Malondialdehyde and Nitric Oxide Levels in Erythrocytes from Patients with Systemic Sclerosis

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Key Words

Nitric oxide · Oxidant/antioxidant status · Systemic sclerosis

Abstract

Objective: To investigate malondialdehyde (MDA) and nitric oxide (NO) levels in the erythrocytes from patients with systemic sclerosis (SSc). **Subjects and Methods:** Twenty-nine patients diagnosed as having SSc and 16 volunteer healthy subjects (as control group) participated in the study. Fasting blood samples were obtained from the patients and healthy subjects in anticoagulated tubes (with EDTA), and the erythrocytes were separated. The MDA and NO levels were measured in the erythrocyte sediments by the spectrophotometric method. **Results:** The levels of MDA and NO were elevated in the erythrocyte sediments of the patients as compared to those of the control group (1.037 ± 0.125 vs. $0.951 \pm 0.114 \mu\text{mol/g Hb}$, respectively, $p = 0.047$ for MDA, and 0.340 ± 0.071 vs. $0.209 \pm 0.074 \text{ mmol/g Hb}$, respectively, $p < 0.001$ for NO). A weak positive correlation was also observed between MDA and NO levels ($r = 0.30$, $p = 0.15$ in the patient group, and $r = 0.27$, $p = 0.49$ in the control group). **Conclusions:** Our results show higher levels of MDA and NO in the erythrocytes of patients with SSc than normal subjects.

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Introduction

Systemic sclerosis (scleroderma, SSc) is a chronic multisystem disorder of unknown etiology, and its process involves immunological mechanisms, vascular endothelial cell activation and/or injury, and production of excessive collagen. It is characterized by thickening of the skin caused by accumulation of connective tissue, and it also involves visceral organs including the gastrointestinal tract, heart, lungs and kidneys. Vascular disorders are among the important features of SSc [1]. There is an immune-inflammatory injury in the microvascular system leading to dysregulation of vascular tone control in SSc. This process results in a decreased blood flow to the involved organs [2].

Nitric oxide (NO) is formed during the conversion of arginine to citrulline catalyzed by NO synthase. It is known that NO has many physiological functions such as that of a vasodilator in the regulation of blood pressure, as a neurotransmitter in the brain, a bactericidal agent during infection and an inhibitor of platelet aggregation [3]. It is also suggested that NO itself is a free radical and may lead to increased free radical production [4]. Malondialdehyde (MDA) is the end product of lipid peroxidation, and its level may give information about the oxidant status in vivo [5]. There are previous studies con-

cerning the levels of NO in serum, urine, exhaled air and monocytes from patients with SSc but not in erythrocytes. Hence, the objective of this study was to measure the levels of MDA and NO in erythrocytes from patients with SSc.

Subjects and Methods

The study was approved by the Ethics Committee, Faculty of Medicine, Ankara University. Twenty-nine patients (25 females and 4 males) clinically diagnosed as having SSc and 16 healthy volunteer subjects (14 females and 2 males) as control group participated in the study. The SSc patients and controls were age matched, with patients 51.7 ± 12.4 years and controls 55.3 ± 7.9 years old. Fasting blood samples were obtained from the participants in anticoagulated tubes (with EDTA). After removal of plasma, erythrocytes were washed 3 times with physiological saline solution and hemolyzed with deionized water; hemoglobin (Hb) levels of the sediments were measured by the cyanomethemoglobin method. After the Hb levels had been adjusted to the same amount, MDA and NO levels were determined. The level of MDA was measured by the thiobarbituric-acid-reactive substances method, while that of NO was measured by the method based on the Griess reaction. Since the nitrate anion does not give this reaction, the samples were treated with cadmium to reduce nitrate anions into nitrite anions before the NO assay. The levels of MDA and NO in the erythrocyte sediment were calculated as micromoles per gram Hb for MDA and millimoles per gram Hb for NO.

Statistics

The data are given as arithmetic mean $\pm$ standard deviation. In the statistical evaluation, Student's *t* test and Pearson correlation analysis were used. Values of $p < 0.05$ were accepted as significantly different.

Results and Discussion

The levels of MDA in controls and patients were 0.951 ± 0.114 and 1.037 ± 0.125 $\mu\text{mol/g Hb}$, respectively. The corresponding levels of NO were 0.209 ± 0.074 and 0.340 ± 0.071 mmol/g Hb . These results indicated elevated levels of MDA and NO in SSc compared to controls. The mean percent increase in NO in SSc was 62% compared to the very small increase of 9% in MDA. There was a weak positive correlation between MDA and NO levels in both groups ($r = 0.30$, $p = 0.15$ in the patient group, and $r = 0.27$, $p = 0.49$ in the control group).

Oxidative stress is one of the reported mechanisms in the pathogenesis of SSc [6–8], and NO is an important molecule which may play potential protective and/or harmful roles in SSc [2].

The significant increases in the levels of NO and MDA in the erythrocytes of SSc patients over those of healthy controls and the weak positive correlations between NO and MDA levels in both groups indicate that there is an increase in NO production in erythrocytes during the disease process. Further, the results of the present study also demonstrate that there is an increase in the erythrocyte MDA level, as an indicator of oxidative stress, in SSc. It is possible that the MDA level is increased owing to the increased NO level or to some other as yet not known factors.

Conclusion

Our results suggest that the NO level in erythrocytes increases in SSc which may lead to oxidant stress and peroxidation in the erythrocytes of these patients.

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