

# The levels of nitric oxide in beta-thalassemia minor

## Beta talasemi minörde nitrik oksit seviyeleri

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### Abstract

**Objective:** The aim of this study was to investigate the relationship between NO (nitric oxide) and beta-thalassemia minor.

**Material and Methods:** A total of 60 patients with beta-thalassemia minor (30 M, 30 F) were included in the study. The control group consisted of 60 healthy subjects (30 M, 30 F). Plasma nitrite/nitrate levels were measured using the Griess reaction method and analyzed by spectrophotometry at 545 nm.

**Results:** Plasma direct nitrite, total nitrite and nitrate levels were  $7.561 \pm 6.19$ ,  $42.548 \pm 7.37$  and  $34.84 \pm 6.24$  in beta-thalassemia minor patients versus  $36.9 \pm 19.8$ ,  $85.9 \pm 35.3$  and  $48.61 \pm 17.35$   $\mu\text{mol/dl}$  in controls, respectively. Plasma direct nitrite, total nitrite and nitrate levels were significantly lower in beta-thalassemia minor patients compared with the control group ( $p < 0.001$ ).

**Conclusion:** These findings confirm that plasma NO levels in beta-thalassemia minor patients are decreased at the time of diagnosis. This may be helpful in assessing the prognosis and follow-up evaluation of patients with beta-thalassemia minor. (*Turk J Hematol* 2008; 25: 187-9)

**Key words:** Thalassemia, nitric oxide, hemoglobin, hemolysis

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### Özet

**Amaç:** Bu çalışmada beta talasemi minor ve nitrik oksit arasındaki ilişki araştırıldı.

**Yöntem ve Gereçler:** Çalışmaya 30 erkek ve 30 kadın olmak üzere toplam 60 beta talasemi minörlü hasta alındı. Kontrol grubu da tamamen sağlıklı 30 erkek ve 30 kadın toplam 60 denekten oluşturuldu. Plazma nitrit/nitrat seviyeleri Griess reaksiyon metodu kullanılarak ölçüldü ve 545 nanometrede spektrometri ile analiz edildi.

**Bulgular:** Beta talasemi hastalarındaki ortalama plazma direk nitrit seviyesi  $\mu\text{mol/dl}$  olarak  $7.56 \pm 6.19$ , total nitrit  $42.54 \pm 7.37$  ve nitrat  $34.84 \pm 6.24$  bulundu. Buna karşılık kontrol grubunda sırasıyla  $36.96 \pm 19.83$ ,  $85.97 \pm 35.28$  ve  $48.61 \pm 17.35$  bulundu. Beta talasemi minörlü hastalarda plazma direk nitrit, total nitrit ve nitrat seviyesi kontrol grubuna göre istatistiksel olarak anlamlı derecede azaldığı tespit edildi ( $p < 0.001$ ).

**Sonuç:** Bu çalışma tanı anında plazma nitrik oksit seviyelerinin beta talasemi minörlü hastalarda azaldığını göstermektedir. Bu bilgi beta talasemi minörlü hastaların takibinde faydalı olabilir. (*Turk J Hematol* 2008; 25: 187-9)

**Anahtar kelimeler:** Talasemi, nitrik oksit, hemoglobin, hemoliz

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## Introduction

Normal individuals have three different hemoglobin electrophoretic patterns: Hb A, Hb A<sub>2</sub>, and Hb F. Thalassemias are characterized by reduced or absent production of one or more globin chains in the Hb A structure, which is a tetramer consisting of two pairs of globin polypeptide chains: one pair of alpha chains and one pair of beta chains [1].

Beta-thalassemia is the classical form of thalassemias and was first described by Dr. Thomas Cooley in 1925. The name thalassemia comes from its being a disorder most common among people of Mediterranean descent. It is an inherited type of anemia caused by the beta-thalassemia genes received from one's parents. This disorder arises from mutations in the gene on chromosome 11, which encodes the globin polypeptide subunits of hemoglobin, resulting in impaired production of beta globin chains [2]. The clinical severity of thalassemia varies tremendously depending on the amount of beta globin chains produced by the defective genes and whether the patient is homozygous or heterozygous. Beta-thalassemias are broadly classified into three groups, based on clinical severity: major, intermedia and minor. Impaired production of beta globin chains leads to a variable accumulation of alpha globin chains in erythroid precursors, which results in decreased production of Hb A and abnormal erythroblast formation. This leads to hemolysis of red cells in the peripheral circulation. Chronic hemolytic anemia, resulting from ineffective erythropoiesis, is the hallmark of all thalassemia syndromes [3].

Nitric oxide (NO), a diffusible intercellular messenger, is produced by most mammalian cells including vascular endothelium, neurons, smooth muscle cells, macrophages, neutrophils, platelets and epithelium [4]. It is also found in the cytoplasm of follicular cells as well as in the endothelial cells of the thyroid gland [5,6]. Immunohistochemical studies have also demonstrated that nitrosamines are found in villous vascular endothelium, and surrounding vascular smooth muscle cells and villous stroma of the placenta [7]. Endothelial dysfunction related to chronic hemolysis is well documented in hemoglobinopathies and may be a result of decreased NO availability [8].

To the best of our knowledge, the relationship between beta-thalassemia minor and NO has not been identified in the literature.

The objective of this study was to demonstrate plasma NO levels in beta-thalassemia minor patients.

## Materials and Methods

### Patients

Patients with beta-thalassemia minor who applied to the Hematology Polyclinic of İnönü University Medical Faculty between January 2005 and December 2006 were included in the study. Of the 60 beta-thalassemia minor patients, 30 were male and 30 were female, with a median age of 26 years. In all patients with decreased mean corpuscular volume (MCV) values and increased red blood cell (RBC) counts, ferritin levels were measured. Patients with iron deficiency anemia and other causes of microcytosis were excluded from the study. After normal serum ferritin levels were documented, hemoglobin electrophoresis was performed. The patients diagnosed as beta-

thalassemia minor by demonstrating increased levels of Hb A<sub>2</sub> on hemoglobin electrophoresis were included in the study. We excluded those with alpha-thalassemia or other hemoglobinopathies. Informed consent was obtained at the beginning of the study from all participants, both the beta-thalassemia minor patients and the healthy control subjects. The control group consisted of 60 healthy subjects (30 M, 30 F, median age: 24 years), NO levels in the blood samples were measured and statistical significance was evaluated.

### Hemoglobin Electrophoresis and Assessment of Proteinuria

Electrophoresis was performed with Hydrogel Hemoglobin agarose gel kits (Hayras Sebia, USA).

### Assay for Nitric Oxide

Plasma nitrite/nitrate levels were measured with the Griess reaction using a spectrophotometer at 545 nm.

### Statistical Analysis

Comparisons between groups of data were performed by Mann-Whitney U test. A value of  $p < 0.005$  was considered significant.

## Results

Plasma direct and total nitrite levels were  $7.561 \pm 6.19$  and  $42.548 \pm 7.37$  in beta-thalassemia minor patients versus  $36.9 \pm 19.8$  and  $85.9 \pm 35.3$  in controls, respectively, and the difference between the two groups was statistically significant ( $p < 0.001$ ). Plasma nitrate level was  $34.84 \pm 6.24$  in beta-thalassemia minor patients versus  $48.61 \pm 17.35$  in controls, respectively, and the difference between the two groups was statistically significant ( $p < 0.001$ ) (Table 1).

## Discussion

Thalassemia describes a group of inherited blood disorders caused by genes received from one's parents [3]. Thalassemia, also called Mediterranean anemia, is among the most common genetic disorders worldwide and is relatively frequent in people of Mediterranean descent. It has a broad clinical spectrum, ranging from the transfusion-dependent state of thalassemia major to the asymptomatic state of thalassemia trait [2].

Hemoglobinopathies characterized by chronic hemolysis are currently considered sources of strong oxidative stress. Reports have shown that the free heme and the red cell membrane elements that are produced during hemolysis have a negative effect

**Table 1. Laboratory findings of thalassemia patients and controls**

	Thalassemia patients n=60	Control group n=60	p value
Hb A (%)	89.787±4.959	96.522±7.675	$p < 0.001$
Hb F (%)	5.593±2.021		
Hb A <sub>2</sub> (%)	3.735±0.974	2.123±0.216	$p < 0.001$
Carbonic anhydrase (%)	1.156±0.354	2.085 ± 0.421	$p < 0.001$
Direct nitrite (µmol/dl)	7.561 ± 6.198	36.963 ± 19.833	$p < 0.001$
Total nitrite (µmol/dl)	42.548 ± 7.379	85.975 ± 35.286	$p < 0.001$
Nitrate (µmol/dl)	34.845 ± 6.249	48.613 ± 17.359	$p < 0.001$

on NO and arginine availability, which in turn promotes vasoconstriction. They also lead to further endothelial dysfunction, resulting in a more pronounced NO reduction [9]. In hemoglobinopathies, NO binds very rapidly to deoxyhemoglobin, forming a stable Hb (Fe<sup>2+</sup>)-NO complex. NO also reacts with and converts oxygenated hemoglobin to methemoglobin and nitrate (NO<sub>3</sub><sup>-</sup>). Rates of intravascular NO scavenging are reduced significantly when hemoglobin is sequestered within red cell membranes [10].

Pathological processes in thalassemia accelerate the destruction of NO, and limit the compensatory increase in NO production. Hemoglobin decompartmentalized from the red cell into blood plasma by intravascular hemolysis reaches steady-state levels of 5-10 µM, at times exceeding 50 µM. This hemoglobin reacts with NO in a rapid, nearly diffusion-limited reaction to produce methemoglobin and inert nitrate. In addition, NO is also consumed by reaction with reactive oxygen species produced as a by-product of the highly expressed enzymatic activities of xanthine oxidase and NADPH oxidase. Lastly, more recent studies suggest that hemolysis leads to uncoupling of endothelial NO synthase activity, likely secondary to heme-mediated oxidative damage to the enzyme, also producing reactive oxygen species. These mechanisms may combine additively to markedly accelerate NO destruction [11]. Arginase activity is elevated in red blood cells of thalassemia patients and is likely related to reticulocytosis, since immature cells and reticulocytes are known to contain a high concentration of arginase. It is therefore likely that erythrocyte release of arginase during hemolysis will limit the availability of arginine to nitric oxide synthase, resulting in a deficiency of NO and dysregulation of arginine metabolism in thalassemia patients through a similar mechanism identified in sickle cell disease. Multiple mechanisms directly and indirectly attributable to hemolysis reduce NO bioavailability in thalassemia. The resulting impairment in NO bioavailability is associated with vasoconstriction, endothelial dysfunction and thrombosis. Autopsy findings in thalassemia, particularly pulmonary thrombi, have been observed in patients who had previously undergone splenectomy [12].

Many factors associated with vascular dysfunction or hemolysis have been shown to affect NO levels. Therefore, patients with the diagnosis of thalassemia minor and no other risk factor were included in the current study and NO levels were measured as soon as blood samples were obtained.

Chronic hemolysis in patients with underlying hemoglobinopathies may be a result of decreased NO availability. However, the relationship between beta-thalassemia minor and NO levels has not yet been proven in the literature. We found that plasma NO levels in beta-thalassemia minor patients were significantly lower than those in controls. The laboratory findings are shown in Table 1. This may be explained by hemolysis-asso-

ciated endothelial dysfunction. In contrast, thalassemia intermedia and thalassemia major are probably associated with more severe degrees of hemolysis. It would have been preferable if the NO levels of the patient group were also compared with thalassemia major patients or Hb S patients as a positive control. Our study population consisted of patients diagnosed with beta-thalassemia minor. This issue may only be fully clarified by further molecular studies.

In conclusion, low NO levels are thought to be related to the chronic hemolysis. Supportive treatment of hemolytic anemia hemolysis may increase the NO bioavailability, which may be helpful in assessing the prognosis and follow-up of the patients. To draw such a conclusion, further prospective studies are needed in a large number of patients.

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