

Original Article

Determination of Plasma Malondialdehyde Values as a Laboratory Index of Lipid Peroxidation Reaction in Multiple Sclerosis Patients and Its Comparison With Normal People

Zahra Goli¹ , Soudabeh Mashayekhi¹, Mohammad Taheri², Mehrdokht Mazdeh³, Mohammad Reza Safari^{2*} 

¹Clinical Chemistry Department, Hamadan University of Medical Sciences, Hamadan, Iran

²Medical Laboratory Department, Hamadan University of Medical Sciences, Hamadan, Iran

³Neurology Department, Hamadan University of Medical Sciences, Hamadan, Iran

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*Corresponding author:

Mohammad Reza Safari,
Email: safarimr2000@yahoo.com



Abstract

Background: Lipid peroxidation reaction and plasma malondialdehyde (MDA) production are important prognoses in the formation and development of neurodegenerative diseases. The link between lipid peroxidation reaction and plasma MDA in multiple sclerosis (MS) patients is poorly understood. In this study, the plasma MDA values of people with MS and normal people were measured.

Methods: In this study, 70 patients with MS and 70 thermally healthy individuals were selected. Their blood samples were poured into the buffer, and the plasma was separated. Then, plasma MDA values were measured through the thiobarbituric acid (TBA) method.

Results: The results showed that the values of plasma MDA in two groups of patients with MS and healthy people are 1.49 ± 0.46 and 0.58 ± 0.15 $\mu\text{mol/L}$, respectively.

Conclusion: The findings of this research revealed that the levels of plasma MDA in MS patients are higher than those in healthy people. Therefore, these patients exhibited elevated oxidative stress.

Keywords: Lipid peroxidation, Multiple sclerosis, Plasma

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Introduction

Multiple sclerosis (MS) is a chronic disease of the central nervous system with a strong tendency to white matter in the brain, spinal cord, and optic nerve, with loss of sensory and motor function as a result of immune inflammation, demyelination, and then axon damage (1,2). During an MS attack, inflammation occurs in areas of the white matter of the central nervous system in areas called plaques. This process continues with the destruction of myelin in the brain and spinal cord, eventually leading to a decrease or lack of function (3,4). The onset of MS occurs in youth aged of 20-40 years, and its prevalence is two to three times higher in women than in men (5,6).

The reaction of lipid peroxidation in biomolecules is one of the cell and tissue damages, and its concentration changes can play an important role in the diagnosis of many degenerative diseases. Determining these values will effectively help to identify the mechanisms involved in various diseases (7,8).

The body naturally produces free radicals during

metabolism. Although free radicals are necessary in certain amounts for body functions, in larger amounts, they cause irreparable damage to the key components of the cell, including membrane lipids, nucleic acids, carbohydrates, and proteins. Hence, in this way, they affect many physiological functions and make a person naturally vulnerable to the aging process, cancer, and other diseases such as degenerative disorders. However, the body itself has a natural antioxidant and anticancer mechanism that protects most cells from oxidative damage (9,10). Therefore, according to the necessity of maintaining the balance of free radicals in the body by antioxidant agents, by maintaining this balance, the body's defense power can be improved to prevent diseases such as degenerative disorders, cancers, and other chronic diseases in humans (11).

Considering the fact that various studies on MS, as a disease whose causes have not been clearly identified, are increasing, this research aimed to measure the lipid peroxidation reaction in patients with MS. Given



that lipid peroxidation is considered a risk factor for degenerative diseases and carcinogenesis, is the level of lipid peroxidation different in MS sufferers compared to that in normal individuals?

Materials and Methods

Two groups of patients and controls (case and control) with 70 patients and 70 healthy people were studied. The test group consisted of individuals with MS who were referred to the MS Support Association and Sina hospital in Hamadan, and their presence of this disease was confirmed by the diagnosis of a neurologist. The control group included healthy people who were matched with the patient group in terms of age and sex. The health criteria of the subjects were having normal blood pressure, no history of diabetes and high blood fat, no heart disease or any other specific disease, and no use of heart drugs and blood sugar- and blood fat-lowering drugs.

After fully informing the patients about the research plan and the questionnaire by the project managers and obtaining written consent from the patients and the control group, sampling of the arm venous blood was conducted. First, 5 mL of blood was taken from each of the studied population, and each sample was immediately poured into tubes containing sodium citrate buffer (Mm 18), and the plasma of each sample was separated by centrifugation. Afterward, 100 μ L of each plasma sample, 100 μ L of ethylenediaminetetraacetic acid (EDTA) solution (10 μ mole), 1.5 mL of trichloroacetic acid (TCA) solution (20%), 1.5 mL of thiobarbituric acid (TBA) solution (0.67%), and 100 μ mole of butylated hydroxytoluene (BHT) solution (50 μ mole) were added, and after mixing with sugar, it was placed in a bain-marie at 97°C for 20 minutes. After cooling, the tubes were centrifuged for 5 minutes at 400 g, and the absorbance of the upper layer of each sample was read in a spectrophotometer at a wavelength of 532 nm. After reading the absorption numbers of the samples in this wavelength, the plasma malondialdehyde (MDA) concentration of each sample was calculated using the relation $A = abc$ where the molar absorption coefficient of MDA is equal to 165 000 mol/cm (8).

Results

In this study, plasma MDA values were measured in 70 people with MS (17-55 years old) and 70 normal healthy people (22-60 years old).

The general information of the participants in the study is summarized in Table 1.

As seen, 78% of patients with MS were women, their disease duration was 4.5 ± 3 , and the age at the onset of the

Table 1. Characteristics of the Two Studied Groups

	Male/Female %	Age	Course of Illness	Age of Disease Onset
Normal group	26/74 %	35 \pm 2	-	-
Patient group	22/78 %	36 \pm 3	4.5 \pm 3	31 \pm 3

disease was 31 ± 3 .

Table 2 presents the statistical analysis of plasma MDA values in two groups of subjects.

The average plasma MDA concentration in the test group and control group was 1.49 μ mol/liter and 0.58 μ mol/L, respectively. Furthermore, the maximum amount of plasma MDA in the test group and the control group was 2.3 and 0.82 μ mol/L, respectively, while the minimum amount of plasma MDA in the test group and the control group was 0.8 and 0.38 μ mol/L, respectively.

The information in Table 3 represents the comparison of plasma MDA values between two groups of sick and healthy people. According to the data in the table, the average values of plasma MDA in these two groups are 1.49 ± 0.46 and 0.58 ± 0.15 μ mol/L, respectively. The observed difference in the amount of plasma MDA at a level above 1% is significant based on the t-test; that is, the observed difference in the amount of MDA is significant at a level above 5%.

Discussion

MS is known as a multi-factorial disease that is caused by various factors. Although this disease is related to the immune system, various mechanisms have been reported for its pathogenesis such as oxidative stress, demyelination, and neuronal cell death (12,13).

The current study compared the serum level of MDA concentration in people with MS and normal people. In this study, a significant increase was observed in the serum level of patients with MS compared to healthy people. In line with this study, Tsikas investigated the serum level of MDA and oxidative stress factors in the serum of patients with relapsing-remitting MS and reported an increase in the serum level of MDA in these people compared to healthy people (13). According to the studies by Liu et al, brain tissue is extremely sensitive to reactive oxygen species, and it leads to lipid peroxidation due to increased oxygen consumption and high concentration of unsaturated fatty acids; as a result, MDA is caused by oxidative stress processes (12). In addition, Wang et al and Padureanu et al reported that lipid peroxidation plays an effective role in the development of neurological diseases such as Alzheimer's and Parkinson's (14,15). Additionally,

Table 2. Statistical Analysis Information of Plasma MDA Values

Statistics (MDA)	MS	Normal
Number	70	70
Mean	1.49	0.58
SD	0.46	0.16
Standard error of the mean	0.11	0.13
Median	1.5	0.55
Mode	1.1	0.49
Minimum	0.8	0.38
Maximum	2.3	0.82

Note. MDA: Malondialdehyde; MS: Multiple sclerosis; SD: Standard deviation.

Table 3. Comparison of Plasma MDA Values Between Two Groups of MS Patients and Healthy People

Studied Groups	N	MDA	Mean Difference	Error Mean Difference	95% CI Mean Difference	t	P Value
MS	70	1.49±0.46	4.32	1.81	39.56-46.84	23.8	0.05 ^a (Significance)
Normal	70	0.58±0.15	4.32	1.81	39.56-46.84	23.8	0.05 ^a (Significance)

Note. MDA: Malondialdehyde; MS: Multiple sclerosis; CI, Confidence Interval.
^a Independent two-sample *t* test.

Hollen et al evaluated the relationship between inflammatory factors and MS and reported a significant relationship between the increase of inflammatory factors and the severity of the disease in MS (16).

According to the analysis of the data obtained in the results section, it was found that the amount of MDA in patients with MS is higher than that in healthy people, and this difference was significant at the level of more than 1% based on the *t*-test. The present study aimed to accurately determine plasma MDA values as one of the main products of radical reactions in MS patients and also to compare this factor with normal healthy individuals. Comparing plasma MDA values between MS patients and healthy individuals in this study revealed that oxidative stress occurs in these patients. Inflammatory reactions are a destructive pathway in MS. Furthermore, oxidative burst related to inflammation in activated macrophages plays an important role in demyelination and tissue damage by free radicals in the pathogenesis of this disease. The inflammatory environment in demyelinated lesions leads to the production of free radicals of the active oxygen group, which ultimately leads to the progression of the disease. Therefore, oxidative stress and inflammation cause the continuation of its cycle and further damage. In addition, the demyelination of neurons and the loss of their outer covering increase vulnerability to the attack of active oxygen free radicals (17,18). Given the importance of increasing radical reactions and considering the concentration of their products such as MDA as an important predictor in the occurrence of many diseases, we aimed to measure the values of this laboratory factor in two groups of patients with MS and healthy people. According to the results of our studies, measuring MDA can be an effective tool for the management of MS.

Authors' Contribution

Conceptualization: Mohammad Reza Safari.

Data curation: Mohammad Reza Safari, Mohammad Taheri, Mehrdokht Mazdeh.

Formal analysis: Mohammad Reza Safari, Mohammad Taheri.

Funding acquisition: Mohammad Reza Safari, Zahra Goli, Soodabeh Mashayekhi.

Investigation: Mohammad Reza Safari, Mohammad Taheri, Mehrdokht Mazdeh.

Methodology: Mohammad Reza Safari, Zahra Goli, Soodabeh Mashayekhi, Saeid Zafari.

Project administration: Mohammad Reza Safari.

Resources: Mohammad Reza Safari.

Software: Mohammad Reza Safari.

Supervision: Mohammad Reza Safari, Mehrdokht Mazdeh.

Validation: Mohammad Reza Safari, Mehrdokht Mazdeh.

Visualization: Mohammad Reza Safari, Mehrdokht Mazdeh,

Mohammad Taheri.

Writing—original draft: Mohammad Reza Safari, Zahra Goli.

Writing—review & editing: Mohammad Reza Safari.

Competing Interests

None.

Ethical Approval

A research project entitled "Determination of plasma malondialdehyde levels as a laboratory index of lipid peroxidation reaction in multiple sclerosis patients and its comparison with normal individuals" with project ID number 9410015448 was approved by the Research Ethics Committee of Hamadan University of Medical Sciences and has the code ethical with the special ID IR.UMSHA.REC.1394.373.

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