A Turkish case of congenital chloride diarrhea with SLC26A3 gene (c.2025_2026insATC) mutation: Diagnostic pitfalls

SLC26A3 gen (c.2025_2026insATC) mutasyonu olan konjenital klor diyareli Türk olgusu: Tanısal güçlükler

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Congenital chloride diarrhea is a rare autosomal recessively inherited disorder characterized by impairment of $Cl^-/HCO3^-$ exchange in an otherwise normal distal ileum and colon. Infrequency of congenital chloride diarrhea makes diagnostics difficult. The typical presentation is watery Cl^- rich diarrhea, hypochloremia, hypokalemia, metabolic alkalosis and failure to thrive. This is a report of a Turkish female infant who was falsely diagnosed with Bartter syndrome when she was two months old. Ibuprofen was commenced at that time. However, severe watery diarrhea, dehydration, failure to thrive, abdominal distention, and electrolyte abnormalities persisted. She was diagnosed with congenital chloride diarrhea based on high fecal Cl^- level and SLC26A3 gene c.2025_2026insATC mutation at the age of eight months. Oral NaCl and KCl supplementation was started. Our patient is now 26 months old. Her growth and development are normal. Early diagnosis and treatment are essential for normal growth and development and prevention of other severe complications of congenital chloride diarrhea.

Key words: Congenital chloride diarrhea

Konjenital klor diyaresi nadir görülen otozomal resesif geçişli distal ileum ve kolonda HCO3/Cl-değişimindeki defekt sonucu ortaya çıkan bir bozukluktur. Nadir görülmesi tanısal güçlüğe yol açmaktadır. Tipik bulguları klordan zengin çok sulu ishal, hipokloremi, hipokalemi, metabolik alkaloz ve büyüme geriliğidir. Burada, 2 aylıkken yanlış olarak Bartter sendromu tanısı konulan ve ibuprofen başlanan; 8 aylıkken sulu ishal, dehidratasyon, büyüme geriliği ve karın distansiyonunun devam etmesi nedeniyle tekrar değerlendirilen bir kız hasta sunuldu. Dışkı klor düzeyi yüksekliği ve SLC26A3 genindeki c.2025_2026insATC mutasyonunun gösterilmesi ile konjenital klor diyaresi tanısı konuldu. Oral NaCl ve KCl desteği başlandı. Hastamız şu an 26 aylık olup büyüme ve gelişmesi normaldir. Normal büyüme ve gelişmenin sağlanması ve Konjenital klor diyaresinin diğer ciddi komplikasyonlarının önlenmesi için erken tanı ve tedavi önemlidir.

Anahtar kelimeler: Konjenital klor diyaresi

INTRODUCTION

Congenital chloride diarrhea (CLD) is an autosomal recessively inherited disease caused by mutations in the solute carrier family 26, member 3 gene (SLC26A3) on chromosome 7q31 encoding for a transmembrane $Cl^-/HCO3^-$ exchanger mainly expressed in the apical brush border of ileal and colonic epithelium. This results in watery, Cl^- rich diarrhea, hypochloremia, hypokalemia, and metabolic alkalosis (1,2). The diarrhea begins in fetal life and causes polyhydramniosis and premature delivery (2). It is more common in Finland, Saudi Arabia, Kuwait, and Poland. Currently, about 260 cases have been reported (3). The estimated incidences of CLD in Finland and Poland are 1 in 20,000 and 1 in 200,000, respectively. Consanguineous marriages in Saudi Arabia and Kuwait cause unusually high local incidences, such as 1 in 5000 (4). In Turkey, CLD incidence is not known and reported cases are scarce. Özen et al. (5) reported a CLD case when the patient was seven years, five months old with severe growth retardation. Our patient was falsely diagnosed with Bartter syndrome at the age of two months and treated with ibuprofen. However, hypochloremic alkalosis,

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hyponatremia and hypokalemia persisted. Watery diarrhea and failure to gain weight directed us to conduct stool chloride analysis in this patient. Increased excretion of fecal chloride led to the diagnosis of CLD. In this report, we also discuss the diagnostic pitfalls during the differential diagnosis of CLD.

CASE REPORT

A female patient with polyhydramniosis was born from healthy nonconsanguineous parents at 32 weeks of gestation with a birth weight of 2330 g, which was appropriate for her age. Maternity hospital records indicated acute gastroenteritis and dehydration in the first week of life. In the 2nd month of life, she was admitted to the local hospital due to severe watery diarrhea, vomiting and failure to gain weight. The patient had hypochloremic metabolic alkalosis, hyponatremia and hypokalemia, with increased levels of plasma renin [18 ng/ml (normal: 0.51-2.64)] and aldosterone [1700 pg/ml (normal: 50-900)]. Sweat chloride level was normal. Stool microscopy was normal; steatocrit, reducing substances, and stool adenovirus-rotavirus antigens were all negative. It was thought that the patient might have CLD; however, stool chloride level was found normal at that time. She was diagnosed with Bartter syndrome and ibuprofen therapy was given. She was followed up at an outpatient clinic irregularly. At eight months of age, the patient was readmitted due to persistent severe diarrhea, dehydration, failure to thrive, and abdominal distention. She had severe malnutrition (weight: 3540 g, <-3 SDS; height 59 cm, <-3 SDS), dehydration and weakness. She was referred to our hospital for investigation of chronic diarrhea after vigorous fluid-electrolyte treatment. On admission to our hospital, arterial blood gas analysis demonstrated pH 7.61, bicarbonate 50.2 mEq/L and base excess 25.9 mmol/L. Plasma electrolytes were Na^+: 132 mEq/L, K^+: 2 mEq/L and Cl^-: 62 mEq/L. Urinary electrolytes were Na^+: 15 mmol/L (normal: 54-150), K^+: 23.9 mmol/L (normal: 20-80), and Cl^-: 14 mmol/L (normal: 110-250). Stool Na^+, K^+ and Cl^- concentrations were 98, 29.4, and 148 mmol/L, respectively. Stool osmotic gap was 32. Abdominal ultrasound showed calcification in renal calices. Her upper gastrointestinal endoscopy was normal. Duodenum biopsy revealed diminished villus/crypt ratio in scattered areas with hematoxylin-eosin staining. Immunohistochemistry with CD10 antibody of the duodenum mucosa showed linear staining and focal loss of brush border (Figure 1). Control biopsy was stained normal (Figure 2). Tissue transglutaminase immunoglobulin (Ig)A and IgG antibodies were negative, with a normal IgA level. DNA analysis for cystic fibrosis was negative for 36 mutations. Our patient was diagnosed with CLD based on high stool Cl level and decreased osmotic gap. A homozygous mutation was found characterized by an insertion of three nucleotides - adenine, thymidine and cytosine - between nucleotides 2025 and 2026 in exon 18 (c.2025_2026insATC) in the SLC26A3 gene. Oral NaCl and KCl replacement therapy (5 mEq/kg/d) was started, and omeprazole was added. Currently under follow-up, she is 26

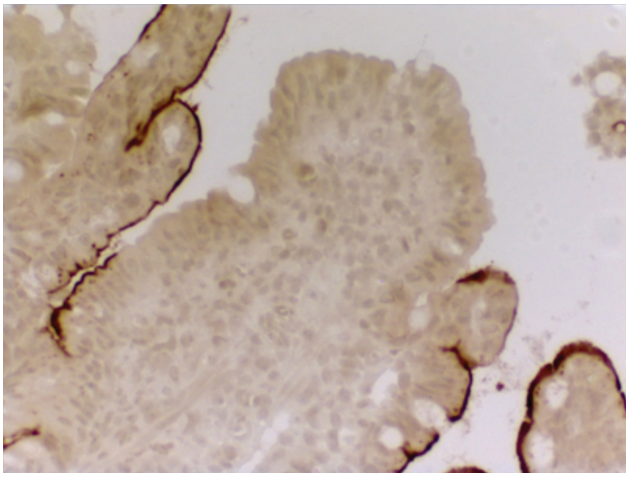

Figure 1. Immunohistochemistry with CD10 antibody of duodenum mucosa showing focal loss of brush border.

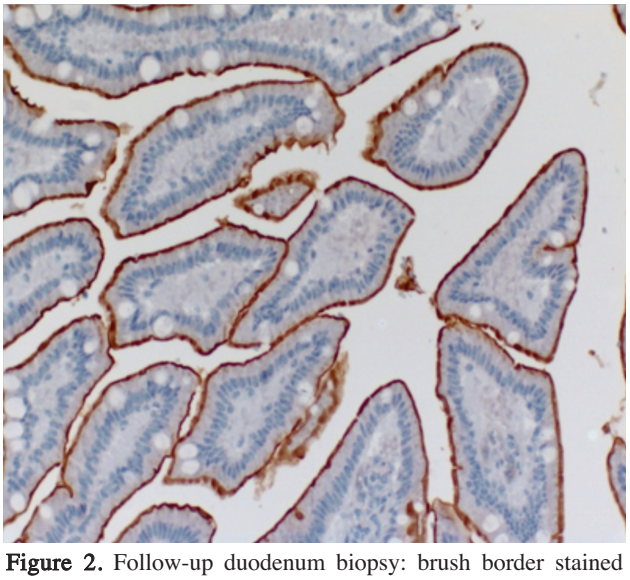

Figure 2. Follow-up duodenum biopsy: brush border stained normal with CD10 antibody.

months old, her weight is 12 kg (50-75 percentile) and her height is 85 cm (25-50 percentile); her developmental milestones are normal for her age.

DISCUSSION

Chloride diarrhea (CLD) was first described in 1945 by Gamble and Darrow. Watery Cl^- rich stool, hypochloremia, hypokalemia, metabolic alkalosis, and failure to thrive are observed in the typical presentation of this disease (6).

The basic defect in CLD is the impairment of $Cl^-/HCO3^-$ exchange in an otherwise normal distal ileum and colon. Active Cl^- re-absorption is defective, massive amounts of Cl^- are lost in the stools, and hypochloremia develops. The respective defect in $HCO3^-$ secretion leads to metabolic alkalosis and the acidification of intestinal content, which further inhibit the absorption of Na^+ through the Na^+/H^+ exchanger. In the intestine, the high luminal electrolyte content leads to diarrhea with osmotic mechanisms. Na^+ and water losses cause secondary hyperaldosteronism and K^+ wastage, resulting in both hyponatremia and hypokalemia. The diagnosis in our patient was confirmed by a stool chloride content that exceeded the sum of fecal sodium and potassium. Stool chloride level exceeds 90 mmol/L (2).

During fetal life, polyhydramnios and dilatation in intestinal loops occur. Patients are born prematurely due to intrauterine diarrhea (2). Congenital CLD in a newborn is a medical emergency requiring early diagnostics and treatment. Affected children can be diagnosed by antenatal ultrasonography demonstrating polyhydramnios and fluid-filled intestinal loops (7). Our patient also had polyhydramniosis and prematurity. However, antenatal ultrasonography was not performed and neonatal diarrhea was misdiagnosed as acute gastroenteritis. Watery diarrhea sometimes cannot be noticed during the neonatal–early infancy period, because the stool in diapers resembles urine (5). This confusing situation may lead to misdiagnosis of CLD and loss of a significant period of time before achieving an accurate diagnosis, as occurred in our patient.

Other clinical presentations are malnutrition, failure to thrive and dehydration. Due to metabolic problems, the impact of malnutrition on brain development and recurrent seizures, neurodevelopmental delay may be seen (8,9). Prolonged dehydration leads to glomerular hyalinosis, diffuse sclerosis and arteriolar changes that lead to proteinuria and reduced renal function (10). Renal hypoplasia, nephrocalcinosis and glomerulonephritis have also been reported in some patients (11). Our patient's ultrasonography showed calcification in renal calices. Some patients require renal transplantation (10).

Congenital CLD can be confused with Bartter syndrome. Bartter syndrome is a group of autosomal recessive disorders that are characterized by markedly reduced or absent sodium chloride transport by the thick ascending limb of Henle. Clinical features of this disorder include hypokalemia, hypochloremic metabolic alkalosis, hypercalciuria with a variable risk of renal stones, and normal blood pressure despite hyperreninemia and hyperaldosteronism. However, these patients do not experience intractable watery diarrhea (12). Our patient was misdiagnosed as Bartter syndrome at the age of two months, and she was treated with oral NaCl, KCl and ibuprofen. Although CLD was suspected, normal Cl^- level of her stool masked the correct diagnosis. In our opinion, this may be attributed to the stool sample being obtained from her soaked diaper instead of by direct sampling to a container. Although our patient's renin-aldosterone levels were high on her first admission, we found them normal. We thought that the normal renin-aldosterone levels were related to intravascular fluid replacement therapy that was performed in the referring hospital.

When metabolic alkalosis is taken as a starting point of the differential diagnosis in a patient, one of the other causes of metabolic alkalosis aside from Bartter syndrome is cystic fibrosis. Infants with cystic fibrosis are prone to develop episodes of hyponatremic, hypochloremic dehydration with metabolic alkalosis, which are biochemical hallmarks of the pseudo-Bartter syndrome (13). The sweat chloride level was normal and cystic fibrosis mutational analysis was negative in our patient. We should be aware that increased concentrations of sweat Cl^- were reported in 12% of CLD patients (7). Other causes of pseudo-Bartter syndrome, such as chronic diuretic usage, chronic administration of a chloride-deficient diet, bulimia, cyclic vomiting, and abuse of laxatives, were absent in our patient (14).

Other causes of intractable watery diarrhea, such as congenital glucose-galactose malabsorption and congenital sodium diarrhea, cause metabolic acidosis instead of metabolic alkalosis. Microvillus

inclusion disease (MID) is a specific disorder of the intestinal brush border and also leads to intractable secretory diarrhea in infants. Electron microscopic analysis is required for its definitive diagnosis. Nowadays, CD10 (a membrane-associated neutral peptidase) immunostaining is a valuable tool for the diagnosis of MID. It was reported that while MID patients had shown prominent cytoplasmic CD10 staining, normal controls and patients with celiac disease, autoimmune enteropathy and allergic enteropathy had shown a linear brush border staining (15). Our patient had diminished villus/crypt ratio in scattered areas of the duodenum mucosa. Immunohistochemistry with CD10 antibody showed linear brush border staining. Local loss of brush border might be explained by severe malnutrition and possible trace element deficiencies and defective regeneration capacity of intestinal epithelium, or concomitant viral gastroenteritis. Celiac disease was excluded by negative tissue transglutaminase antibodies and catch-up growth of the patient after NaCl and KCl replacement.

High fecal Cl^- concentration (>90 mmol/L) is the standard for diagnosis of CLD, although DNA analysis is also available (6). Congenital CLD is caused by at least 30 different mutations in the solute carrier family 26 member 3 gene (SLC26A3). The V317del mutation has been found in 98% of all Finnish CLD-associated chromosomes. So far, no evidence of the phenotype-genotype correlation has been found (4). The clinical course of CLD, however, is variable, and may rather depend on environmental factors and compensatory mechanisms than mutations (4).

SLC26A3 encodes for a transmembranic $Cl^-/HCO3^-$ (or OH^-) exchanger mainly expressed in the apical brush border of the ileal and colonic epithelium (7). In our patient, a homozygous mutation in the SLC26A3 gene was found: an insertion of three nucleotides - adenine, thymidine and cytosine - between nucleotides 2025 and 2026 in exon 18 (c.2025_2026insATC). This mutation predicts an in-frame addition (insertion) of the amino acid isoleucine between codons 675 and 676. This mutation is the Polish founder mutation for CLD, and it is found in 47% of the Polish CLD-associated chromosomes (4). To our knowledge, our patient is the first reported Turkish case in whom CLD mutation analysis was performed.

Congenital CLD is usually lethal if untreated, and those who survive with an undiagnosed disease are likely compensating their diarrheal losses by salty diet (5). The therapy consists of a combination of NaCl and KCl, and the recommended dose of Cl- is 6 mmol/kg/d for children under three years of age (NaCl:KCl=2:1) (16) Cholestyramine has been shown to reduce the diarrhea but the effect fades over a few weeks (17). Omeprazole therapy is associated with reductions in the volume and frequency of the stool (18). Our patient was administered 5 mEq/kg/d NaCl and KCl and 1 mg/kg/d omeprazole therapy.

Although CLD is a very rare disorder, it should be included in the differential diagnosis of persistent watery diarrhea, hypochloremia, hypokalemia, and metabolic alkalosis in childhood. Its antenatal diagnosis is also possible with ultrasonography. Early diagnosis and treatment are essential for normal growth and development and prevention of other severe complications of CLD.

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