and the total pressure of the gas in the PA spectrometer was measured (with a working temperature of 23–25^0 C).

The assessment of breath ethylene was performed for one specific CO_2 laser line [10P (14)] where we have the maximum absorption coefficient (α = 30.4 cm^{-1} atm^{-1}, λ = 949.479 cm^{-1}) [11–13].

Figure 2 presents the levels of ethylene biomarker experimentally evaluated for 9 patients subjected to radiation treatment with a high dose fraction (2–8 Gy/fraction), depending on the cancer type. The breath measurements before the radiotherapy are compared with the breath measurements immediately after the radiation treatment.

We note that immediately after the radiotherapy, the patients suffer an increase in the generation of oxidants and in accordance with the clinical practice, this increase is sufficient to assess the body response to the treatment [14].

So, as it shown in Fig. 2, it is obviously that at patients with cancer and particularly at those exposed to radiation treatment, the ethylene level is increased, proving the presence of oxidative attack.

Radiotherapy may intensify the oxidative attack and this seems to be due to multiple factors including an increase in the production of agents from oxidative metabolism, and a decrease in anti-oxidant defenses.

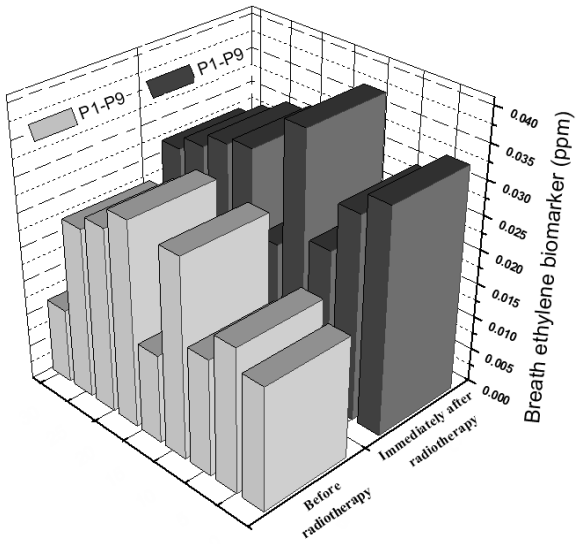

Fig. 2 – Breath ethylene concentration in exhaled breath for nine patients (P1-P9) before radiotherapy and immediately after radiotherapy.

Figure 3 compares the breath ethylene biomarker levels immediately after the radiation treatment and at 15 minutes from the treatment.

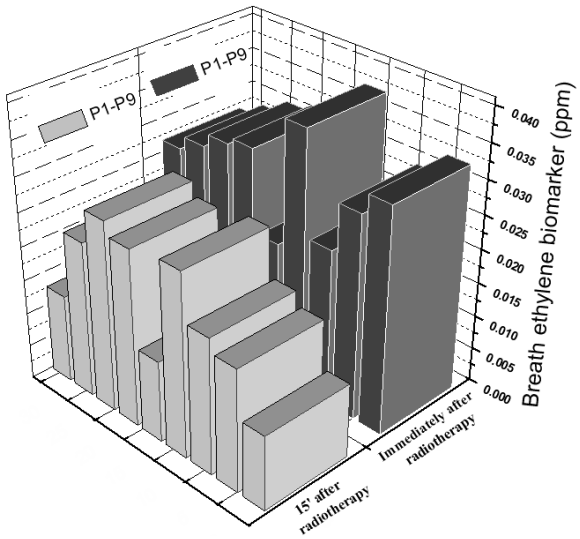

Fig. 3 – Breath ethylene concentration in exhaled breath for nine patients (P1-P9) immediately after radiotherapy and at 15 minutes from the radiotherapy.

An unexpected decrease was noted in the breath air after 15 minutes, the level of ethylene biomarker being (in most of the breath exhaled cases) even lower that the normal baseline of the patients (the ethylene measured before radiotherapy). The unexpected decrease could be explained as an organism reaction to the increased concentration of oxidative damage: the higher the level of damage, the higher the radio-resistivity developed by the patient. Further research is required in order to verify this hypothesis.

4. CONCLUSIONS

Breath ethylene biomarker of oxidative attack and DNA damage can be used for monitoring stress inside the human body, but also they may have the potential both to act as marker of disease development risk and to assess efficacy of radiotherapy.

We have monitored the evolution of the oxidative attack before, immediately after and at 15 minutes from the radiotherapy using the exhaled ethylene as a biomarker.

These measurements were only possible because of the high sensitivity of our LPAS system, sensitivity that was obtained through successively improvements in optics, laser source and electronics (faster response, low noise equipment).

The LPAS system provides a very useful assessment of oxidative damage and production of ethylene and demonstrates that it is perfectly suitable as diagnostic tool by assessing the breath compounds.

With the relevant characteristics of high sensitivity and specificity, LPAS holds a great potential for medical diagnostics and future steps are to be taken in order to bring this method near the patient bad. More comprehensive studies are necessary to establish the exact biomarker correlation with the radioresistance, considering age, sex, and treatment received by the patients. Also, efforts for technological improvement of the system will be done, in order to increase compactness and ease of maneuvrability.

As future work, we intend to extend the number of patients and conditions that the LPAS system can analyze.

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