
The Levels of Sera Malondialdehyde, Erythrocyte Membrane Na⁺-K⁺/Mg⁺⁺ and Ca⁺⁺/Mg⁺⁺ Adenosine 5' Triphosphatase in Patients with Sickle Cell Anemia

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ABSTRACT

The various membrane abnormalities of sickle erythrocytes may result from excessive accumulation of oxidant damage. We measured the sera levels of malondialdehyde, products of lipid peroxidation, Na⁺-K⁺/Mg⁺⁺ Adenosine 5' triphosphatase (ATPase) and Ca⁺⁺/Mg⁺⁺ Adenosine 5' triphosphatase, erythrocyte membrane enzymes, in patients with sickle cell anemia and compared their levels with that of normal controls. MDA, Na⁺-K⁺/Mg⁺⁺ and Ca⁺⁺/Mg⁺⁺ ATPase levels of control groups were 0.90 ± 0.04 nmol/mL, 168 ± 12.9 and 140.6 ± 8.2 nmol Pi/mg prot/hour, respectively. MDA, Na⁺-K⁺/Mg⁺⁺ and Ca⁺⁺/Mg⁺⁺ ATPase levels of patients were 2.02 ± 0.01 nmol/mL, 127.0 ± 8.4 and 110.0 ± 9.6 nmol Pi/mg prot/hour, respectively. Our experimental results showed that there was a significant increase in MDA levels of patients with sickle cell anemia as compared with that of the controls. However, erythrocyte membrane Na⁺-K⁺/Mg⁺⁺ and Ca⁺⁺/Mg⁺⁺ ATPase levels of patients with sickle cell anemia were significantly lower than the, Na⁺-K⁺/Mg⁺⁺ and Ca⁺⁺/Mg⁺⁺ ATPase levels of normal controls.

Key Words: MDA, ATPase, Sickle cell.

Turk J Haematol 2000;17(1):23-26.

INTRODUCTION

Although deoxygenating induced sickling is most easily demonstrated property of sickling erythrocytes, numerous membrane abnormalities have also been described. These include: the "frozen" spectrin/actin shell of irreversibly sickling RBC^[1]; an abnormal orientation of lipid bilayer phospholipids^[2]; a deficient calcium ATPase^[3]; and a propensity for hemoglobin S RBC to adhere to vascular endothelium^[4]. Although it seems likely that some of these membrane abnormalities contribute to the overall pathophysiology of sickle cell disease, their etiology remains obscure. Hb S RBC membranes are deficient vitamin E^[5], which might be the result of, or a predisposing factor towards, abnormal peroxidation of membrane lipid. Compared with normal erythrocytes, sickle erythrocytes spontaneously generate approximately twice as much superoxide, peroxide and hydroxyl radical. In addition, Hb S RBC contain increased amount of malondialdehyde, a by-product of lipid peroxidation and evidence of abnormal amino group cross-linking by malondialdehyde has been demonstrated in lipid extract of HbS RBC membranes^[6]. The purpose of this study was to determine the sera levels of malondialdehyde (MDA), erythrocyte membrane $\text{Na}^+\text{-K}^+\text{/Mg}^{++}$ Adenosine 5' triphosphatase ($\text{Na}^+\text{-K}^+\text{/Mg}^{++}$ ATPase) and $\text{Ca}^{++}\text{/Mg}^{++}$ Adenosine 5' triphosphatase ($\text{Ca}^{++}\text{/Mg}^{++}$ ATPase) to show membrane damage.

MATERIALS and METHODS

Subject: 20 patients with sickle-cell anemia and healthy control subject, both aged between 16-35 years, were included in the study.

Determination of serum MDA: The levels of MDA were determined according to the methods of Ohkawa et al^[7].

Determination of $\text{Na}^+\text{-K}^+\text{/Mg}^{++}$ and $\text{Ca}^{++}\text{/Mg}^{++}$ ATPase:

Preparation of erythrocyte membrane: The erythrocyte membrane prepared by the procedure of Beutler^[8].

ATPase measurement: Measurement of ATPase specific activity is based on the principal of

inorganic phosphate released in one hour each milligram protein at the presence of 3 mM ATP, added to the incubation medium^[9]. The inorganic phosphate released from the ATP to the incubation medium was measured according to the method suggested Ames BN^[10].

Protein measurement: The protein quantity contained in the sample was determined according to the method developed by Lowry and his colleagues^[11].

The results of ATPase enzymes systems were stated in nmol Pi/mg prot/hour.

Statistical analysis: The results were expressed in terms of arithmetic means (X) \pm standard deviation (SD). The statistical significance of the difference between the means was evaluated by "student-t-test"^[12].

RESULTS

MDA, $\text{Na}^+\text{-K}^+\text{/Mg}^{++}$ and $\text{Ca}^{++}\text{/Mg}^{++}$ ATPase values of both controls and patients with sickle cell were given Table 1. MDA, $\text{Na}^+\text{-K}^+\text{/Mg}^{++}$ and $\text{Ca}^{++}\text{/Mg}^{++}$ ATPase levels of controls were 0.9 ± 0.04 nmol/mL, 168.5 ± 12.9 , 140.6 ± 8.2 nmol Pi/mg prot/hour, respectively. MDA, $\text{Na}^+\text{-K}^+\text{/Mg}^{++}$ and $\text{Ca}^{++}\text{/Mg}^{++}$ ATPase levels of sickle cell were 2.02 ± 0.01 nmol/mL, 127.0 ± 8.4 , 110.0 ± 9.6 nmol Pi/mg prot/hour, respectively.

DISCUSSION

Increasing evidence suggests that in vivo lipid peroxidation may be an important factor in sickle cell anemia^[13]. Sickle erythrocytes and their membranes are susceptible to endogenous free-radical-mediated oxidative damage that correlates with the proportion of irreversibly sickled cell^[14]. Moore et al. examined normal and sickle cell erythrocyte membranes for significant differences in their ATPase activities, thiobarbituric acid reactive products formed (measured relative to malondialdehyde), membrane protein polymerization, and number of protein-free sulfhydryl groups when treated with 0.5 mmol/L t-buthylhydroperoxide (tBHP) for 30 minutes. Isolated sickle cell membranes treated with tBHP produced significantly greater inhibition in both their basal and calmodulin-stimulated $\text{Ca}^{++}\text{/Mg}^{++}$ ATPase acti-

Table 1. The levels of MDA, Na⁺-K⁺/Mg⁺⁺ and Ca⁺⁺/Mg⁺⁺ ATPase in patients with sickle cell anemia and control group

	n	MDA (nmol/mL)	Na ⁺ -K ⁺ /Mg ⁺⁺ ATPase (nmol Pi/mg prot/hour)	Ca ⁺⁺ /Mg ⁺⁺ ATPase (nmol Pi/mg prot/hour)
Sickle cell anemia	20	2.02 ± 0.01	127.0 ± 8.4	110.0 ± 9.6
Control	20	0.90 ± 0.04	168.0 ± 12.9	140.6 ± 8.2
p		p < 0.001	p < 0.001	p < 0.001

The results were expressed in terms of arithmetic means (X) ± Standard deviation (SD), N: Number of sample, p: Value of significance with difference of each group.

vities (75% inhibition in both cases) compared with that of control membranes in addition, there was significantly more malondialdehyde formed from sickle cell membranes compared with control membranes^[15].

In our study, increased MDA levels in sera were investigated effect on erythrocyte membrane ATPase. We found that there were % increasing the levels of MDA, % inhibition the levels of Na⁺-K⁺/Mg⁺⁺ and % inhibition the levels of Ca⁺⁺/Mg⁺⁺ ATPase in patients with sickle cell anemia compared with that of controls. Our results may indicate that there is a relationship (direct or indirect) between the levels of MDA and decrease of, Na⁺-K⁺/Mg⁺⁺ and Ca⁺⁺/Mg⁺⁺ ATPase levels.

Salil et al observed that a reduction in the levels of cholesterol in sickled erythrocyte membranes^[16]. The loss of cholesterol from the membrane may enhance its susceptibility to free radical attack, since normally the unsaturated lipids are protected from free radical attack by the presence of cholesterol in the biomembranes^[17].

These data suggest that an excessive accumulation of oxidant damage in sickle erythrocyte membranes might contribute to the accelerated membrane senescence of these cells. They further indicate that accumulation of oxidant damage could be a determinant of normal erythrocyte membrane senescence.

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