Beta Talasemi Taşıyıcılığına Eşlik Eden Demir Eksikliği Anemisi

Beta Thalassemia Trait Accompanying Iron Deficiency Anemia

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ÖZET

Amaç: Anemi sıklıkla karşılaşılan bir halk sağlığı problemidir. Bu çalışmada beta talasemi taşıyıcılığında demir eksikliği anemisinin birlikteliği bir takım biyokimyasal ve hematolojik indeksler kullanılarak araştırıldı.

Gereç ve Yöntem: Laboratuvarımıza gönderilen numuneler içinden (n = 895) hematolojik indeksler ve HbA2 değerlerine göre beta talassemi taşıyıcısı olan 128 tam kan numunesi seçildi. Hemoglobin zincir analizi yüksek basınçlı sıvı kromatografisi metodu ile çalışıldı. Tam kan sayımı, serum demir, total demir bağlama kapasitesi, serum ferritin düzeyi, Mentzer İndeksi ve transferrin saturasyon indeksi geriye dönük olarak değerlendirildi.

Bulgular: Değerlendirmeye alınan tüm taşıyıcıların; % 63'ü kadın, % 23'ü erkek ve % 14'ü 16 yaş altı çocuktu. Ferritin düzeyi düşüklüğü erkeklerde ve çocuk yaş grubunda sadece birer taşıyıcıda görülmesine rağmen kadın taşıyıcılarda bu oran % 28'di. Kadın taşıyıcıların % 20'sinin, erkek taşıyıcılarının % 3'ünün ve çocuk yaş grubu taşıyıcıların % 6'sının Mentzer İndeksi 13'ün üzerinde bulundu. Kadın taşıyıcıların % 14'ünde, erkek taşıyıcıların % 10'unda, çocuk yaş grubu taşıyıcıların % 11'inde transferrin saturasyon indeksi % 10'un altında saptandı.

Sonuç: Özellikle erişkin kadın ve çocuk beta talasemi taşıyıcılarının tedavisi sırasında demir eksikliğinin de birlikte olabileceği göz önünde bulundurulmalıdır.

Anahtar kelimeler: Talasemi; demir eksikliği anemisi; ferritin

ABSTRACT

Objective: Anemia is a commonly encountered health problem. In this study, togetherness of beta thalassemia trait with iron deficiency anemia was investigated using by some biochemical and hematological cell count indices.

Material and Methods: The 128 specimens of whole blood samples (n = 895) that were sent to our laboratory were selected as beta thalassemia carriers by hematological indices and HbA2 values. The

hemoglobin chain analysis was performed by high performance liquid chromatography method. Complete blood count, serum iron, total iron binding capacity, serum ferritin level, Mentzer Index and transferrin saturation index were retrospectively evaluated.

Results: In all carriers included in the review, 63 % were females, 23 % were men and 14 % were children under the age of 16. Although low ferritin levels were observed in only one carrier of each men and children group, in female carriers this was found as 28 %. Mentzer Index was found higher than 13 in 20 % of female, 3 % of men and 6 % of children carriers. Transferrin saturation index was found lower than 10 % in 14 % of female, 10 % of men and 11 % of children carriers groups.

Conclusion: Especially in female and child beta thalassemia carriers, it should be taken into account the possible togetherness with the iron deficiency during the treatment period,.

Keywords: Thalassemia; iron deficiency anemia; ferritin

INTRODUCTION

Anemia was a public health problem in the world and in our country that has been shown to be the most important 8th disease especially for girls and women (1, 2). Iron deficiency anemia (IDA) and thalassemia emerge as the most common causes in hypochromic microcytic anemia subgroup (3). 15-64 % of women and 1.9-14 % of men in Asian and South American countries, especially in regions with low socioeconomic status and high birth rates are affected from this disease (4,5). In addition to these population and regions, hemoglobinopathies are common in the regions of Mediterranean, southwest of Europe and middle of Africa (3). The rate of high HbA2 levels of β-thalassemia carriers was reported as 1.2-10.8 % in Turkey (6).

Hemoglobin (Hb) production disturbances in different polypeptide chains, determinates the types of thalassemia and shows different clinical and biochemical aspects. β-thalassemia minor is the common heterozygous form. The basic defect of β-thalassemia in hemoglobinopathies, as the most common autosomal recessive trait and single gene disorder in the world, is the synthesis defect in the β globin chains of hemoglobin (7). IDA indicates a wide range of symptoms such as impaired tissue oxygenation, low birth weight, fatigue, and low job performance. The complete treatment in β-thalassemia with iron deficiency anemia is very important to prevent the complications (8).

Recently, a number of studies have focused on the subjects about discriminating algorithms for screening on iron deficiency anemia and thalassemia trait such as hemoglobinization of reticulocytes (Ret-He) and Ret-He/RBC-He ratio as additional parameters (9). Urrechaga et al. have also reported the percentage of hypochromic and microcytic red blood cells as the distinguishing new parameters for differential diagnosis (10). The purpose of this study was to investigate the association of β-thalassemia trait and iron deficiency anemia using some biochemical and hematological indices because of the importance of the differential diagnosis.

MATERIAL AND METHODS

In this study, 895 patients with a presumptive diagnosis of hypochromic microcytic anemia results between November 2011 and December 2012 were retrospectively analyzed from the archive of the laboratory electronic records. 193 of total 895 patients who had HbA2 levels over 3.5, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values below 80 fL and 27 pg respectively, were considered as 'β-thalassemia carrier'. 128 beta-thalassemia carriers who were examined for all tests of this study and had no other diseases according to the routine tests were included to the study. Patients were grouped as female (n= 80), men (n= 30) and children under 16 years of age (n= 18). Female patients with β-thalassemia trait were divided into two groups according to ferritin levels;

Group I: carriers with normal ferritin levels without IDA, Group II: carriers with low ferritin levels with IDA. Patients who had altered white blood cell indices were excluded from analysis as the potential bias to ferritin values as an acute phase protein.

EDTA blood samples were used for the complete blood counts and hemoglobin chain analysis, and serum samples were used for iron, total iron binding capacity (TIBC) and ferritin levels. Complete blood counts (22 parameters) were analyzed by automated analyzer Cell-Dyn 3500 (Abbott Diagnostics, USA) with optical laser scatter and calculation methods. Hemoglobin chain analysis was performed by the D10 Dual Program high performance liquid chromatography method (Bio-Rad Laboratories, Inc., Cl, USA). Iron and TIBC levels were studied by colorimetric spectrophotometric method by Abbott Architect C16000 (Abbott Diagnostics, USA) and ferritin levels were studied by electrochemiluminescence method (Advia Centaur XP, Siemens, Germany) in fully automated analyzers. Mentzer Index was calculated using the formula: 'MI = MCV/Red blood cell (RBC)'. Transferrin saturation index was calculated by 'TS (%) = serum iron/TIBC x 100' formula.

Hematological indices (MI and TS) and ferritin values were evaluated to determine the association of iron deficiency and β-thalassemia trait after selecting the beta-thalassemia carriers by complete blood counts and hemoglobin chain analysis. Lower limit reference values of ferritin levels have been accepted for interpreting in favor of iron deficiency anemia as 10 ng/mL for female group, 15 ng/mL for male group and 12 ng/mL for children group for achieving the highest specificity as the cut off values (10).

For statistical analysis, SPSS 15 for Windows XP (SPSS Inc, Chicago, IL, USA) program was used. Normally distributed data were expressed as mean ± standard deviation (SD) and non-normally distributed data were expressed as the median (minimum-maximum). One-Way analysis of variance and Sample T tests were used for comparing parametric tests among groups. Kruskal-Wallis and Mann-Whitney U tests were performed for non-parametric tests. P values <0.05 were considered statistically significant. Error level 0.05/3 = 0.016 was taken for ferritin test while using Bonferroni post-hoc analysis.

RESULTS

In all, demographic information of 128 β-thalassemia carriers was presented in Table 1. Hematological, biochemical data and Mentzer Index were shown in Table 2. HbA2 levels were significantly lower in thalassemia carriers with IDA compared to patients without IDA (n = 24; 4.9 % ± 0.7, n = 104; 5.4 % ± 0.6, respectively, p = 0.001). In female carriers, these ratios were 4.9 % ± 0.7 (n = 22), 5.4 % ± 0.6 (n = 58), respectively (p = 0.006). Iron, ferritin and TS % levels were higher in men than women and children groups during that time, TIBC were found as lower. However low ferritin levels was observed in only one carrier in men and children group, 28 % (22/80) of female carriers had low ferritin levels. Ferritin levels of female thalassemia carriers with IDA and without IDA were presented in Table 3. Mentzer Index were found as >13 for 20 % of female carriers, 3 % carriers of men, and 6 % of children. Ferritin levels of 63 female carriers (MI < cut-off value) with MI: 11.0 ± 1.0 were 24 ng/mL (2-215) and significantly different (p <0.001) than 17 female carriers with MI: 14.7 ± 1.4 who had ferritin levels as 56 ng/mL (2-255). Transferrin saturation were found <10 in 14 % of female carriers, 10 % of men and 11 % of children carriers. Ferritin levels of 67 female carriers with TS: 24 % (10-64) were found 33 ng/mL (4-255), while 13 female carriers (TS < cut-off value) with TS: 5.0 % (2.3-9) were found 6 ng/mL (2-133) as significantly different (p = 0.003). 50 % of female thalassemia carriers with IDA (n = 11) had TS <10 %, yet 9 % of this group (n = 2) were found as MI >13.

Table 1. Demographic information of β-thalassemia carriers.

Variable	β-thalassemia carriers (n=128)n (%)	Age, y [median (min-max)]
Male	30 (23)	26 (18-78)
Female	80 (63)	37 (16-81)
Child	18 (14)	5.5 (1.0-15)

Table 2. Hematological and biochemical parameters and Mentzer Index of β-thalassemia carriers (n = 128).

Variable	**Male** (n = 30)	**Female** (n = 80)	**Child** (n = 18)	***p***
HbA_2 (%)	5.4 ± 0.6	5.3 ± 0.7	5.6 ± 0.7	0.489
Hb (g/dL)	12.3 ± 1.2	10.7 ± 1.2	10.7 ± 0.7	<0.001
RBC ($x10^6/\mu L$)	6.0 ± 0.5	5.2 ± 0.5	5.6 ± 0.4	<0.001
MCV (fL)	62.8 ± 4.3	62.1 ± 4.4	57.0 ± 4.3	0.687
MCH (pg)	20.6 ± 4.3	20.8 ± 5.3	19.0 ± 1.4	0.817
RDW (%)	18.9 ± 1.4	19.0 ± 3.2	19.0 ± 1.8	0.164
Mentzer Index	10.0 ± 1.6 (13 ≠)	11.8 ± 1.8 (13 ≠)	10.4 ± 1.3 (13 ≠)	<0.001
Iron (μg/dL)*	92 (12-215)	64.5 (9-149)	67 (14-133)	0.012
TIBC (μg/dL)*	297 (193-484)	320 (165-535)	303 (244-432)	0.149
Ferritin (ng/mL)*	65 (7-196)	27 (2-255)	38 (10-205)	0.004
TS (%)*	31 (4-72) (10 ≠)	22 (2.3-64) (10 ≠)	22 (3.6-44) (10 ≠)	0.006

Data were expressed as mean ± SD. *, median (min-max); ≠, cut-off value; TIBC, total iron binding capacity; TS, transferrin saturation index.

Table 3. The groups of female thalassemia carriers (according to their ferritin levels)

Variable	**Group I** (without IDA, n = 58)	**Group II** (with IDA, n = 22)	***p***
Ferritin (ng/mL)*	45 (13- 255)	7.0 (2.0-10)	<0.001
Iron (μg/dL)*	82 (21-149)	31 (9.0-149)	<0.001
TIBC (μg/dL)*	313 (165-514)	380 (217-535)	<0.001
TS (%)*	26 (8.0-64)	9.0 (2.3-45)	<0.001
RBC ($x10^6/\mu L$)	5.3 ± 0.6	5.2 ± 0.6	0.837
MCV (fL)	58 ± 4.5	63 ± 3.7	<0.001
MCH (pg)	18 ± 1.9	21 ± 5.9	0.037
RDW (%)	20 ± 3.4	18 ± 3.0	0.010
Mentzer Index*	11 (9.0-19)	11 (8.6-15)	0.032

Data were expressed as mean ± SD. *, median (min-max); TIBC, total iron binding capacity; TS, transferrin saturation index.

DISCUSSION

There are three main clinical forms of thalassemia as homozigot thalassemia major, homozygous/heterozygous compound intermedia and heterozygous thalassemia minor. In worldwide, the rate of thalassemia minor carrier was observed as 5.1 %. The early prevalence studies have been initiated in the 70s (11). Screening of a total of 377,339 healthy people within 5 years the rate of thalassemia minor carrier was determined as 0.7 - 13.1 % (12).

Beta-thalassemia disease associated with iron deficiency emerges as an even bigger problem more common in women, especially in childhood. If thalassemia trait cannot be correctly diagnosed in some cases, patients may expose unnecessary iron overload (13). Sometimes iron deficiency directly affects HbA2 synthesis in bone marrow and the thalassemia trait existence is marred (14). Post-treatment levels after 8 weeks were reported as increased levels according to some studies (15,16). However in childhood iron deficiency does not affect HbA2 synthesis (2), some authors have reported that iron deficiency may be a source of interference for HbA2 determination (17). Although HbA2 measurement plays a key role in differential diagnosis of iron deficiency anemia and thalassemia, yet there is no international standardization of HbA2 determination. Then, the test that should be

selected firstly is complete blood count. Red blood cell count generally increases in thalassemia minor, by contrast decreases in iron deficiency according to the degree of anemia. RBC distribition width (RDW) in thalassemia is generally normal, in contrast, they are typically over 17 % in IDA due to unequal distribution of iron in the bone marrow (2,9,18). MCV values below 80fl have higher diagnostic value for thalassemia trait than the MCH below 27 pg. MCV/RBC values known as the Mentzer Index are usually below 13 in thalassemia and over in iron deficiency (19). In children, it has shown to be reliable with 98 % sensitivity when the ratio 13 was accepted as the cut off value for the diagnosis of thalassemia, nevertheless it can detect iron deficiency with 82 % sensitivity (3). Another important test of iron deficiency anemia was ferritin as an early laboratory finding. Serum ferritin level is the most powerful parameter for the diagnosis of IDA after bone marrow aspiration (2,20). Transferrin saturation index is seen generally below 10 % in IDA and while usually normal in thalassemia trait (2, 20).

Along with the widespread use of ferritin measurements, the possible association of iron deficiency anemia and thalassemia was begun to attract attention (13). In this study, ferritin levels were found low in 18 % of all carriers in favor of iron deficiency anemia. This ratio in women carrier group was observed as 28 %. MCV and MCH values were significantly lower and RDW levels were significantly higher in female patients without iron deficiency than those with iron deficiency. Iron deficiency anemia has been reported in 62 % of infants (2), 18 % of women in fertile age and 4 % of men (4, 21). Similarly, according to some studies, low ferritin levels were found in fertile β-thalassemia women carriers than men carriers as might be due to dietary habits and also to excessive menstrual blood loss (4,13,21). IDA frequency has been identified as 30 % in 91 Indian women, contrarily, 3.4 % in 59 male carriers (13). Ferritin levels of 74 % of β- thalassemia fertile women carriers in Pakistan were normal, 12 % of total were high and 14 % of total were low that support iron deficiency anemia (22). According to another study, thalassemia trait with iron deficiency anemia has been reported as 2.7 % in 150 children (2).

In our study, in thalassemia carriers with iron deficiency, transferrin saturation was observed more sensitive than MI and can better distinguish the carriers without iron deficiency from the carriers with iron deficiency. It has been reported that iron deficiency might mask the diagnosis of thalassemia and HbA2 levels have barely reached to the carrier level (5.2 ± 0.3 %) after 20 weeks treatment (16). In present study, it was found that HbA2 levels were significantly lower in thalassemia carriers with iron deficiency compared to without iron deficiency. It is possible that the transcription and/or translation may be decreased in δ gene or there was a competition between the β chain of HbA and δ chain of HbA2 for the limited amount of iron (15). Although ferritin levels as an acute phase reactant were high in malignancy and chronic inflammatory disease, it is very valuable in the diagnosis of IDA in these patients. However the limitation of this study was the numbers of child population that can not reflect the actual proportions, there had been low ferritin levels in only a child carrier.

Hematological indices are not enough for diagnosis of iron deficiency. Ferritin levels should be measured in patients. Beside this, for suspected cases of thalassemia diagnosis, analysis should be repeated after 16-20 weeks of iron therapy (15). Hemoglobin chain analysis should be performed in microcytic anemia with resistant to iron therapy. Because iron deficiency will aggravate the clinical occurs in the thalassemia trait and it can be treated, this association should not be overlooked.

In conclusion, it was put forward that the importance of considering the association of iron deficiency anemia and thalassemia for women carriers in this study. The treatment of iron deficiency would be beneficial for prevention of complications in the treatment

process of β-thalassemia. Molecular genetic analysis should be tried to diagnose some thalassemia mutations in which patients HbA2 levels are less than 3.5 % and in where iron deficiency shadows this. Further studies should be performed with large numbers of populations and for some other situations such as malignancy and chronic inflammatory disease. And also, further studies should be planned for patients with iron deficiency and screened for hemoglobin chain analysis.

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