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Laser-based spectrometer for optical trace gas detection in young adults with autism

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ABSTRACT

Autism is known as a complex neurodevelopmental disability that involve a combination of impairments in communication, reciprocal social interaction, and stereotypic behaviors. Many studies linked oxidative stress (OS) with the etiopathogenesis of Autism Spectrum Disorders (ASD), but the literature reports somewhat contradictory results. The aim of our study was to evaluate ethylene as a by-products of OS in human body, in people with autism. We used a laser-based spectrometer for optical trace ethylene detection. The results indicated that OS was not significantly increased in this disorder. As a by-product of OS exhaled ethylene from adults with autism presented very small concentration differences compared to healthy controls. The possible responsible factor for the normality between the concentrations of breath ethylene at young adults with autism and control adults could be explained by the antioxidants intake together with the lifestyle and dietary patterns. Additional studies are needed to determine carefully which antioxidant will have the greatest therapeutic benefit considering the importance of oxidative stress in many biological reactions.

1. Introduction

Autism is a spectrum condition, a behavioral disorder, with hallmark communication and social deficits [1]. The routes to obtain a precise diagnosis are very difficult because the characteristics of autism may vary from one person to another [2]. There are different ways a person can be affected by ASD and so, having a diagnostic decision is a complex process [1]. Conclusive diagnosis of ASD can be made by a team of professionals experienced in the field like psychologist, psychiatrist, neurologist, developmental pediatrician, or similar qualified medical professional [3]. Many autism biomarkers have been proposed for diagnostic of ADS [4] but at present there ar no validated biomarkers for clinical practice use [5].

It has been suggested that OS may play a role in etiopathogenesis of ADS [6,7].

The etiology of this disorder remains elusive, but OS and the understanding of the potential role of lipid peroxidation (LP) - a degradation of cell membrane by free radicals - in the etiopathogenesis of autism would be very useful for therapeutic or preventive strategies into clinical settings [6–9].

OS in biological molecules occures when the balance between reactive oxygen species (ROS) produced and the cell's antioxidant defense mechanisms is modified and is performed the function of free radical inactivation. Reactive oxigene species include ions, free radicals and peroxides. During times of environmental stress ROS levels can increase dramatically which can results in significant damage to cell structures, especially in absence of antioxidant defenses, such as the enzymes superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase or antioxidant vitamins A, C and E and polyphenol antioxidants. ROS are highly reactive molecules and when interact with polyunsaturated fatty acids of lipid membranes may induce LP [10]. The end product of LP, ethylene, have been considered to be a marker of OS [11].

Several studies have been investigated the association between OS and ASD sometimes with contradictory results [6,10,12–14].

Newer breath analysis techiques, focused on detection of OS markers show promisse in unreveling new informations in ASD.

Analysis of exhaled breath is a noninvasive sampling technique easy to performe and safe for the patients, having no undesirable side effects [15]. The results are achieved in near real time and data are immediately available to the clinicians, helping in the treatment decision-making process, reducing the number of visits to the clinic.

Human breath is mainly composed of nitrogen, oxygen, carbon dioxide, water vapors and other gases found in much smaller proportions like volatile organic compounds (VOCs), estimated as parts per trillion (ppt) or parts per billion (ppb) by volume of the exhaled breath. Part of these VOCs are of endogenous origin produced within the body, while several hundred others are exogenic, taken up from the environment [16]. VOCs derived from cellular metabolism are transported with

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the blood to the alveoli of the lung, for release through exhalation as important breath biomarkers to distinguish healthy and diseased state [17].

Analysis of breath biomarkers is still in an exploratory area presenting challenges in the field of research and the progress is closely related with the detection techniques.

The field of breath analysis has gained considerable research attentionin recent years, due in part to the relatively recent developments of real-time trace gas sensor technologies. There are a variety of gas sensing techniques including gas chromatography (GC), mass spectrometry (MS) or combinations of the two (GC-MS), chemiluminescence, Fourier transform infrared spectroscopy (FTIR) and electrochemical sensors, which do not meet all the requirements, and only in some cases, researchers have succeeded in identifying VOCs that are specific to certain diseases. Laser-based gas detection systems can offer analysis at high sensitivity and selectivity, and often in real-time. Therefore they introduced in applications where there is a need for rapid, specific and precise measurements of trace gases. In addition to high sensitivity and necessary selectivity for analyzing multicomponent mixtures by the use of line-tunable CO_2 lasers, laser photoacoustic spectroscopy (LPAS) systems also provides high accuracy and precision and good temporal resolution.

CO_2 laser photoacoustic spectroscopy is a well known technique for measure trace gases at ppm (parts-per-million) or ppb level. Analysis of exhaled breath using laser-based spectrometer its suitable for molecular identification and quantification to provide the pathophysiological status of the body.

The present study uses CO_2 laser photoacoustic spectroscopy to compare the level of OS (given by the exhaled ethylene) from young adults with autism with the level of OS from individuals with healthy physiological state.

2. Experimental

2.1. Participants and sampling

Breath samples were investigated at the Optics and Lasers in Life Sciences, Environment and Manufacturing Laboratory from National Institute for Laser, Plasma and Radiation Physics.

The young people with autism were recruited and informed consent was obtained from the team with experience at the C.S.C.C.H.S. Center, Calarasi, Romania (all gave their informed consent to participate in this research, which was approved by the institutional review boards of institutions). The trial protocol was reviewed by ward consultants at the C.S.C.C.H.S. Center, and patients matched for age and gender.

The diagnosis of autism was made based on the criteria of autism disorders as evaluated in the Complex Evaluation Service of the C.S.C.C.H.S. Center.

A total of nine young adults (7 males and 2 females, age range from 23 to 26 years) who had been previously diagnosed as suffering from autism and nine subjects without any history of psychiatric illness or other diseases, were selected as a control group (9 males, age range from 25 to 33) and included in the study. It is important to mention that the adults with autism prior to the study (a month before) took some vitamin complex.

The non autistic subjects were non- or ex-smokers, non-alcoholic, non-renal, non-diabetic and free from psychiatry disorders, somatic diseases or brain tumors and had never been treated with antidepressant or antipsychotic medications.

Prior to the analysis of breath, the subjects avoided for at least 3 h before or at any time during the breath sample collection: alcohol and coffee, food or beverages and to refrain from exercise in the morning. On the day prior the test, they stop all consumption of products such as onions, leeks, eggs, and garlic.

The collection of breath samples was made in 0.75-l aluminum-coated bags, designed to collect multiple samples from subject and hold a sample for maximum 6 h. All of the collected samples were analyzed within 3 h after sampling over a period of 1 months. A total of 10 samples per subject were collected over this period.

2.2. Photoacoustic setup

Laser photoacoustic spectroscopy is essentially based on the generation of an acoustic wave in a gas excited by a modulated laser beam. This is a technique where laser radiation is modulated at a wavelength that overlaps with the spectral feature of the target species. A fraction of the ground-state molecular population of the target molecule is excited by absorption of the incident laser radiation (see Fig. 1).

Between vibrational levels and from vibrational states to rotational degrees of freedom occur energy exchange processes. The absorbed energy by a vibrational-rotational transition is almost completely converted to kinetic energy by collisional de-excitation of the excited state. At the modulation frequency, the kinetic energy is converted into a periodic local heating. During expansion and contraction of the gas in a closed volume a pressure variation which leads to the formation of a standing acoustic wave in the resonator. These acoustic waves are measurable with sensitive microphones [17–21].

A typical setup of a CO_2 laser photoacoustic spectroscopy was used to analyzed the breath ethylene from the young adults with autism (see Fig. 2).

The entire spectroscopic system is formed of a line-tunable CO_2 laser and a photoacosutic cell where the gas is detected and analyzed, in combination with a flow-through system [18,19,20].

The CO_2 laser is continuous wave, line-tunable and frequency-stabilized, emitting radiation in the 9.2–10.8 μm region on 52 different vibrational-rotational lines with powers varying between 0.5 and 6.5 W depending on the emmited laser transition.

The CO_2 laser beam is modulated in intensity by a high quality low vibration noise and variable speed mechanical chopper (DigiRad C-980 or C-995) operating at the appropriate resonant frequency of the cell (564 Hz), is focused by a ZnSe lens, and then is introduced in the photoacoustic cell. In the resonator tube wall carefully are embedded four microphones (Knowles electret - EK-23024) where the acoustic wave is detected and generate a corresponding signal (voltage) which is fed into a lock-in amplifier (Stanford Research Systems model SR 830). The lock-in amplifier gives the amplitude and the phase of the photoacoustic signal synchronized to the chopper phase. The amplitude of the photoacoustic signal is proportional with the concentration of the absorbing molecules. A powermeter measures the laser beam power after the photoacoustic cell. Its digital output is introduced in the data acquisition interface module together with the output from a lock-in amplifier. All experimental data are processed and stored by a computer. The modular software architecture aimed at controlling the experiments, collecting data, and preprocessing information. It helps to

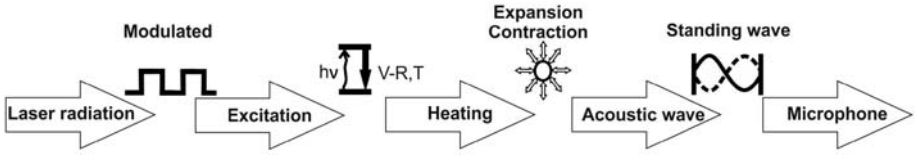

Fig. 1. Schematic of the physical processes during optical excitation of molecules in photoacoustic spectroscopy.