Erythrocyte Arginase Levels in Down Syndrome and in Healthy Children¶

DOWN SENDROMLU VE SAĞLIKLI ÇOCUKLARDA ERİTROSİT ARGİNAZ DÜZEYLERİ

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Abstract

Objective: Several different tissues in which the urea cycle does not take place harbor arginase activity. It has been reported that both liver and erythrocyte arginase levels were low in cases with mental retardation, cerebral palsy and hyperargininemia. The purpose of this study was to determine the erythrocyte arginase levels in cases with Down Syndrome (DS) and in healty children.

Material and Methods: Enzyme levels were measured in 20 children with DS (6.32 ± 1.40 years) and in 20 age-matched healthy children (5.8 ± 1.06 years). Erythrocyte arginase activity was measured by the modified Shimke method.

Results: No significant differences were detected between erythrocyte enzyme levels of the children with DS (22.86+0.67 U/g.Hb/min) and those of healthy children (21.89+1.03 U/g. Hb/min).

Conclusion: It was concluded that the level of erythrocyte arginase enzyme could not be considered as a useful marker for assessing the level of mental retardation children in DS.

Key Words: Down syndrome, mental retardation, erythrocyte arginase

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Özet

Amaç: Üre döngüsünün yer almadığı birçok doku arginaz aktivitesine sahiptir. Karaciğer ve eritrosit arginaz aktivitesinin, mental retardasyon, serebral palsi ve hiperargininemili olgularda düşük düzeyde olduğu bildirilmiştir. Bu çalışmanın amacı, Down Sendrom (DS)'lu ve sağlıklı çocuklarda eritrosit arginaz seviyelerini belirlemek ve DS'li gibi sık rastlanılan mental retardasyonlu olgularda bu enzimin durumunu ortaya koymaktır.

Gereç ve Yöntemler: Enzim seviyeleri 20 DS'lu (6.32 ± 1.40 yaş) ve 20 aynı yaşta sağlıklı çocukta (5.8 ± 1.06 yaş) ölçüldü. Eritrosit arginaz aktivite ölçümünde modifiye Shimke metodundan yararlanıldı.

Bulgular: DS'lu (22.86 ± 0.67 U/g. Hb / dakika) ve sağlıklı çocukların (21.89 ± 1.03 U/g. Hb / dakika) eritrosit arginaz düzeyleri arasında önemli bir farklılık saptanamadı.

Sonuç: Eritrosit arginazının, DS'li çocukların mental retardasyon seviyesinin değerlendirilmesinde yararlı bir belirteç olarak kullanılamayacağı sonucuna varıldı.

Anahtar Kelimeler: Down sendromu, mental retardasyon, eritrosit arginazı

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Arginase (L-arginine ureahydrolase; EC 3.5.3.1) is a cytoplasmic enzyme that hydrolyses L-arginine to urea and ornithine by catalysing the last step of the urea cycle.[1] The human arginase gene is located on chromosome 6q23.[2] It is mainly produced by the liver, but it can also be detected in other cells and tissues such as erythrocytes, leucocytes, platelets, skeletal and heart muscle, brain, intestines, kidney, pancreas, lung, breast, thyroid, salivary glands, skin, testes, plasma and fibroblasts.[3-5]

Besides liver arginase, the level of erythrocyte arginase has been reported to be less in general in patients with mental retardation when compared to those of healthy subjects.[6] On the other hand, mental retardation is the hallmark of the Down syndrome (DS) (trisomy 21). To our knowledge, however, no study has been reported with regard to a possible relationship between erythrocyte arginase status and DS with mental retardation.

The aim of this study was to find out whether erythrocyte arginase levels in children with DS differ from those of healthy control subjects, and

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whether any significant differences could be used to assess the level of mental retardation in children with DS.

Material and Methods

This study comprised a total of 20 children with DS (11 male and 9 female children; mean age: 6.32±1.40) admitted to Ondokuzmayıs University Mentally-Disabled Children Research and Education Centre, Samsun, Turkey. A total of age-matched and otherwise healthy children admitted to the Department of Pediatrics for routine check-ups served as controls (13 female and 7 male children; mean age: 5.80±1.06). Exclusion criteria for both study and control groups included conditions such as an acute infection in the last month, a history of chronic or metabolic disease, or a history of drug usage. Consent for blood sampling and further laboratory studies was obtained from the parents. Blood samples were centrifuged at 2500 rpm for 10 minutes, and hemolysate obtained from the erythrocytes was stored at –70ºC until the determination of arginase activity.[7] DS was confirmed by chromosomal analysis at the Medical Genetics Division. IQ assessments of the children with DS were performed by two methods, namely the Wechsler Intelligence Scales for Children (WISC) for those older than six years old and the Peabody for those younger than six. IQ levels in children with DS ranged between 40-60.

All chemicals used in assay were purchased from Sigma and Merck and were analytical grade. The determination of erythrocyte arginase activity was performed by modified Schimke method using erythrocyte lysate.[8] Ornitine originating from the hydrolysis of arginine was determined by end-point ninhydrine reaction.[9] Briefly, 250 uL of erythrocyte lysate diluted 1:4 was placed into a clean tube containing 250 uL of glycine-KOH and 250 uL of $MnCl_2$. The reaction mixure was vortexed and placed into a shaking water bath at 51^0C for 10 minutes. At the end of the incubation, 250 uL of arginine-KOH was added into all tubes, followed by vortexing and incubation at 37^0C in a water bath for 5 minutes. Enzymatic reaction was stopped by adding 500 uL of % trichloroacetic acid. The tubes were immersed in ice-cold water for 15 minutes, and then centrifuged at 2500 rpm for 20 minutes at 4^0C. The supernatant was saved for ornithin assay. For this, 25 uL of the supernatant was added into a clean tube containing 525 uL of ddw and 1500 uL glacial acetic acid. The mixure was vortexed well, and boiled for 60 minutes. It was then cooled down to room temperature. Optical density was measured at 515 nm using a spectrophotometer (UV-160A, SHIMADZU). A standard curve was also obtained by using a serial dilution of ornithin prepared at 0.125-4 mmol concentration. Enzyme unit were calculated by using the standard curve. Specific arginase activity in unit was expressed as µmol ornitine that forms at 37ºC per g of Hb in every one minute (U/g.Hb/min).

Hemoglobine (Hb) determinations were performed with the use of Cell Dyne 1700 (Abbott corp).

Statistical analysis was carried out by using SPSS 9.0. The erythrocyte arginase levels were evaluated statistically by Mann-Whitney U test. Results were given as mean ± SD, and $p<0.05$ was accepted as significant.

This study was conducted in accordance with the requirements of the Helsinki Declaration of 1975 as revised in 1996.

Results

Erythrocyte arginase levels are summarized in Table 1. No statistically significant difference was obtained between the two groups ($p>0.05$).

Discussion

Several investigators have reported apparent changes in the activities of erythrocyte enzymes in children with DS. Almost four decades ago, it was

Table 1. Erythrocyte arginase levels in children with DS and healthy control groups (U/g.Hb/min)

	No of cases	mean	standard error	p value
DS	20	22.86	0.67	>0.05
Control	20	21.89	1.03	>0.05

first reported by Baikie et al. that the level of erythrocyte phosphohexokinase activity resulting from gene dosage effect in patients with DS was approximately 50% higher than in healthy controls.[10] Sinet *et. al* reported in two successive studies that the levels of superoxide dismutase-1 and glutathione peroxidase activities from the erythrocytes of patients with DS were higher than those of healthy controls.[11] Further studies have reported between 15%-60% increases in several erythrocyte enzymes such as acetylcholine esterase, glutamic oxaloacetic transaminase, glucose-6- phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, adenosine deaminase, and catechol-O-methyl transferase activities and 60%-70% decreases in erythrocyte membrane sodium-potasium adenosine triphosphatase activity.[12,13] Furthermore, Watterberg *et. al* also reported a decreased dopamine β-hydroxylase activity in erythrocytes in patients with DS.[14]

In some reports, both arginase activities derived from the urea cycle as well as from erythrocytes in children with mental retardation have been found to be less than that of healthy children.[12] Furthermore, it has been reported that the erythrocyte arginase levels in two boys, aged 9 and 5 4/12, with cerebral palsy were decreased as compared to healthy controls.[15] In children with hyperargininemia, an inherited disorder of urea cycle in which mental retardation is one of the facets of the disease, erythrocyte arginase activity is either totally absent or less compared to healthy children.[16-18] In a case report by Endres et al,[16] among 16 healthy children with a mean erythrocyte arginase of 62.2±28.4 μmol/g.Hb/min, an 8 months-old boy had no erythrocyte arginase activity at all. Similarly, Prasad et al[17] also found no erythrocyte arginase activity in two male siblings with hyperargininemia.[19] On the other hand, Konarska et al[19] reported that erythrocyte arginase levels in chidren with hyperargininemia were less than one-tenth of the levels of healthy controls.

Although the reports summarized above indicate decreased erythrocyte arginase activities in patients with mental retardation, none of them included those children with mental retardation in DS. In our study, we found no statistically significant differences between children with mental retardation in DS and healthy controls with regard to erythrocyte arginase levels.

We conclude that erythrocyte arginase activity with its many known and speculative biochemical functions was not useful at least clinically as a predictor in the diagnosis of mental retardation originating from DS.

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