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Different presentations of patients with transcobalamin II deficiency:

A single center experience from Turkey

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Running head: Presentations of Transcobalamin II deficiency

Key words: Transcobalamin II, vacuolization, immune deficiency, neurological impairment,
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Abstract

Objective: Transcobalamin II (TC) deficiency is a rare autosomal recessive disease characterized by decreased cobalamin availability, which in turn causes accumulation of homocysteine and methylmalonic acid. The presenting clinical features are failure to thrive, diarrhea, megaloblastic anemia, pancytopenia, neurologic abnormalities and also recurrent infections due to immune abnormalities in early infancy.

Materials and Methods: Here, we report the clinical and laboratory features of six children with TC deficiency who are all molecularly confirmed.

Results: The patients were admitted between 1 and 7 months of age with anemia or pancytopenia. Unexpectedly, one patient had a serum vitamin B12 level lower than normal range and the other one had not significantly elevated serum homocysteine levels. Three patients had lymphopenia, four had neutropenia and two had also hypogammaglobulinemia suggesting the consideration of TC deficiency in the differential diagnosis of immune deficiency. Hemophagocytic lymphohistiocytosis was also detected in one patient. Furthermore, two patients had vacuolization in the myeloid lineage in bone marrow aspiration, which may be an additional finding of TC deficiency. The hematological abnormalities in all patients resolved after parenteral cobalamin treatment. In the follow-up, two patients showed neurological impairment such as impaired speech and walking. Among our six cases who were all molecularly confirmed, two had the mutation which was reported in the TC deficient patients of Turkish ancestry. Also, a novel TCN2 gene mutation was detected in one of the remaining cases.

Conclusion: TC deficiency should be considered in differential diagnosis of infants with immunological abnormalities as well as cytopenia and neurological dysfunction. Early recognition of this rare condition and initiation of adequate treatment is critical for the disease control and better prognosis.

Key words: Transkobalamin II, vacuolization, immune deficiency, neurological impairment, hemophagocytic lymphohistiocytosis

Transkobalamin II eksikliği olan hastaların farklı sunumları: Türkiye'den tek merkez deneyimi

Öz

Amaç: Transkobalamin II eksikliği homosistein ve metilmalonik asit birikimine neden olan kobalamin yararlanımının azaldığı nadir bir otozomal resesif hastalıktır. Erken dönemde gelişme geriliği, diyare, megaloblastik anemi, pansitopeni, nörolojik anormallikler ve ayrıca immün bozukluklara bağlı tekrarlayan enfeksiyonlarla karakterizedir.

Gereç ve Yöntemler: Bu çalışmada, transkobalamin II eksikliği tanısı moleküler olarak konfirme edilen altı hastanın klinik ve laboratuvar özellikleri sunulmuştur.

Bulgular: Hastalar 1-7 ay arasında anemi veya pansitopeni ile başvurdu. Beklenmedik şekilde, bir hastanın serum vitamin B12 düzeyi normalden düşüktü ve diğer bir hastanın serum homosistein düzeyi belirgin düzeyde yüksek değildi. Üç hastada lenfopeni, dört hastada nötropeni ve iki hastada ayrıca hipogamaglobulinemi olup bu bulgular immün yetersizliğin ayırıcı tanısında transkobalamin II eksikliğinin düşünülmesi gerektiğini göstermektedir. Bir hastada hemofagositik lenfohistiyoitoz saptandı. Ayrıca, iki hastanın kemikiliği aspirasyonunda myeloid seride vakuolizasyon olup transkobalamin II eksikliğinin ek bir bulgusu olabilir. Parenteral kobalamin tedavisinden sonra tüm hastaların hematolojik anormallikleri düzeldi. Takipte iki hastada konuşmada ve yürümede bozulma şeklinde nörolojik anormallikler saptandı. Tümü moleküler olarak doğrulanmış altı vakamızdan ikisinde, transkobalamin II eksikliği olan Türk ırkına ait hastalarda bildirilen mutasyon mevcuttu. Ayrıca, kalan vakalardan birinde yeni bir TCN2 gen mutasyonu tespit edildi.

Sonuç: Transkobalamin II eksikliği sitopeni ve nörolojik bozuklukların olduğu gibi immünolojik anormalliklerin de olduğu infantlarda ayırıcı tanıda düşünölmelidir. Bu nadir hastalığın erken tanınması ve uygun tedavinin başlanması hastalığın kontrolü ve daha iyi bir prognoz açısından kritiktir.

Anahtar Sözcükler: Transkobalamin II, vakuolizasyon, immün yetersizlik, nörolojik bozukluk, hemofagositik lenfositosis

Introduction

Transcobalamin II (TC) deficiency is a rare autosomal recessive disease in early infancy caused by mutations in the TCN2 gene [1]. TC is a transport protein for vitamin B12 and facilitates its cellular uptake by receptor mediated endocytosis [1]. A deficiency of TC results in a lack of vitamin B12 entry into cells leading to intracellular cobalamin depletion [2]. The presenting clinical features are failure to thrive, diarrhea, pancytopenia, neurologic abnormalities and infections due to immunodeficiency [3, 4, 5, 6].

The diagnosis is suspected by the presence of clinical and laboratory features [5]. Patients with TC deficiency have elevated homocysteine and methylmalonic acid levels [2, 7]. Treatment with parenteral vitamin B12 is highly effective on clinical and biological signs. It is important to establish the diagnosis of TC deficiency as early as possible because a significant delay in diagnosis, and therefore the treatment, the neurological abnormalities can be irreversible [8].

In the present study, we report the clinical and laboratory features of six children diagnosed with TC deficiency in our hospital.

Materials and Methods

We retrospectively reviewed the medical records of six children with a diagnosis of TC deficiency in our hospital. Complete blood count, serum vitamin B12, folic acid, homocysteine and immunoglobulin levels, percentage of lymphocyte subsets, bone marrow aspiration and molecular analysis results were obtained from medical charts. Treatment regimens given to patients were recorded. The clinical and laboratory findings of our patients were shown in Table 1.

Results

Case 1

A 2-month-old male baby was admitted with complaints of fever, cough, diarrhea and respiratory distress. The parents were first-degree cousins. The constellation of clinical features such as prolonged fever and splenomegaly and laboratory findings (cytopenia in peripheral blood, elevated ferritin, triglyceride and liver enzymes and hemophagocytosis in the bone marrow) suggested the diagnosis of hemophagocytic lymphohistiocytosis (HLH). Cytomegalovirus (CMV) PCR was found to be positive and he was given ganciclovir therapy. Intravenous immunoglobulin was added to the therapy due to the presence of hypogammaglobulinemia. Percentage of lymphocyte subsets were in the normal ranges. A second bone marrow aspiration demonstrated megaloblastic changes in the erythroid series. The patient's serum vitamin B12 level was normal; however, serum homocystein level (23 $\mu\text{mol/l}$) was significantly higher than normal. A genetic deficiency of TC was suspected and a homozygote TCN2 gene mutation was detected in molecular analysis. This 5304-bp deletion began 1516 bp into intron 7 and ended 1231 bp into intron 8. The deletion included all of exon 8 and caused a frame shift to produce premature stop four codons into new reading frame. The patient was treated with intramuscular vitamin B12, which was followed by improvement in both clinical and laboratory findings. This case was published as a case report in the literature [9].

Case 2

A 6-month-old girl presented with complaints of failure to thrive, vomiting and diarrhea. A diagnosis of sepsis or metabolic disease was suspected and antibiotic therapy was started empirically. She had anemia, neutropenia and thrombocytopenia. Serum vitamin B12 level was found to be normal; however, serum homocysteine was 53 $\mu\text{mol/l}$. Megaloblastic

changes and vacuolization were prominent in myeloid lineage in the bone marrow aspiration. Immunological evaluation revealed hypogammaglobulinemia. Percentage of lymphocyte subsets were in normal range. A genetic deficiency of TC was suspected. The molecular analysis revealed c.1106+1516_1222+1231del mutation. This mutation is not listed in the Human Gene Mutation Database Cardiff. A 5304 bp deletion that begins 1516bp into intron 7 and ends 1231bp into intron 8. The deletion includes all of exon 8 and causes a frameshift to produce a premature stop four codons into the new reading frame. The patient was treated with intramuscular vitamin B12 and oral folic acid, which was followed by improvement in hematological response but a speech deficit was detected at two years of age in the follow-up.

Case 3

A 7-month-old boy presented with complaints of poor feeding, diarrhea and petechiae. He was the child of first-degree cousins. He had pancytopenia. Serum vitamin B12 level was found to be normal and serum homocysteine level was high at borderline (16 $\mu\text{mol/l}$). Bone marrow was hypocellular and megaloblastic changes were prominent in the myeloid lineage. A genetic deficiency of TC was suspected. The molecular analysis revealed c.1106+1516_1222+1231del mutation. This mutation is not listed in the Human Gene Mutation Database Cardiff. A 5304 bp deletion that begins 1516bp into intron 7 and ends 1231bp into intron 8. The deletion includes all of exon 8 and causes a frameshift to produce a premature stop four codons into the new reading frame. The patient was treated with intramuscular vitamin B12 which was followed by improvement in hematological response but a delay in walking was detected at two years of age in the follow-up.

Case 4

A 5-month-old girl was admitted with failure to thrive, poor feeding, vomiting and diarrhea. She was the child of first-degree cousins and had a history of sibling death. Laboratory evaluation showed pancytopenia which required transfusions and lymphopenia and hypogammaglobulinemia. Percentage of lymphocyte subsets were in normal range. Serum

vitamin B12 level was low (136 pg/ml) but serum homocysteine level could not be measured. CMV PCR was found to be positive. Severe combined immunodeficiency was suspected. Intravenous immunoglobulin, ganciclovir treatment, antibacterial and antifungal prophylaxis were given. However, bone marrow aspiration showed prominent vacuolization in the myeloid lineage which suggested Pearson syndrome and prominent megaloblastic changes in myeloid lineage. However, molecular analysis did not support the diagnosis of Pearson syndrome. A genetic deficiency of TC was suspected. The patient was treated with intramuscular vitamin B12 and oral folic acid with clinical and hematological improvement. After her family discontinued vitamin B12 therapy, she showed relapse with severe pancytopenia. Then, vitamin B12 treatment was restarted. The molecular analysis revealed homozygous TCN2 gene mutation.

Case 5

A 1-month-old male baby presented with irritability, fever and poor feeding. He had cleft palate and lip. He was the child of first-degree cousins. A diagnosis of sepsis was suspected and antibiotic therapy was started empirically. Complete blood count revealed macrocytic anemia which required transfusions in the follow-up. Serum vitamin B12 and folic acid levels were found to be normal. Bone marrow aspiration showed megaloblastic changes in the myeloid lineage. Serum homocysteine level was 45 $\mu\text{mol/l}$. A genetic deficiency of TC was suspected. Homozygous deletion of TCN2 gene was detected in exon 8. The patient was treated with intramuscular vitamin B12, which was followed by clinical and hematological response.

Case 6

A 2-month-old male baby presented with complaints of irritability, fever, oral aphthous ulcers and diarrhea. A patent ductus arteriosus was found in echocardiography. Laboratory evaluation revealed pancytopenia. Serum vitamin B12 and folic acid levels were found to be normal. Serum homocysteine level could not be measured. Bone marrow aspiration was remarkable for megaloblastic changes in erythroid and myeloid cell precursors. A genetic

deficiency of TC was suspected. The molecular analysis revealed homozygous TCN2 gene mutation; c.106C>T (p.Q36*) (p.Gln36*). The patient was treated with intramuscular vitamin B12 and oral folic acid. He has been asymptomatic in the follow-up.

Discussion

Here, we report six patients with the diagnosis of TC deficiency in our institution. The present study extends our understanding about this rare disease and provides additional data for the clinical and laboratory manifestations. The presenting clinical features are macrocytic anemia, pancytopenia, failure to thrive, gastrointestinal symptoms and neurologic dysfunction [4, 5, 10]. In addition to aforementioned clinical features, recurrent infections can be seen in TC deficiency due to immunological abnormalities. Specific immune abnormalities include hypogammaglobulinemia, specific antibody deficiency, neutropenia and low T and/or B cell counts [3, 5, 11]. Consistent with these data, three patients had lymphopenia, four had neutropenia and two had hypogammaglobulinemia in the present study suggesting the consideration of TC deficiency in the differential diagnosis of immune deficiency.

Hemophagocytic lymphohistiocytosis (HLH) is a syndrome characterized by uncontrolled immune response with hyperinflammation [12, 13]. Acquired HLH can develop as a result of infections, malignancy and autoimmunity. In addition, HLH is associated rarely with congenital metabolic disorders [13, 14]. Case 1 was reported by Unal et al. in the literature as the first case of TC deficiency presenting with HLH [9]. Defects in cobalamin metabolism were suggested to lead HLH due to defects in DNA synthesis, secondary NK cell functions and immune regulation [15]. Hypogammaglobulinemia, lymphopenia and neutrophil dysfunction have been reported in TC deficiency [3, 5, 11]. Therefore, it can be considered that immune changes caused by a lack of TC in our patient led to the development of a secondary HLH. Potential causative mechanism of HLH induced by defects of cobalamin metabolism merit further investigation.

Despite early onset cobalamin treatment and close monitoring of hematological parameters, patients with TC deficiency have been reported to show neurological deterioration including

intellectual disability, attention deficits, tremor, myoclonus, ataxia, delay in language and motor skills. Epilepsy responsive to antiepileptic medication was also reported [4, 5]. The most common neurological complication in the literature was speech deficit [5]. Consistent with the literature, case 2 had speech deficit and case 3 had impairment in walking despite intramuscular cobalamin treatment. The starting age and mode of cobalamin treatment influence neurological outcome [4, 5]. The patients who were treated early have had a better outcome than those inadequately treated. Nevertheless, the natural course of the disease over time might also result in late onset neurological symptoms [4, 5, 8]. We initiated the treatment with cyanocobalamin 1 mg intramuscular daily in the first week and the therapy was continued with cyanocobalamin 1 mg every second day in the second week. In the follow-up, weekly injections of cyanocobalamin 1 mg were administered based on the clinical and hematological response. In association with cyanocobalamin therapy, folic acid supplementation was also given.

To date, serum vitamin B12 levels have been reported to be normal in cases with TC deficiency [4, 10, 16]. This is due to the fact that the majority of vitamin B12 in circulation is bound to haptocorrin rather than transcobalamin. As a result, patients with TC deficiency do not show a low level of circulating vitamin B12 [1, 2]. However, Schiff et al, reported TC deficient cases with low vitamin B12 levels [8]. In the present study, case 4 had the unusual feature of a low serum cobalamin level similar to the cases reported in Schiff's study. Homocysteine and methylmalonic acid levels are the metabolic markers of vitamin B12 deficiency [7]. The diagnosis of TC deficiency is confirmed by the measurement of these metabolic markers, although they rarely can be within normal limits [10]. Consistent with this data, the homocysteine levels of Case 3 were in the upper limit of normal. Therefore, our findings indicate that a normal or low serum vitamin B12 level or a slightly elevated homocysteine level does not exclude TC deficiency. Thus, molecular analysis should be done in order to establish a firm diagnosis in the subjects with suggested TC deficiency but with normal plasma homocysteine levels.

Previous two studies reported vacuolization in the bone marrow aspiration of TC deficient patients [10, 17]. Similar to these previous studies, vacuolization in myeloid lineage was seen in the bone marrow aspiration of Case 2 and 4 in the current study and Pearson syndrome was suspected. Vacuolization is an important finding in Pearson syndrome which may be

related to defect in mitochondrial DNA synthesis [18]. However, it may be an additional finding in TC deficiency, resulting from a defect of cobalamin metabolism.

Trakadis et al, reported a series of 30 patients with TC deficiency. Molecular testing was performed on 20 families and 17 different mutations were identified. 16 families had homozygous and four had compound heterozygous mutations. Different intragenic deletions were identified in 15 out of the 20 families [5]. Four mutations resulted in exon skipping [19]: two involved exon seven and one involved exon four; the fourth was a large deletion involving exons 1 to 7. Trakadis et al, reported that a founder mutation may be present in the patients of Turkish ancestry, as all presumably unrelated probands of Turkish ancestry shared the same homozygous mutation (c.1106+1516_1222+1231del) [5]. This common mutation among the Turkish population was also detected in our cases 2 and 3 who were living in the same town. In this mutation, the deletion includes all of exon 8 and causes a frameshift to produce a premature stop four codons into the new reading frame. The other mutation in Case 6 (c.106C>T (p.Q36*) (p.Gln36*)) produces a premature stop four codons into the new reading frame.

In conclusion, TC deficiency often presents early in life with multisystem involvement. Therefore, it should be considered in differential diagnosis of infants with cytopenia and neurological dysfunction as well as immunological abnormalities. Early recognition of this rare disease and initiation of adequate treatment is critical for the disease control and better prognosis.

Author's Contribution:

Data Collection: Feryal Karahan, Asuman Akar, Selma Ünal, Analysis or Interpretation: Selma Ünal, Tuğba Arıkoğlu, Literature Search: Tuğba Arıkoğlu, Writing: Tuğba Arıkoğlu, Critical Revision: Selma Ünal, Semanur Kuyucu

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Table 1. Clinical manifestations and laboratory findings of the patients.

	Case 1	Case 2	Case 3	Case 4	
Age (mo) at presentation	2	6	7	5	
Sex	Male	Female	Male	Female	
Consanguinity	First-degree cousins	Absent	First-degree cousins	First-degree cousins Two siblings ex	First
Symptoms at presentation	Fever, cough, diarrhea, respiratory distress	Vomiting, diarrhea, failure to thrive	Poor feeding, diarrhea petechiae	Vomiting, diarrhea, failure to thrive	Fev p
Additional findings	CMV PCR: (+) Hemophagocytic syndrome	Impaired in speech	Impaired walking	CMV PCR: (+)	Clef
Hb (g/dL)	10.4	9.6	6.9	6.2	
Hct (%)	29.2	27.1	21.2	17	
RBC (x10 ¹² /L)	3.2	3.1	2.4	1.9	
WBC (x10 ⁹ /L)	3.67	5.19	3.47	1.00	
MCV (fL)	89	85	88	84	
Platelets (x10 ⁹ /L)	81	45	62	1	
ANC (x10 ⁹ /L)	1.54	1.08	0.50	0.08	
ALC (x10 ⁹ /L)	1.86 (3.5-13.1)	3.35 (3.8-9.90)	2.82 (3.8-9.90)	0.91 (3.7-9.6)	6.

IgG mg/dl IgA mg/dl IgM mg/dl IgE IU/ml	(at 2 months) 212 (376-685) 26 (9-30) 20 (36-77) <17	(at 6 months) 255 (304-1231) 84 (7-123) 20 (32-283) 5	NA	(at 5 months) 183 (294-1165) 42 (13-72) 52 (33-154) <2	(at 12 months) 183 (294-1165) 42 (13-72) 52 (33-154) <2
CD3 CD4 CD8 CD19 CD16+56	80% (60-85%) 66% (41-68%) 29% (9-23%) 13% (4-26%) 4% (3-23)	81% (50-77%) 66% (33-58%) 13% (13-26%) 14% (13-35%) 4% (2-13%)	NA	80% (48-75%) 52% (33-58%) 25% (11-25%) 16% (14-39%) 1% (2-14%)	80% (48-75%) 52% (33-58%) 25% (11-25%) 16% (14-39%) 1% (2-14%)
Vitamin B12 (normal: 197-866 pg/ml)	568	1281	479	136	136
Homocysteine (normal: 5- 15µmol/L)	23	53	16	NA	NA
Bone marrow examination	Hypocellular, increase in myeloid lineage	Vacuolization and megaloblastic changes in myeloid lineage	Hypocellular, megaloblastic changes in myeloid lineage	Vacuolization, megaloblastic changes in myeloid lineage	Megaloblastic changes in myeloid lineage
TCN2 mutation	c.940+283_286delTGA;c.940+303_1106+764del2152insCTGG	c.1106+1516_1222+1231del	c.1106+1516_1222+1231del	Homozygous deletion of TCN2 gene was detected in exon 8	Homozygous deletion of TCN2 gene
Treatment regimen	Ganciclovir and IVIG at first admission Cyanocobalamin i.m oral folic acid	Cyanocobalamin i.m oral folic acid	Cyanocobalamin i.m oral folic acid	Ganciclovir and IVIG at first admission Cyanocobalamin i.m oral folic acid	Cyanocobalamin oral folic acid

ANC: absolute neutrophil count, ALC: absolute lymphocyte count, CMV: cytomegalovirus, Hb: hemoglobin, Htc: hematocrit, IVIG: intravenous immunoglobulin; i.m: intramuscular; MCV: mean corpuscular volume; NA: not available, RBC: red blood cell, WBC: white blood cell