

Investigation of Correlation between Neurological Diseases and Breath Hexanal

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Abstract

Breath analysis is a method that has the potential to be used as a non-invasive method for the diagnosis of diseases, in the planning of drug use, and in indicating the health status of the patient, and of which importance increases with each passing day. The aim of this study is to examine neurological diseases from breath hexanal. In our study, the breaths of a total of 29 individuals consisting of 9 multiple sclerosis patients, 8 Parkinson's patients and 12 healthy individuals in the control group were examined by means of the thermal desorption gas chromatography mass spectroscopy method. According to the results obtained, hexanal molecule, known as the biomarker for lipid peroxidation, was observed in patients with a statistically significant difference compared to the control group with multiple sclerosis patients as $p=0.048$. No correlation was found between Parkinson's patient and the control group.

Keywords: Breath analysis; Multiple sclerosis; Parkinson's disease; Hexanal

Introduction

The breath analysis method has the potential to be used in the diagnosis of diseases, in the follow-up of treatment processes, and in the investigation of metabolisms. The diagnosis of diseases by the breath analysis will provide a great convenience for both patients and doctors. For example, the diagnosis and treatment process of MS disease can be painful and disturbing for patients. During the initial diagnosis of MS disease, cerebrospinal fluid (CSF) is taken with a needle having a depth of approximately 8-10 cm. It is known that in patients with MS, CSF contains immunoglobulins synthesised in the central nervous system. In the event that the disease is diagnosed by breath, a risk-free, painless and an easy method will be provided for patients and doctors. The main mechanisms that play a role in the formation of neurodegenerative diseases are oxidative stress, mitochondrial dysfunction and inflammation [1]. The brain is very sensitive to oxidative damage due to the high amount of polyunsaturated fatty acid, high-speed metabolic activity and low antioxidant defence capacity [2].

There is not much study about breath analysis of MS and Parkinson's diseases. In a study in which lipid peroxidation is predicted to increase with the damage of the myelin sheath consisting of the lipid structure at the time of the attack [3], ethane and pentane molecules which are the biomarkers of lipid peroxidation were examined from breath. At the time of the MS attack, it was determined that the breath pentane levels become to be higher, but the ethane molecule levels don't increase. It was interpreted that the reason underlying the non-increase of ethane molecule levels may be due to the low unsaturated fat concentration in the brains of MS patients. It was emphasized that the pentane molecule levels increased in direct proportion to the increased oxidative stress. Tisch et al. studied breath air of Alzheimer's patients and Parkinson's patients to distinguish these diseases from breath and showed remarkable results accuracy of 85, 78 and 84%, respectively [4]. In another study, the levels of hexanal molecules in the breath of MS patients were found higher than the control group and it was indicated that the hexanal molecule could be used with 80.4% accuracy in the diagnosis of MS disease [5]. According to our study hexanal molecule is higher in MS patients like Ionescu et al. The concentrations of the breath molecules of the healthy individuals in the literature are shown in the Table 1.

The present study has been done for better understanding the

MS Patients	
Gender (male/female)	3/6
Age (years)	Between 13 and 49 years
	Average:32,9 years
	Standard Deviation (SD): 9,7
BMI (kg m ⁻²)	Between 16,5 and 37,5
	Average :24,3
	SD: 5,6
Parkinson's Patients	
Gender (male/female)	6/2
Age (years)	Between 40 and 72 years
	Average:54,1 years
	Standard Deviation (SD): 12,2
BMI (kg m ⁻²)	Between 19,3 and 33
	Average:23,6
	SD: 4,4
Healthy Control Group	
Gender (male/female)	10/2
Age (years)	Between 22 and 42 years
	Average:27,8 years
	Standard Deviation (SD): 7,3
BMI (kg m ⁻²)	Between 21 and 26,4
	Average :24,2
	SD: 1,5

Table 1: Demographic, clinical and laboratory features of 13 MS patients, 8 Parkinson's patients, and 12 healthy volunteers.

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metabolism of the diseases under investigation and for convenient and non-invasive diagnosis of the diseases in question. In our study, hexanal molecules present in the breath of a total of 29 individuals consisting of 9 multiple sclerosis patients (MS), 8 Parkinson's patients and 12 healthy individuals in the control group were examined by means of the thermal desorption gas chromatography mass spectroscopy (TD-GC/MS) method. Statistically significant differences were observed between the hexanal molecule and MS patients and the control group. No correlation was found between the hexanal levels present in the breaths of Parkinson's patients and the control group. MS attacks are currently diagnosed by MR. During the patient's waiting for MR, the attack status can damage the patient. During the MS attacks, the non-invasive analyses of the amounts of hexanal, molecules found in the breath can be a significant study and it can provide an immediate intervention for attacks.

Methods

The information about the patients and the control group of which breath samples were taken is given in Table 1. Patients with a respiratory disorder such as active upper respiratory tract infection, etc., patients with severe neurological deficits that would prevent the evaluation of the patients, patients with cognitive impairment and coexistent depression and/or another systemic disorder, and patients with an EDSS score of over 6.5 for MS patients were not included in the study. Information on the age, height, weight, medications used, smoking and alcohol consumption of the participants was obtained from the forms filled in. The study was conducted with the patients of Suleyman Demirel Research and Application Hospital and approved by the ethics committee of the hospital. Written consents were obtained from all volunteers regarding their participation in the study.

In our study, the amounts of hexanal in the breaths of MS and Parkinson's patients were examined by being compared with the control group. Statistical calculations were performed by the 'One-Way ANOVA' test using OriginPro 2017 program.

Breath collection

The exhaled breath samples were collected from each patient and control group before meal (12:00 a.m.). For TD-GC/MS analysis, breath samples were collected by Bio-VOC system, and transferred to Tenax-TA 200-mg polymeric thermal desorption tubes (Markes, Bio-VOC). Tenax-TA tubes has low affinity for water, and suitable for trapping of volatile and semi-volatile compounds from breath and vapor. Tenax-TA tubes were store at $< 4^{\circ}\text{C}$ to minimize the evaporation of VOCs and preserved for about maximum 15 days which is a suitable time to preserve VOCs.

Thermal Desorber-Gas Chromatography / Mass Spectroscopy

GC is commonly used for the breath analysis together with MS. In this study, TD-GC/MS was used for the analysis of related breath molecule levels (Agilent Technologies 7890A 5975C). Volatile organic compounds (VOCs) were thermally desorbed by the thermal desorber injected into GC/MS. The conditions for the thermal desorber are shown in Table 2.

A non-partitioned mode was applied to the TD system. The GC/MS injector temperature was set at 250°C and the flow rate was set at 20 psi. The column oven temperature was held at 60°C for 4 min and then raised to 180°C by increasing by 20°C per min. After being held at 180°C for 15 min, the temperature was again raised to 215°C at a rate

Pre-desorption settings Split on in standby	
Flow path temperature	200°C
Minimum transporter pressure	5 psi
Pre-purification time	1 min
Tube / sample desorption settings	
Tube desorption time	5 min
Tube desorption temperature	250°C
Trap Settings	
Pre-trap fire purge	Minimum 1 min of trap flow
Trap low	-10°C
Trap heating rate	Maximum
Trap capture / minute	3 min
Split	Open
Trap maximum	300°C

Table 2: Thermal desorption unit settings

of $4^{\circ}\text{C}/\text{min}$. It was held at this temperature for 20 min. The column oven was also heated up to 240°C by 4°C per min. It was held at this temperature for 35 min. The analytes were then injected into a capillary column, which was DB-5 non-polar, and of which length was 30 m and internal diameter was 0.25 mm. The selected ion monitoring (SIM) mode was used to obtain the sub-detection limits. The SIM mode allows us to get much more effective results in the full scan mode. Helium gas (99.999%) was used as a transporter with a flow of approximately 20 psi in the analytical column. The ionisation of the individual compounds was carried out by electron impact ionisation at 70 eV.

For the SIM mode calibration, it is necessary to manually inject and introduce standards at different concentrations to the TD-GC/MS device. The same temperature program was then applied for the analysis of a sample and the molecules in the breath mixture were identified. Limit of detection (LOD) is found as 0.7 ppb. In the study, the calibration curves were observed to be linear.

Results

The results obtained in our study are given in Figure 1. According to the results below, upon comparing the results of MS and Parkinson's patients and the healthy control group, the levels of molecules examined were lower in the control group when compared to the patients. According to these results, upon comparing the amount of hexanal in the breaths of MS patients and healthy volunteers, it was seen that the hexanal amounts in the breaths of MS patients were significantly higher ($p=0.048$).

No correlation of the molecules we examined in MS, Parkinson's patients with age and BMI was found. The results obtained for MS patients are shown in Figure 2 as an example.

Discussion

Metabolisms related to hexanal molecule

Lipid peroxidation metabolism: During lipid peroxidation, along with the increase in the formation of reactive oxygen species (ROS) that cause the increase in oxidative stress and neurotoxicity in the brain, an increase in lipid peroxidation markers such as malondialdehyde (MDA) and hexanal, deterioration in mitochondrial functions, and a decrease in antioxidant enzymes such as reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GRd) are observed [6].

The amount of lipid in nerve tissues is especially high. Lipid peroxidation is important in many neurological diseases due to lipid-rich membranes of nerve cells. At the first stage of lipid peroxidation, lipid radical is formed by removing an H atom containing an electron from conjugated double bonds in fatty acids. The lipid radical forms the lipid peroxide radical (LOOH) by reacting with oxygen. LOOH degradation occurs with the ion catalysis of the transition metals. The cell membrane and organelle lipid peroxidation can be stimulated by free radicals and increases in the presence of transition metals. The formation of the hydroxyl radical (OH·) from hydrogen peroxide (H₂O₂) can initiate a chain reaction. As a result of these reactions, aldehydes (e.g. hexanal) emerge as end products. Also 5-HPETE (5-Hydroperoxyeicosatetraenoic acid) is the compound incorporated in the aldehyde structure (Figure 3) [7,8].

The lipid peroxidation metabolism progressing without enzymes is a very harmful chain reaction. In these reactions, the membrane structure is directly damaged and the produced reactive aldehydes indirectly damage other cell components. This can cause many diseases [9,10]. Hexanal lipid peroxidation during lipid peroxidation is the molecule that occurs in the breath as a biomarker [11-15].

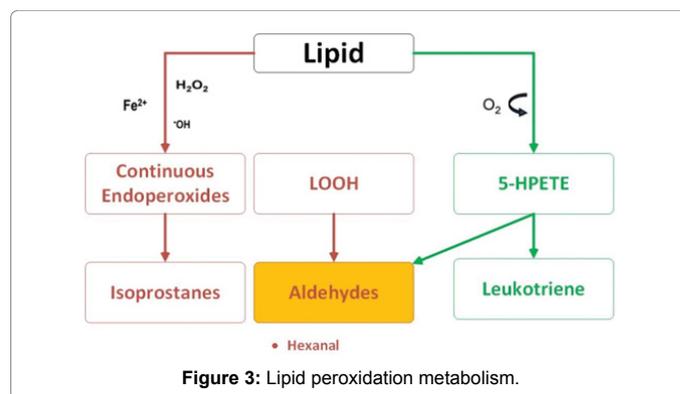


Figure 3: Lipid peroxidation metabolism.

Multiple sclerosis

In myelinated nerves, nonpolar lipids allow the rapid spread of depolarization waves as electrical isolators. In MS, the theory that the lesion formation develops as these lipids damage the immune system is widespread. Lipids also assist mitochondria in the electron transport function. In communication, recognition, and immunity cases in all cells, lipids also play important roles.

MS is a repetitive or progressive inflammatory demyelinating disease of the central nervous system. The attack of the immune system to the neural myelins at the time of the attack of the patient leads to disorders in the nerve conduction. The formation of plaques with demyelinating in the brain and sometimes in the spinal cord in MS causes pyramidal, sensory, cerebellar, rarely autonomic and extrapyramidal system disorders [16]. Zhornitsky et al. reported that 24-hydroxycholesterol and 27-hydroxycholesterol, which are the intermediate products of MS disease and lipid peroxidation, cause cell damage. These molecules play a role in the MS progression. It is reported that MS disease progresses as cell damage and neurotoxicity increase [17]. Lazar et al. determined that lipid peroxidation is effective in some neurodegenerative diseases such as Alzheimer's disease, MS and Huntington's disease [18]. There are other studies that prove that lipid peroxidation is higher in MS patients [19-23].

Parkinson's disease

The main symptoms of Parkinson's disease are the slowness and stiffness of walking and movements, difficulty in initiating movements and in fine works, dull face, muscle stiffness, hand and leg tremor while resting, deterioration of posture, balance, and reflexes, bending forward of the body [24].

The most characteristic feature of Parkinson's disease is the occurrence of degeneration and cell loss in a cell group called 'substantia nigra' which is bilaterally symmetrically located in the area called the brain stem. At least 60-70% of the cells have to be lost for the development of Parkinson's disease. In fact, the disease starts long before the symptoms emerge. Dopamine is a neurotransmitter produced in the area designated to be substantia nigra. With the damage to this region, dopamine deficiency and difficulty in initiating the movements and progressing them in a harmonious way occur. The greater the dopamine deficiency of the patients is, the greater the distresses about the movement are. However, there is no direct correlation between the excess tremor and the decrease in dopamine. Parkinson's disease is a slowly progressing disease. Studies have been conducted on the fact that in Parkinson's disease, lipid peroxidation increases specifically in the 'substantia nigra' region and causes the degenerative death of cells in this region [25-27].

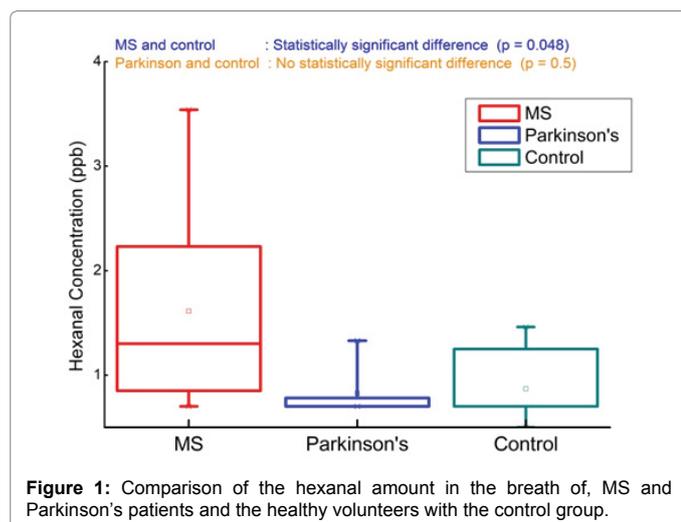


Figure 1: Comparison of the hexanal amount in the breath of, MS and Parkinson's patients and the healthy volunteers with the control group.

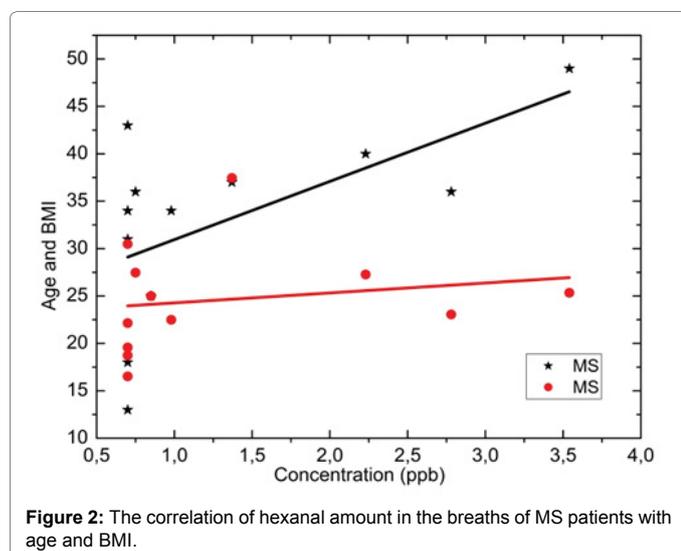


Figure 2: The correlation of hexanal amount in the breaths of MS patients with age and BMI.

In our study, no statistically significant correlation was observed between the levels of hexanal molecules in the breaths of the patients and the healthy control group, although there are studies on the presence of lipid peroxidation in Parkinson's patients.

Conclusion

As far as we know, neurological diseases have not been studied extensively with the methods of breath analysis. In this study we conducted, the relationship between hexanal molecules and neurological diseases as MS and Parkinson's was examined. Statistically significant differences were observed between the hexanal molecule in breath of MS patients and the control group. The high levels of hexanal molecule in MS patients may indicate a high level of lipid peroxidation in these diseases. No correlation was found between the hexanal levels in the breaths of Parkinson's patients and the healthy control group. MS attacks are currently diagnosed with MR. During the patient's waiting for MR; the attack status can damage the patient. Immediate results given diagnose techniques and studies for MS are a need in this area.

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