

Molecular Screening of Chronic Diarrhea: A 2-Year Single-Center Experience

Emine Ergül Sarı¹, Hasret Ayyıldız Civan^{2*}, Andreas Janecke³, Hasan Önal¹, Gokhan Baysoy⁴ and Dilek Güller⁵



¹Department of Pediatrics; Bakırköy Dr. Sadi Konuk Training and Research Hospital, Turkey

²Department of Pediatric Gastroenterology, Hepatology and Nutrition; Bakırköy Dr. Sadi Konuk Training and Research Hospital, Turkey

³Department of Pediatrics I& Division of Human Genetics, Medical University of Innsbruck, Austria

⁴Department of Pediatric Metabolism and Nutrition; Çam and Sakura City Hospital, Turkey

⁵Department of Pediatric Gastroenterology, Hepatology and Nutrition, Medipol University Mega Hospital, Turkey

*Corresponding author: Hasret Ayyıldız Civan, Bakırköy Dr. Sadi Konuk Eğitim ve Araştırma Hastanesi, İstanbul, Turkey

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ABSTRACT

Aim: Congenital diarrheal disorders (CDDs) represent a group of enteropathies caused by different pathomechanisms that typically have their onset in the neonatal period, and that carry significant morbidity and mortality rates. We aimed to evaluate the clinical, prognostic and molecular features of patients who presented with chronic diarrhea and were diagnosed with CDDs as a result of genetic analysis.

Methods: The study comprized family and nutritional history, detailed clinical history, and physical examination findings in patients who presented with intractable or episodic diarrhea. Routine laboratory tests, detailed stool tests, gastroscopy and colonoscopy were performed for all cases.

Results: As a result of genetic testing; 1 patient was diagnosed with DGAT-1 enzyme deficiency, 1 patient with microvillus inclusion disease (MVID), 1 patient with trichohepato-enteric (THE) syndrom type 2, 1 patient cystic fibrosis, 2 patients with enteric anendocrinosis (EA), 1 patient with congenital tufting enteropathy, 1 with patient Mednik syndrome and 1 patient was diagnosed with glucose-galactose malabsorption. Four of the cases died during the follow-up period.

Conclusions: In cases presenting with chronic diarrhea, awareness of the CDDs should be increased and molecular analysis should be included in the diagnostic algorithm after carefully taken detailed history and routine examination. Thus, it will contribute to the reduction of morbidity and mortality rates by enabling CDD-specific treatment.

Introduction

Diarrheal disorders are the second most common leading cause of death worldwide with an estimated 750,000 deaths annually in children under 5 years old. Diarrhea is defined as stool volume >10 g/kg/day in infants, and >200 g/kg /day in adolescents [1,2]. The terms “persistent” and “chronic” are often used interchangeably,

but according to the World Health Organization (WHO) persistent diarrhea persists for more than two weeks and chronic diarrhea usually exceeds 1 month (> 3 times / day watery) without acute onset [2]. In children chronic diarrhea can occur due to large number of disorders including infections, postinfection syndrome,

diet related factors (allergy or intolerance), inflammatory bowel disease, malabsorption, cystic fibrosis, immune deficiencies and congenital diarrheal disorders (CDDs) [3]. Moreover, numerous etiologies, overlapping symptoms, and similar clinical presentations make the diagnosis and management of the disease difficult. Therefore, detailed medical history and physical examination findings are crucial in the evaluation of cases [4]. CDDs are rare, mostly autosomal recessive inherited enteropathies that typically early onset with varying severity and prognostic features [5]. In the etiology of CDDs which are associated with high risk of mortality and morbidity; Over 30 CDD disorders and 35 responsible genes have been identified that cause defects in nutrients and electrolytes digestion, absorption, enterocyte structures, intestinal immune-related homeostasis and enteroendocrine cell development [6]. Thus, molecular based diagnosis is required and clearly plays a key role in the CDD diagnosis. Therefore, we aimed to evaluate the clinical, prognostic and molecular features of patients who were presented with chronic diarrhea and diagnosed with CDDs as a result of genetic analysis [7].

Materials and Methods

This study was performed with the Institutional Review Board protocol approval date 18/02/2019 and number 2019/13 in Istanbul Dr. Sadi Konuk Training and Research Hospital, Department of Pediatric Gastroenterology, Hepatology and Nutrition, between 01/06/2017 to 01/06/2019. A total number of 9 patients (5 girl - 4 boy), who were presented with dehydration, severe diarrhea, weight loss for more than 4 weeks and diagnosed with CDDs, evaluated retrospectively. During the application, detailed clinical history was taken for the primary diagnostic assessment in cases. The information including demographic data, disease onset time, nutritional history, consanguinity, family atopy, asthma, eczema history, death of another brother or sister, presence of similar disease in family, maternal history of polyhydramnios, history of

stool loose in the first 24 hours, mucus and blood content in gaita, stool volume and frequency, vomiting, and recurrent lung infection were questioned in interviews with the parents.

In the physical examination of the patients, abdominal distention, perianal hyperemia, rash, pretibial edema, severe gland dermatitis, skin dryness, signs of rickets, ADEK vitamin deficiency, and other syndromic findings were evaluated. Detailed biochemical analysis, complete blood count, ESR, and allergy panel were analysed in all cases. Where necessary, celiac antibodies, skin prick test and sweat test were examined. In addition to detailed morphological and microscopic assessment in stool, bacteriological evaluation was performed by gaita culture. Genetic examination was performed in patients who could not be diagnosed as a result of detailed clinical history, routine laboratory tests, gastroscopy, colonoscopy, and histopathological evaluation. Blood cell count analysis was performed on patients' venous blood samples. Haematological parameters were analyzed using a hematology analyzer (Cell-Dyne 3700, Abbott, Abbott Park, IL, USA). Biochemical analysis performed from serum samples by electrochemiluminescence immunoassay on Beckman Coulter Unicel DXI 800 analyzer. CDDs-related mutations were examined with the help of real-time Polymerase Chain Reaction (PCR) technique through genomic DNA isolated from the patients' peripheral blood.

Results

The mean age of 9 CDD-patients (5 female and 4 male) included in our study was 19 months (Ranged = 1-84). The oral intake was stopped in 6 patients who applied with watery diarrhea and followed up for 24 hours. Three of the patients were diagnosed with osmotic diarrhea that stopped with fasting, and 3 patients were diagnosed with secretory diarrhea which continued with fasting. In addition, 3 patients applied with fatty stool diarrhea and diagnosed with osmotic diarrhea due to the high steatocrit (Table 1).

Table 1: CFTR Mutations identified in patients.

No	Gender	Age Onset	Consanguinity	Stool	Histopathology	Mutation
1	Female	1 month	+	Secretory	Normal	MYO5B
2	Male	1 month	-	Secretory	Normal	EpCAM
3	Female	1 month	+	Osmotic	Normal	DGAT-1
4	Female	1 month	+	Osmotic	Normal	SLC51A
5	Female	1 month	+	Osmotic	Normal	Neurogenin-3
6	Male	1 month	+	Osmotic	Normal	Neurogenin-3
7	Male	1 month	+	Osmotic	Normal	CFTR
8	Female	1 month	+	Secretory	Normal	SKIV2L
9	Male	1 month	+	Secretory	Normal	AP1S1

Case 1

First patient was presented with secretory diarrhea. Family history revealed consanguineous parents and death of MVID-diagnosed brother. There was no oral intake and no response to formula feeding. Thus, parenteral nutrition (PN) was maintained. Genetic examination revealed a homozygous mutation in MYO5B gene and patient diagnosed with MVID. She died due to sepsis at the age of 5-months.

Case 2

Second patient was also presented with secretory diarrhea which continued with fasting and without response to formula feeding. He has a history of death brother due to the CDD-like disease but there was no consanguinity among the parents. The patient has poor anthropometric status. Additionally, histopathological findings in gastroscopy and colonoscopy were normal. Genetic analysis identified a homozygous mutation in the EPCAM gene and patient diagnosed with congenital tufting enteropathy. He continuous on TPN administration and oral table food, while awaiting bowel transplant.

Case 3

She was presented with peripheral edema and osmotic fatty diarrhea which stopped with fasting. Family history revealed consanguinity in parents. Gastroscopic and colonoscopic examination showed no abnormal finding. There was low plasma lipid level, lymphopenia, fecal alpha 1 antitrypsin, increased steatocrit and normal fecal elastase levels in routine examinations. Genetic analysis revealed a homozygous mutation in DGAT-1 gene and patient diagnosed with DGAT-1 enzyme deficiency. She still maintains her treatment with low-fat diet + high MCT and high protein table foods.

Case 4

A 7-years-old girl presented with distention and episodes of diarrhea which onset and maintained since the first days of her life. There was no response to regular formula regimens, which was started as a results of positive reducing substance in stool (Ph=4). Genetic analysis identified a homozygous SCL5A1 mutation, and she was diagnosed with glucose-galactose malabsorption. The patient is being followed up with administration of lactose-free milk, glucose-poor and fructose-based diet.

Case 5 and 6

Our 5th and 6th cases are brother and sister born to consanguineous parents. They were presented with watery diarrhea onset in early infancy which stopped with fasting. Gastroscopic and colonoscopic examination findings were normal. Molecular analysis revealed homozygous mutation in the Neurogenin-3 gene,

and they were both diagnosed with EA. Since both of the patients are TPN dependent, their hospitalization continues.

Case 7

Our 7th case was presented with osmotic fatty diarrhea which stopped with fasting. During postnatal 4th month he was under the birth weight. Family history revealed consanguinity in parents. In routine examinations steatocrit was positive and fecal elastase was < 100 mg/g. Molecular genetic analysis has identified homozygous mutations in the CFTR gene and he was diagnosed with Cystic fibrosis. Although PERT (pancreatic enzyme replacement therapy), ADEK vitamin supplementation, pulmozym, pulmonary rehabilitation support and hypercaloric formula regimens was started, he died due to sepsis.

Case 8

Our 8th case was presented with hair anomalies, facial dysmorphism, hepatomegaly, cholestasis and failure to thrive. She was also born to consanguineous parents. Gastroscopic and colonoscopic examination showed no abnormal finding. Genetic examination revealed a homozygous mutation in SKIV2L gene and patient diagnosed with THE syndrom type 2. She died due to sepsis.

Case 9

Our last case presented with secretory diarrhea, ichthyosis, and weight loss. Family history revealed consanguinity in parents. There was no response to variety of formula regimens. Genetic analysis identified AP1S1 mutation, and he was diagnosed with Mednik syndrome. He received TPN administration but died due to sepsis.

Discussion

CDDs are severe and rare group of life-threatening enteropathy with high mortality. Supportively Ye et al. screened 137 CDDs-children with an average symptom onset age of 28 days; they reported successful rate of molecular analyzes as 64.2% (88/137) and the overall mortality rate was 14.6%. In recent years, an increasing number of new genomes associated with CDDs have been identified. In addition, according to the classification suggested by Berni Canani published in 2010, CDDs are grouped in 4 categories considering the type of defect; the first group includes digestive-absorption defects and nutrient-electrolyte transport defects. Glucosegalactose malabsorption (SLC5A1 gene) and cystic fibrosis (CFTR gene) are defined in this group [8]. Glucose-galactose malabsorption is an extremely rare disorder in enterositis, which occurs with sodium-coupled transport defect of glucose and galactose. It is an autosomal recessive (OR) inherited disorder caused by mutation in SLC5A1, which is the Na⁺

/ glucose cotransporter gene. In a study, Anderson et al. revealed no abnormal findings by routine laboratory, imaging, endoscopy and histopathological evaluation in a patient at the age of 37-days who presented with weight loss, hyperbilirubinemia, metabolic acidosis and congenital diarrhea. However, in molecular analysis, a homozygous SLC5A1 mutation has reported [9]. Similarly, in our 4th case; a 7-years-old girl presented with episodes of diarrhea which onset in the first days of her life. Genetic analysis identified a homozygous SCL5A1 mutation, and she was diagnosed with glucose-galactose malabsorption. She is being followed up with administration of lactose-free milk, glucose-poor and fructose-based diet.

Disorders in CDD classification group 2 include differentiation and polarization defects of enterocytes; MVID (MYO5B gene), congenital tufting enteropathy (EPCAM gene) and syndromic diarrhea are defined in this group [8]. MVID is a rare OR inherited disease which can cause severe congenital diarrhea with high morbidity and mortality. Sadiq et al. reported a 17-day-old, female infant presented with severe diarrhea with unknown etiology, metabolic derangements and failure to thrive; despite detailed stool examination, abdominal usg, duodenal biopsy and flexible sigmoidoscopy examinations, researchers failed to diagnose. Even though researchers tried various treatment and formula regimens, none of them was effective to stop diarrhea. Finally, by applying molecular methods, homozygous MYO5B gene mutation was detected in the patient, and she was diagnosed with MVID. As the bowel transplantation is only treatment method reached consensus for MVID; it was reported that patient was maintained on TPN while awaiting bowel transplant [10]. Similarly in our first case presented with secretory diarrhea; she had history of consanguinity in parents and death of MVID-diagnosed brother. Genetic examination revealed a homozygous mutation in MYO5B gene and patient diagnosed with MVID. But she died due to sepsis at the age of 5-months. Similarly, congenital tufting enteropathy (CTE) formed by OR inherited EPCAM gene mutation; causes severe diarrhea in the infant as a result of villous and epithelial tufts changes [11]. Kahvecioğlu et al. reported no abnormal findings on routine evaluation in a patient presented with watery diarrhea (10 times a day) in postnatal 4th day. Researchers noted a history of death brother and consanguinity in parents. They started phosphorus supplementation on admission, but patient died due to cardiac arrest on the postnatal 35th day. The patient was diagnosed with CTE as a result of postmortem histopathological evaluations [12]. Consistently in our study, our second case, who had a history of death brother due to the CDD-like disease, was also presented with secretory diarrhea. Genetic analysis identified a homozygous mutation in the EPCAM gene, and he diagnosed with CTE. He continuous on TPN administration and oral table food, while awaiting bowel transplant.

Syndromic diarrhea or tricho-hepato-enteric (THE) syndrome is caused by mutations in the SKIV2L and TTC37 genes. Patients are presented with severe diarrhea in addition to the characteristic phenotype [13]. Vardi et al. reported a 4-month-old girl with congenital diarrhea, failure to thrive and history of consanguinity in parents; Researchers confirmed SKIV2L gene mutation as a result of molecular analysis [14]. In accordance with published data, our 8th case was presented with hair anomalies, facial dysmorphism, hepatomegaly, cholestasis and failure to thrive. She was also born to consanguineous parents. Genetic examination revealed a homozygous mutation in SKIV2L gene. She died due to sepsis. Disorders in CDD classification group 3 include differentiation and function defects of enteroendocrine cells; enteric anendocrinosis (EA) or congenital malabsorptive diarrhea (NEUROG3 gene) are defined in this group. In addition, the last CDD classification group includes dysregulation in intestinal immune responses [8] OR inherited Neurogenin-3 (NEUROG3) gene mutation results in EA characterized by severe malabsorptive diarrhea and intestinal enteroendocrine cell deficiency. German-diaz et al. reported Neurogenin-3 mutation in an 11-day-old boy presenting with severe dehydration, metabolic acidosis and severe watery diarrhea. At the end of the follow-up period, he was 16 years old and reported to continue TPN once a day alongside gluten free regular home diet [15]. Similarly, our 5th and 6th cases were presented with watery diarrhea onset in early infancy. Molecular analysis revealed homozygous mutation in the Neurogenin-3 gene, and they were both diagnosed with EA. Since both of the patients are TPN dependent, their hospitalization continues.

DGAT1 and DGAT2 (diacylglycerol acyltransferase) catalyze triglyceride biosynthesis and OR mutations causing DGAT1 functional loss result in severe diarrhea and protein-losing enteropathy [16]. Ratchford et al. identified a homozygous DGAT1 mutation in a 7-month-old girl presented with severe diarrhea. TPN was started and changed to a low-fat diet of table foods by 13 months of age. At the end of the study, researchers reported normal values of D vitamin, calcium, IgG, albumin and TG [17]. She was presented with peripheral edema and osmotic fatty diarrhea which stopped with fasting. Family history revealed consanguinity in parents. Gastroscopic and colonoscopic examination showed no abnormal finding. There was low plasma lipid level, lymphopenia, fecal alpha 1 antitrypsin, increased steatocrit and normal fecal elastase levels in routine examinations. Genetic analysis revealed a homozygous mutation in DGAT-1 gene and patient diagnosed with DGAT-1 enzyme deficiency. She still maintains her treatment with low-fat diet + high MCT and high protein table foods. Consistently, our 3th case was presented with osmotic fatty diarrhea which stopped with fasting. Genetic analysis revealed a homozygous mutation in DGAT-1 gene. She still maintains her treatment with low-fat diet + high MCT and high protein table foods.

MEDNIK syndrome is caused by OR inherited mutation in the AP1S1 gene [18]. Incek et al. reported a 10-year-old girl presented with diarrhea, ichthyosis, and sensorineural deafness. Her family history revealed consanguinity in parents and genetic analysis identified a homozygous mutation in AP1S1 gene. Researchers started zinc sulphate during follow-up period [19]. Similarly, in our last case presented with secretory diarrhea, ichthyosis, and weight loss. Family history revealed consanguinity in parents. Genetic analysis identified AP1S1 mutation. He received TPN administration but died due to sepsis.

Conclusion

In conclusion, although chronic diarrheal diseases may present overlapping symptoms, CDDs are closely associated with poor prognosis and high mortality. Therefore, in cases presenting with chronic diarrhea, awareness of the CDDs should be increased and molecular analysis should be included in the diagnostic algorithm after carefully taken detailed history and routine examination. Thus, it will contribute to the reduction of morbidity and mortality rates by enabling CDD-specific treatment.

Conflict of Interest

No conflict of interest with any institution/organization.

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Hasret Ayyıldız Civan. Biomed J Sci & Tech Res



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