Three cases of a rare disease, congenital chloride diarrhea, summons up the variation in the clinical course and significance of early diagnosis and adequate treatment in the prevention of intellectual disability

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SUMMARY: Gürakan F, Baysoy G, Wedenoja S, Uslu N, Özen H, Özaltın F, Höglund P. Three cases of a rare disease, congenital chloride diarrhea, summons up the variation in the clinical course and significance of early diagnosis and adequate treatment in the prevention of intellectual disability. Turk J Pediatr 2011; 53: 194-198.

Congenital chloride diarrhea (CLD) (OMIM #214700) is a rare, autosomal recessive disease that is characterized by increased chloride loss in stool. As a result of electrolyte loss, surviving patients might have some complications, one of them being mental retardation. Here, we present three new Turkish patients with new mutations in the SLC26A3 gene. Although the clinical picture of the patients might be similar, consequences of the disease and complications might differ greatly among patients. Pediatricians should be aware of CLD as a potentially fatal or disabling disease if untreated. History of polyhydramnios, watery diarrhea, failure to thrive, poor growth, soiling, metabolic alkalosis and hypokalemia/hypochloremia should be an alarming set of findings for the diagnosis. Salt substitution therapy started early in life prevents early complications, allows normal growth and development, and favors good long-term prognosis.

Key words: congenital chloride diarrhea, mental retardation, SLC26A3 gene mutation.

Congenital chloride diarrhea (CLD) (OMIM #214700) is a rare, autosomal recessive disease that is characterized by increased chloride loss in stool[1]. The gene responsible for the disease is localized on chromosome 7q31 and was confirmed as a down-regulated in colonic adenoma gene that is currently known as SLC26A3[2,3]. This gene encodes a chloride transporter mainly expressed in the apical brush border of the ileum and in colonic surface epithelia. The functional defect characteristic in CLD begins *in utero* causing polyhydramnios, and often prematurity[3]. Among 260 reported cases of CLD, most come from Finland, Poland, Saudi Arabia, and Kuwait. Although consanguineous marriages are common in Turkey, only one previous report of a Turkish patient was found[4]. Herein, we present three novel Turkish cases of CLD, and illustrate the genetic analyses of their SLC26A3 genes and their clinical pictures. Mutation analysis of the patients and their parents was performed with DNA extracted from peripheral venous blood samples. After the DNA samples were obtained, they were amplified by polymerase chain reaction (PCR) and exon-specific primer pairs. The PCR products were sequenced using dye-terminator chemistry. Informed consent for DNA analysis was obtained from the parents.

Case Reports

Case 1

An eight-year-old boy was admitted to Hacettepe University Children's Hospital with

the complaint of chronic diarrhea. He was born as the first child of consanguineous parents. His parents and three-year-old brother were healthy. He was born at term with 2500 g birth weight and polyhydramnios was noted during pregnancy. Exchange transfusion was performed on the 3rd day of life due to hyperbilirubinemia. The early childhood period was uneventful apart from diarrhea beginning soon after birth and he was hospitalized at the age of 3 months because of failure to thrive. At the time of admission at 8 years of age, he was having 3 or 4 watery bowel movements per day without blood or mucus. There was also soiling and night- time defecation. Academic performance and physical examination were normal. His weight was at 25th percentile and length was at 50-75th percentile. Laboratory findings revealed hypokalemia and slight metabolic alkalosis. Serum sodium (Na), potassium (K) and chloride (Cl) levels were 142 mEq/L (normal: 135-145), 3 mEq/L (normal: 3.5-5.5), and 97 mEq/L (normal: 90-102), respectively. Venous blood pH was 7.45 (7.35-7.45) with HCO_3 of 33.1 mmol/L (normal: 24-31). Fecal pH was 6.5, and reducing sugar and steatocrit were negative. Acute phase reactants, immunoglobulin levels, sweat chloride level, antigliadin and endomysium antibodies, and cow's milk-specific RAST were all negative. Fecal electrolytes revealed a Na level of 61 mEq/L, K level of 51 mEq/L, and Cl level of 165 mEq/L. High fecal chloride with concurrent chronic diarrhea established the diagnosis of CLD. Omeprazole and KCl treatments were started. He did not benefit from omeprazole and had enuresis and encopresis.

Mutation analysis showed one homozygous mutation in the SLC26A3 gene: a deletion of three nucleotides, thymine-cytosine-thymine, with an insertion of a cytosine between nucleotides 1624 to 1626 in exon 15 (c.1624_1626delTCTinsC). This variation predicts a change of a serine by a proline at codon 542 and a frameshift followed by the 11th codon being the stop codon (p.S542PfsX11). This is a novel mutation. The father of this patient was found to be a carrier of this novel mutation; other family members were unavailable for genetic analysis.

Case 2

An 11.5-year-old boy was admitted to our hospital for the first time at the age of 6 months. His main symptom was watery diarrhea starting from the 2nd month of life. He was born from consanguineous parents following an uneventful pregnancy at the 32nd week of gestation with a birth weight of 2320 g. He had one healthy four-year-old sister. Rectal biopsy was performed in the neonatal period due to inability to pass meconium while he was followed up in the Neonatal Intensive Care Unit (NICU). Biopsy revealed nonspecific changes and thereafter he started to defecate normally. However, he had to be hospitalized at the ages of 2 and 4 months because of vomiting, diarrhea and dehydration. He was admitted to our hospital at the age of 6 months with moderate dehydration, malnutrition, hypokalemia (2.2 mEq/L), hyponatremia (120 mEq/L), proteinuria, glycosuria, and hypochloremic (59 mEq/L) metabolic alkalosis (pH 7.53, HCO_3 44.6 mmol/L). Urinary Cl was 60 mEq/day and Na was 73 mEq/day. With intravenous hydration therapy, his clinical and laboratory findings improved, and he was discharged in 10 days with the diagnosis of Bartter syndrome. Because his symptoms persisted, he was hospitalized again in 15 days. Laboratory findings were similar to those on the first admission. Indomethacin treatment was started for the diagnosis of Bartter syndrome; however, he did not benefit from this treatment. Antigliadin and anti- endomysium antibodies and occult blood in the stool were negative. Stool pH was 6.5 and reducing sugar was absent in the stool. Stool Na was 68 mEq/L, Cl was 23 mEq/L and K was 144 mEq/L. Cow's milk-specific IgE was negative. He was hospitalized for 2 months. His biochemical abnormalities were corrected with intravenous hydration and oral potassium. The patient was followed-up in another city and he admitted again at the age of 11 years. It was learned that he was hospitalized several times up to the age of 9 years because of dehydration and electrolyte abnormalities. Diarrhea, occasional vomiting, enuresis, polyuria, and night-time encopresis were the complaints at the last admission. He was found obese and mentally retarded (IQ: 45). Laboratory findings revealed a stool Cl level of 161 mEq/L, Na level of 68 mEq/L and K level of 72 mEq/L. Blood Na was 144, K 3.2 and Cl 103 mEq/L. Urinalysis and blood

gases were normal. After the diagnosis of CLD, oral salt, potassium, and lansoprazole treatments were started. He is still using these treatments with no apparent benefit on the diarrhea. Due to a diagnosis of moderate mental retardation, he attends a special class for mentally disabled children.

Mutation analysis showed one homozygous mutation in the SLC26A3 gene: a substitution of a guanine by an adenine at nucleotide 1954 in exon 17 (c.1954G>A). The c.1945G>A mutation predicts a change of an aspartic acid by an asparagine at codon 652 (p.D652N). This mutation has been detected in two patients with CLD before (Wedenoja, personal communication). This mutation was found in both parents in heterozygous form.

Case 3

A 12.5-year-old boy was admitted to our hospital at the age of 11.5 years with diarrhea and growth retardation. He was born at the 32nd week of gestation with a birth weight of 1760 g from consanguineous parents. Polyhydramnios was present. He was hospitalized in the neonatal period due to sepsis, necrotizing enterocolitis and hyperbilirubinemia for 2 months. Watery diarrhea and vomiting were apparent after hospital discharge. He was rehospitalized at the age of 8 months with diarrhea and malnutrition. Hyponatremia (122 mEq/L), hypokalemia (1.8 mEq/L) and hypochloremia (68 mEq/L) with metabolic alkalosis (pH: 7.58, HCO_3: 36.2 mmol/L) were found. He was diagnosed as Bartter syndrome and treated accordingly. He was admitted to several hospitals before coming to our clinic. Among the diagnostic tests, stool pH was 6.5, and reducing sugar and steatocrit were absent. Stool examination, celiac serology, and upper gastrointestinal endoscopy were found to be normal. At the time of admission, his growth was markedly retarded (height z-score -4.1, weight z-score -2.1), and his cognitive performance was in agreement with mild mental retardation (IQ: 59) (Table I). His blood Na was 140, K was 3.0, and Cl was 99 mEq/L, while stool electrolytes were 101, 28, and 169 mEq/L, respectively. He was diagnosed as chloride-losing diarrhea, and oral salt, potassium chloride, lansoprazole, butyrate complex, and cholestyramine treatments were started. Diarrhea did not improve with these treatments, and he is currently using oral salt, potassium chloride and lansoprazole. He is attending normal school, but needs support to complete his schoolwork, while his academic performance remains poor.

Mutation analysis showed a homozygous substitution of a guanine by a thymine at nucleotide 559 in exon 5 (c.559G>T) in the SLC26A3 gene. The mutation c.559G>T denotes a point mutation leading to replacement of an amino acid glycine at position 187 with a stop codon (p.G187X). This c.559G>T (p.G187X) mutation is the previously characterized Arabic founder mutation for CLD[5]. Both parents were found to be carriers of this mutation.

Discussion

Congenital chloride diarrhea is a rare disease reported with occasional patients worldwide. Diagnosis after a typical neonatal period is often delayed in low-incidence regions in view of the lack of knowledge and experience of pediatricians. Antenatally, CLD is characterized by polyhydramnios, caused by watery diarrhea. Postnatally, the fecal concentration of chloride is typically high (>90 mmol/L) with fecal anion gap (e.g. the sum of fecal Na^+ and K^+ is lower than the fecal Cl^-). Infants develop rapid dehydration, hypochloremia, hyponatremia, hyperbilirubinemia, and later hypokalemia with metabolic alkalosis. Failure to pass meconium, distended abdomen and visible movements of intestinal loops are early signs that address the pathology in the intestinal region, and may sometimes result in a suspicion of acute surgical condition, such as Hirschsprung disease in our Patient 2[6].

Biochemical abnormalities in CLD patients are similar to those in pseudo-Bartter syndrome apart from urinary chloride content, which is low in untreated CLD but high in all forms of Bartter syndrome. Thus, it is important to obtain a correct urinary specimen by adequate placing of urinary bags and by binding the non-absorbing side of the diaper at the same time to collect stool separately from urine. Our Patient 2 possibly presented with high "urinary" chloride because of wrongly collected urinary specimen, and was initially diagnosed with Bartter syndrome, delaying correct diagnosis of CLD for 11 years.

Table I. Clinical and Biochemical Characteristics of the Patients

	Case 1	Case 2	Case 3
Birth weight (g)/week of gestation	2500/37	2320/32	1760/32
Neonatal period	Exchange transfusion for hyperbilirubinemia	Phototherapy, respiratory distress syndrome, investigated for Hirschsprung disease	Exchange transfusion for hyperbilirubinemia, sepsis, NEC
Weight (kg) (percentile)	24 (25p)	57.9 (>95p)	19.9 (<5p)
Height (m) (percentile)	1.29 (50-75p)	1.53 (90-95p)	1.27 (<5p)
BMI (kg/m^2) (percentile)	14.42 (10-25p)	24.73 (>95p)	12.33 (<5p)
Serum Na/K/Cl (mEq/L) in infancy	ND	120/2.2/59 (6 months)	128/1.8/68 (8 months)
Current serum Na/K/Cl (mEq/L) (without specific treatment)	139/2.7/96	140/3.1/102	140/3/99
Stool Na/K/Cl (mEq/L)	84/38.8/162	68/23/144	101/28.4/169.5
Renal function at last visit (BUN/creatinine) (mg/dl)	26/0.6	14.3/0.56	20.4/0.47
Renin (5-27.8) (pg/ml)	17	ND	2.81
Aldosterone (29.4-161.5) (pg/ml)	62	ND	152
Mental retardation	No	Yes	Yes
Soiling	Yes	Yes	Yes

BMI: Body mass index. BUN: Blood urea nitrogen. ND: No data. NEC: Necrotizing enterocolitis.

Although approximately 30 different mutations in SLC26A3 have been identified to date, no phenotype-genotype correlation characteristic of SLC26A3 has been delineated[7]. It was suggested that compensatory mechanisms such as activation of the renin-aldosterone system or consumption of salty foods might explain at least partly the observed differences in the clinical presentation of patients[8]. Three different mutations were described among our set of patients. One had a known mutation, the Arabian founder mutation [c.559G>T (p.G187X)], whereas the other two (previously unpublished mutations) had a novel mutation c.1624_1626delTCTinsC and c.1945G>A (p.D652N), which was previously seen in two patients with CLD (Wedenoja, personal communication).

Noteworthy, all of our patients had delayed diagnosis and two had delayed development/mental retardation. As the mutational background of CLD in these patients was similar to previously known patients who had had normal development (Arabic founder mutation and 2 unreported patients), in addition to the fact that these patients did not carry syndromic features suggestive for a genetic etiology (other than CLD), the observed intellectual disability was most likely associated with recurrent episodes of dehydration and hyponatremia during an early age. In countries like Finland, where the disease is more frequent, early diagnosis and regular salt supplementation therapy have been shown to prevent in addition to imminent danger of death due to dehydration, also associated mental retardation[9]. Again, we have no explanation for the finding that the two patients in this series of three patients with delayed diagnoses of CLD appeared to have more severe clinical course associated with repeated hospital admissions and even developmental delay, whereas the third patient appeared to develop normally even without salt supplementation therapy.

In the long term, incidence of renal impairment is high, and may not be totally prevented by diligent salt and fluid substitution therapy[10]. Although undiagnosed and untreated for the first decade, our three patients had normal renal function, which may be related to their young age. No curative treatment for the diarrhea

has been found. In accordance with previous reports, cholestyramine was found to reduce the amount of diarrhea moderately but only temporarily[9]. Attempts using lansoprazole (1 mg/kg/day, n=2), omeprazole (1 mg/kg/day, n=1) and butyrate (100 mg/kg/day, n=1) were unhelpful[11].

Replacement therapy with peroral NaCl and KCl has been shown to ensure normal growth and development in children, and partially prevent complications, such as renal insufficiency. Over-substitution must be avoided because it may even increase diarrhea by an osmotic mechanism. Sufficient excretion of chloride in urine, normal acid-base and electrolyte balance are good indicators in the adequate amounts of salt substitution therapy.

In conclusion, all pediatricians should be aware of CLD as a potentially fatal or disabling disease if untreated. History of polyhydramnios, watery diarrhea, failure to thrive, poor growth, soiling, metabolic alkalosis and hypokalemia/hypochloremia should be an alarming set of findings for the diagnosis, which can be confirmed by fecal measurement of chloride content. It is worth remembering that fecal chloride may be lower in untreated CLD because of severe dehydration, so for suspected cases, re-testing should be available[12]. Salt substitution therapy started early in life prevents early complications, allows normal growth and development, and favors good long-term prognosis.

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