PURINE CATABOLIC ENZYMES AND NITRIC OXIDE IN PATIENTS WITH IRON DEFICIENCY ANEMIA

MUSTAFA ORAN[1], MURAT AYDIN[2], RAFET METE[3], FETI TÜLÜBAŞ[2], OKAN AVCI[1], AHSEN YILMAZ[2], AHMET GÜREL[2]
[1]Namık Kemal University Faculty of Medicine, Department of Internal Medicine, Tekirdag - [2]Namık Kemal University Faculty of Medicine, Department of Biochemistry, Tekirdag - [3]Namık Kemal University Faculty of Medicine, Department of Gastroenterology, Tekirdag, Turkey

ABSTRACT

***Introduction**: To clarify the mechanism of oxidative damage,which affects many tissues and organ systems in iron deficiency anemia, comes into prominence in the treatment and avoiding the complications of the disease. In the present study, serum uric acid, ADA, XO, and nitric oxide (NO) levels were measured in patients with iron deficiency anemia and healthy controls in order to ascertain the function of the above parameters in oxidative stress metabolism.*

***Materials and method**: A total of 36 patients (27 female and 9 male with a mean age of 31.1 ± 6.9 years) with iron deficiency anemia and 36 healty controls (29 female and 7 male with a mean age of 29.8 ± 7.7 years) were included in the study. The patients blood parameters, serum iron level, total iron binding capacity and ferritin levels were determined by using commercial kits.Serum adenosine deaminase, xanthine oxidase and nitric oxide measurements were performed spectrophotometrically with manual methods.*

***Results**: The levels of xanthine oxidase and uric acid in patients with iron deficiency anemia were found to be significantly lower than healthy controls (p=0.01 and 0.003, respectively), while nitric oxide levels were found to be significantly higher (p = 0.003). Adenosine deaminase levels in patients with iron deficiency anemia were significantly lower than in healthy controls, but the difference was not statistically significant.*

***Discussion**: It is known that, oxidative stress increases in iron deficiency anemia due to both increased oxidant molecules and the inadequate capacity of the antioxidant enzyme systems. Decreased levels of xanthine oxidase and uric acid and increased nitric oxide in patients with iron deficiency anemia compared to healthy population is considered to be a result of increased oxidative stress.*

***Key words**: Iron deficiency anemia, Adenosine deaminase, Xanthine oxidase, Uric acid, Nitric oxide.*

***Received** October 12 2013; **Accepted** November 29, 2013*

Introduction

Iron is one of the most important minerals in the human body and is essential for many of the body's metabolic functions. It plays a vital role in the metabolism, particularly in the following three stages: hematopoiesis, oxidative stress, and cellular immunity[1, 2]. The need for iron is higher in young women than in other age groups due to higher blood loss. Iron deficiency anemia (IDA) is a significant public health problem which is often encountered in developing countries[3].

Free radicals and reactive oxygen species occur within a cell as a result of energy metabolism. The disruption of the balance between these free radicals, reactive oxygen species and the antioxidant system can lead to oxidative stress and result in cellular damage[4]. The free radicals that can not be neutralized cause irreversible damage in all of the organelles, especially in the cell membrane, and this leads to the oxidation of protein, carbohydrate, and DNA, which are the basic building blocks, and also to lipid peroxidation. Severe cell and tissue damage can occur, ranging from simple dysfunction to apoptosis[5, 6].

Nitric oxide (NO) is a molecule that is synthesized by nitric oxide synthase (NOS) in the endothelium. Its functions range from vasodilatation to the release of inflammatory cytokines. Additionally, nitric oxide plays a role in non-specif-

ic immunity, the regulation of cell function and communication, and cardiovascular homeostasis[7, 8].

Xanthine oxidase (XO) and adenosine deaminase (ADA) are enzymes that participate in purine catabolism. Uric acid is the end product of purine degradation and its synthesis is catalyzed by the enzyme XO. Uric acid is a potent antioxidant and shows its effects through the inactivation of superoxide radicals and the prevention of vitamin C oxidation and iron chelation[9, 10].

It is important to clarify the mechanisms of oxidative damage and how it can affect tissues and organ systems in the case of iron deficiency anemia (IDA). This investigation will help in treating the disease and avoiding its complications. In the present study, serum uric acid, ADA, XO, and nitric oxide (NO) levels were measured in patients with iron deficiency anemia and healthy controls in order to ascertain the function of the above parameters in oxidative stress metabolism.

Materials and method

Study Group

A total of 36 patients (27 female and 9 male with a mean age of 31.1 ± 6.9 years) with IDA who were admitted to the Internal Medicine Clinic of Namik Kemal University Educational and Research Center and 36 healthy controls (29 female and 7 male with a mean age of 29.8 ± 7.7 years) were included in the study. The IDA diagnosis was based on World Health Organization (WHO) diagnostic criteria[11].

Routine laboratory test results and the medical history of the participants were reviewed. Patients with the following conditions were not included in the study: hematological disease causing anemia of a different type to IDA, endocrine or metabolic disease, acute or chronic infection, any inflammatory or autoimmune disease, hypertension and atherosclerotic cardiovascular disease, renal disease, and pregnancy. The control group was selected from healthy volunteers of a similar age and sex without acute or chronic disease.

Informed consent was obtained from all participants and the study was conducted with the permission of the Ethics Committee of the Faculty of Medicine at Namik Kemal University.

Biochemical Measurements

The blood parameters of the patients were measured by analyzing blood samples using a Pentra Nexus Dx 120 (England) device. The blood was taken in K2 tubes containing EDTA and mixed for 15 minutes. For biochemical measurements, venous blood, taken in the morning after 8 to 10 hours of fasting, was used. Uric acid, iron and total iron binding capacity (TIBC) tests were performed with a Cobas C 501 Roche (Japan) biochemistry analyzer using commercial kits. Ferritin levels were measured with a Cobas E 601 Roche (Japan) hormone analyzer.

Measurement of Serum ADA Activity

Serum adenosine deaminase activity was estimated spectrophotometrically by the Giuisti method, which is based on the indirect measurements of ammonia formation produced when ADA acts in excess of adenosine. (12) The results were expressed as units per liter (U/L) and were calculated as mean ± standard deviation. Assays were conducted with no access to clinical information.

Measurement of Serum XO Activity

Plasma XO (EC 1.2.3.2) activity was measured spectrophotometrically by the formation of uric acid from xanthine through an increase in absorbance at 293 nm. (13) One unit of activity was defined as 1 mmol of uric acid formed per minute at 37 ºC, pH 7.5, and the results were expressed in units per liter of plasma (U/L).

NO Determination

As NO is very difficult to measure in biological specimens, sample nitrite and nitrate levels are used as an index of NO production. This method is based on the Griess reaction[14] Samples were initially deproteinized with Somogyi reagent. The total nitrite (nitrite + nitrate) was measured following the conversion of nitrate to nitrite with copperized cadmium granules using a spectrophotometer at 545 nm. A standard curve was established with a set of serial dilutions of sodium nitrite and linear regression was performed by using the peak area from the nitrite standard. The resulting equation was then used to calculate the unknown sample concentrations. The results were expressed as μmol/L.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) Version 17.0 (Chicago, IL) was used for all analyses. Quantitative variables were expressed as mean ± standard deviation. The student's t-test was used for comparisons between groups and a P value

of <0.05 was considered statistically significant.

Results

Hemoglobin, iron, and ferritin levels, in addition to mean corpuscular volume (MCV) were lower, while the total iron-binding capacity (TIBC) was higher in patients with IDA compared to healthy volunteers. The levels of XO and uric acid were significantly lower statistically in patients with IDA than in the healthy controls (p = 0.01 and 0.003, respectively). NO levels were significantly higher in the patient group (p = 0.003). ADA levels were significantly lower in patients with IDA than in the healthy controls, but the difference was not statistically significant (p = 0.133). All biochemical measurement results are presented in Table 1.

	Control			Anemia			p
ADA (U/L)	55,53	±	14,10	50,52	±	13,47	0,133
XO (U/L)	0,28	±	0,13	0,20	±	0,11	0,010
NO (μmol/L)	81,66	±	17,63	101,61	±	31,18	0,003
UA (mg/dl)	4,43	±	0,84	3,76	±	1,02	0,003
Iron (μg/dl)	74,46	±	20,51	30,80	±	13,76	0,000
TIBC (mg/dl)	249,57	±	79,20	397,74	±	51,76	0,000
Ferritin (ng/ml)	48,88	±	24,85	7,74	±	5,06	0,000
HGB (g/dl)	13,08	±	1,10	11,14	±	1,14	0,000
MCV (fl)	89,80	±	4,49	77,75	±	5,91	0,000

Table 1: Biochemical measurement results in Anemia and control group.
ADA: Adenosine deaminase, XO: Xanthine oxidase, NO: Nitric oxide, UA: Uric acid, TIBC:Total iron binding capacity HGB:Hemoglobin, MCV: mean corpuscular volume.

Discussion

Red blood cells are equipped with an extremely effective antioxidant defense system consisting of highly active antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT)[15, 16]. In the literature, there are differing conclusions regarding how these enzyme activities, which protect cells from oxidative damage, are affected by iron deficiency anemia. In a study conducted by Yoo et al.[17], oxidative capacity was found to be significantly higher, while total antioxidant capacity and CAT activity were found to be lower in patients with IDA. Similarly, in a study conducted by Kurtoğlu et al.[18], SOD, GSH-Px, and CAT activity were found to be lower in patients with IDA. Conversely, in a study conducted by Baccinet et al., SOD and CAT activity were found to be higher in patients with DEA[19].

It has been shown that hypochromic erytrocytes are more easily damaged than normal erytrocytes when exposed to hydrogen peroxide[20]. This result may indicate that there is vulnerability in the antioxidant defense system of hypochromic erythrocytes. Nevertheless, the accumulation of excess iron causes oxidative damage by creating hydroxyl radicals[4]. It has been shown that ferritin may protect against oxidative stress through free iron chelation[6].

Additionally, the decrease in the production of iron-containing proteins such as cytochrome, myoglobin, catalase, and peroxidase may contribute to increased oxidative stress in patients with IDA[19].

Although there is no study that evaluates XO in IDA, some studies evaluated XO in various patient groups and found increased oxidative stress. Contrary to the findings of those studies, the results of the present study have shown that XO activity is lower in patients with IDA, and this is linked to increased oxidative stress[20, 21]. Uric acid levels were lower in the patient group, which is consistent with decreased XO levels.

Further studies are needed to evaluate the role played by decreased iron in the case of a decrease of XO in IDA patients.

Uric acid is the second most potent antioxidant in plasma after albumin, and shows its effect by inactivating superoxide radicals and preventing iron chelation and the oxidation of vitamin C[9]. It has been reported that uric acid, at normal levels, cleans up toxic reactants and protects against oxidative stress[23]. Although the pathophysiological mechanism is not clear, high uric acid levels are known to be significantly associated with inflammation, endothelial dysfunction, antiproliferative effects, high intracellular oxidative stress, and subclinical atherosclerosis[24, 25]. Our search of the literature revealed no study conducted on IDA and uric acid. In our study, serum uric acid levels in patients with anemia were lower than in healthy controls. The reason for the decrease in uric acid levels may be twofold. Firstly, there may be excessive consumption of uric acid during the neutralization of oxygen free radicals and secondly, the synthesis of uric acid may be reduced. In fact, the activity of XO, which

is the synthesis enzyme of uric acid, was found to be lower in the IDA group. Studies reporting that there is a positive correlation between ferritin and the level of uric acid,[26] and also between iron overload and uric acid[27] seem to support our thesis.

It has been reported that in the case of IDA, the activity of ADA in the thymus and spleen tissue decreased, and there was a correlation between the levels of iron and ADA. (28) Accordingly, the literature states that the T-cell subgroups in patients with iron deficiency anemia are affected[29], and that infectious diseases are more prevalent in these patients than in the normal population[30, 31]. In our study, although the level of ADA was lower in the IDA group than in the control group, no statistical significance was found. This result is partly consistent with the literature.

In our study, serum NO levels were found to be higher in the IDA group than in the control group. Clinical and experimental studies support our conclusion. Choi et al. have reported that serum NO levels in 369 adult patients with anemia whose Hb value was under 8 g / dl, increased 7.5-fold compared to the control group[32]. The reason for this is that anemia increases NO activity by leading to tissue hypoxia, and this thesis has been proven by different researchers using experimental models. (33-36) In another study, the nitrite and nitrate levels in children with iron deficiency were reported to be higher than in healthy volunteers[37]. Since NO is inactivated by uric acid[38]. and there is a negative correlation between NO and uric acid[39], increased NO levels might be a consequence of decreased uric acid levels.

Although the present study was conducted in a small patient group, its findings concluded that decreased XO and uric acid levels may contribute to increased oxidative stress in IDA patients. Studies evaluating the ADA,XO activity,NO and uric levels before and after iron treatment would be useful to further elucidate the underlying mechanism of increased oxidative stress in IDA patients.

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MURAT AYDIN
Namik Kemal University Department of Biochemistry
Namık Kemal MahallesiKampusCaddesi No:1
59100-Merkez-Tekirdag
(Turkey)