Gastroenterology and Hepatology From Bed to Bench.
2023 **RIGLD**, Research Institute for Gastroenterology and Liver Diseases

CASE REPORT

Tufting enteropathy: a rare anatomical cause of small bowel diarrhoea in infants with mild or no villous abnormality

Prasenjit Das[1], Rohan Malik[2], Sanjeevani Kaul[2], Govind K Makharia[3]

[1]Departments of Pathology, All India Institute of Medical Sciences, New Delhi, India
[2]Pediatric Gastroenterology, All India Institute of Medical Sciences, New Delhi, India
[3]Gastroenterology & Human Nutritions, All India Institute of Medical Sciences, New Delhi, India

ABSTRACT

The causes of intractable diarrhoea in infancy are varied, and can be classified into enteropathic and non-enteropathic groups. Congenital tufting enteropathy (CTE) is a rare cause of enteropathic form of intractable diarrhoea in infants requiring nutritional supplementation. We herein report a case of CTE in a one-year-old female child who presented with recurrent abdominal distension, frequent watery diarrhoea and marked stunted growth soon after birth. A systematic clinical, laboratory and pathological evaluation brought out the etiology, followed by genotypic confirmation. Histological examination revealed mild villous abnormality with presence of epithelial tufts both in the villous and crypt surface, in the duodenum and rectal biopsies supported by complete loss of MOC31 staining. Deep sequencing revealed homozygous 3' splice mutation at intron 5 of the EPCAM gene (c.556-14A>G). She was given TPN support and discharged with weight gain under home-based parenteral nutrition supplement. This case brings out the need for a multidisciplinary team approach to reveal underlying the cause of infantile intractable diarrhoea and report a favorable outcome with nutritional supplementation.

Keywords: Tufting, Enteropathy, Congenital, Diarrhoea, Infant, *EPCAM.*

(Please cite as: **Das P, Malik R, Kaul S, Makharia GK. Tufting enteropathy: a rare anatomical cause of small bowel diarrhoea in infants with mild or no villous abnormality. Gastroenterol Hepatol Bed Bench 2023;16(2):225-229. https://doi.org/10.22037/ghfbb.v16i1.2731).**

Introduction

Intractable diarrhoea of infancy was first reported by Reifen et al to describe a chronic, severe, unexplained congenital diarrhoeal disorder (CDD) in infants and young children requiring nutritional support (1). This disorder presents in two groups of children, one with 'normal crypt-villous axis', and in other with 'altered crypt-villous axis'. Infective causes of diarrhoea and congenital defects in transport of sodium, chloride, bile acid, glucose, enterokinase deficiencies, and allergy to milk proteins/ supplements while are a few examples of the former type of infantile diarrhoea; the second group, also known as congenital diarrhoea and enteropathies (CODEs) (2), comprises of various anatomical mucosal defects as congenital tufting enteropathy (CTE), microvillous inclusion disease (MVID), and autoimmune enteropathy (AIE) (3, 4). All these enteropathies are commonly seen in children younger than 6 months age. On the other hand, celiac disease (CeD) is seen in post-weaning period and rarely is responsible for intractable diarrhoea, unless there is a clinical crisis. Over the last few decades our understanding regarding the causes of intractable diarrhoea in infants and young children has improved with the availability of genetic analyses. Today the anatomical pathologists play an important role in identifying the epithelial mucosal defects, narrow down differential diagnoses and guide further investigations for confirmation (3). In this article, we are reporting a case of CTE presenting as protracted diarrhoea in an infant where the histopathological examination followed by genetic analyses unfolded the diagnoses,

Received: 11 January 2023 *Accepted*: 01 March 2023
Reprint or Correspondence: **Prasenjit Das,** *Department of Pathology, Gastrointestinal & Hepatopancreatobiliary Pathology Section, All India Institute of Medical Sciences, New Delhi -110029, India.*
E-mail: prasenaiims@gmail.com
ORCID ID: 0000-0002-2420-8573

minutely described management undertaken and reviewed the literature.

Case report

A one-year-old female child, first full-term born child out of a third degree consanguineous married couple, presented with intermittent, self-resolving abdominal distension since one month of her age, followed by episodes of loose stool since two and half months of age. For the intermittent abdominal distention she was first admitted at 35 days of life, and again at 3 months of age along with loose stools during the later occasions which were managed conservatively. Thereafter there were multiple episodes of hospital admissions due to loose stools, often for 5 to 10 episodes per day, watery in consistency, not blood stained, non-bilious and non-foul smelling. There was no history of vomiting, constipation, history of recurrent infection or neonatal sepsis, excessive thirst, lethargy, or any episode of fever. She had poor weight gain since 2 months of age, with no neck control. On examination, at one-year of age, her vitals were normal, she was alert and active with no neck rigidity, irritable but consolable and severely malnourished (-7.9 SDS for age), with microcephaly (-5.4 SDS for age), and severe stunting (-6.19 SDS for age). There were loose skin folds, sparse scalp hair, and hollow cheekbones. No pallor, icterus, cyanosis or clubbing were identified. Abdomen was distended but soft and non-tender, with non-palpable liver and spleen and centrally located inverted umbilicus and normal bowel sounds. On admission her hemoglobin was 10.2 gm%, with normal serum albumin and liver function tests and procalcitonin level (0.05 ng/ml). Blood culture and urine cultures were sterile and C-reactive protein level was 0.5 mg/L. Thyroid function tests were also within normal limits. No similar illness was reported in family members including her two later siblings, with no histology of sibling death.

Based on overall features, clinical possibilities kept were carbohydrate malnutrition, pancreatic insufficiency, immunodeficiency disorders and possible anatomical mucosal defect. As there was no worsening of symptoms on adding feeds, possibility of carbohydrate malabsorption was considered less likely. Possibility of human immunodeficiency virus infection

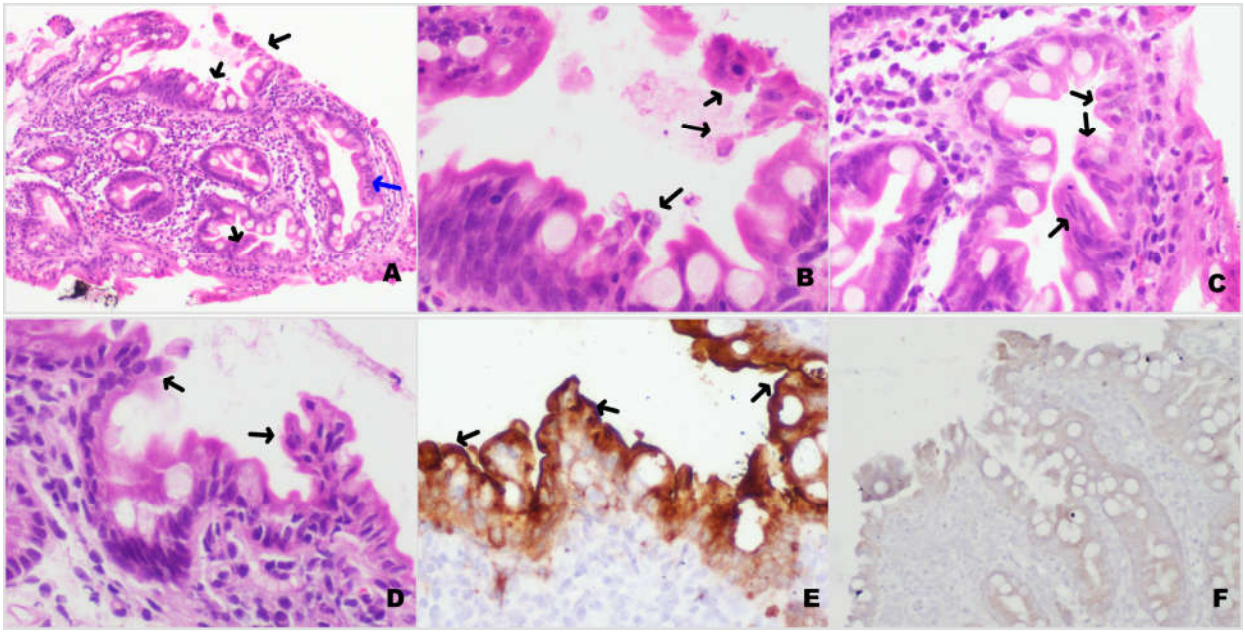

Figure 1. Duodenal biopsy shows irregularly dilated duodenal crypts (blue arrow) with multiple epithelial tufts (black arrows) present both on the villous and crypt surface epithelium. Moderate mononuclear cell infiltrate was noted in lamina propria [A x 100]. High power images of the villous mucosal surface [B x 400] and crypt mucosal surface [C x 400] show multiple tear-drop shaped epithelial tufts (arrows). Rectal biopsy also showed surface mucosal epithelial tufts (arrows) in the index patient [D x 200]. CD10 stain revealed normally present apical epithelial layer of microvilli in duodenum (arrows) [E x 200]. MOC31/EpCAM stain was completely negative in the duodenal mucosal epithelium [F x 200].

was ruled out both in the child and mother. Immunoglobulin levels were within normal limits. Fecal elastase level was normal (163 microgram/ gm), making pancreatic insufficiency a less likely consideration. Barium enema was normal with no sign of mechanical obstruction. Upper gastrointestinal tract endoscopic biopsy (UGIE) as well as rectal biopsies were done. Duodenal biopsy showed a crypt: villous ratio of 1:2, however, intra-epithelial lymphocytes (IELs) were not increased. Moderate mononuclear cell infiltrate was noted in lamina propria (Figure A). Luminal parasites or mucosal eosinophilia were not seen. However, there were prominent enterocyte tufting formed by small tear-drop like epithelial cell clusters, noted on villous mucosal surface covering up to 40-45% of the surface epithelial area (Figures A-C). The mucosal microvillous layer was present on apical surface both in the villi and crypts. Rectal biopsy also revealed surface epithelial tufting covering up to 40% of mucosal surface area (Figure D). Crypts also showed the epithelial tufting with focally irregular dilated crypt lumen (Figure C). Features of microscopic colitis were not seen. CD10 immunohistochemical stain (Clone 56c6, 1:100, BioCare, CA, USA) showed a normal microvillous layer on villi and crypt surfaces (Figure E). EpCAM /MOC31 (1:200, BioSB, CA, USA) stain was completely lost in the epithelial layer (Figure F). Based on overall features, possibility of a histological diagnosis of CTE was suggested. Thereafter blood sample was sent for clinical targeted exome sequencing using an Illumina platform, which showed homozygous 3' splice variation at intron 5 of the EPCAM gene (c.556-14A>G), confirming our diagnosis.

The parents were explained about the limited treatment options and possible outcome in the index case, and child was started on management for severe acute malnutrition, vitamin, MgSO4, folic acid, antibiotics supplements were added with fluid replacement were made for loose stools. Routine hemogram, biochemical, electrolyte and blood sugar monitoring were started with daily weight monitoring. As on oral supplementation she did not attain weight gain, parenteral nutrition (PN) was started using amino acids, dextrose, and lipid supplements. However, she developed deep vein thrombosis on day 20 in the right external iliac vein, hence, a peripherally inserted central catheter (PICC) line was placed and enteral nutrition elemental formula was started. The child was discharged with a weight of 3.3 kg and on formula feeds. The parents were explained regarding long term need of home-based PN, and the child was discharged with naso-gastric tube and the Hickman catheters in situ and the parents were taught about line care; however, after 2-months at home the child succumbed to sepsis. The parents were advised regarding segregation analysis and to follow up under genetics in the subsequent pregnancy.

Discussion

Herein, we reported a case of CTE in a one-year-old female child who presented with recurrent abdominal distension, pain abdomen, watery loose stools and growth retardation soon after birth and symptoms persisted even in resting bowel condition. A detailed clinical and biochemical workup followed by histopathological examination of the UGIE duodenal and sigmoidoscopic rectal biopsies revealed the diagnostic clue of CTE, and ruled out other mimickers. A targeted deep sequencing an EPCAM gene revealed homozygous splice mutation on intron 5 (c.556-14A>G) confirming the diagnosis.

CTE, also referred as intestinal epithelial dysplasia (IED), is an autosomal recessive disorder presenting since first few weeks of life (median age of presentation 2-4 weeks). However, rare late presentation in adolescence have been described (5). Though CTE is very rare, it is more common in families with consanguineous marriages, like the index case. Hence, the incidence of CTE has been registered more in Middle-East, Turkey and North Africa (3). In a large study on 137 cases of CODEs from China, only two cases (1.5%) of CTE were identified (2). Extraintestinal manifestations as choanal atresia, esophageal atresia, punctuated keratosis (in more than 60% cases), dysmorphic facial features and chronic arthritis can be identified in syndromic CTE (3). Reported cases showed no or mild villous abnormality in duodenal biopsy, with crypt hyperplasia, focally cystically dilated crypts, usually no increase in IELs, and variable density of lamina propria inflammation, like the index case. However, epithelial tufts are characteristic. The epithelial tufts appear as closely packed enterocytes with overlapping of nuclei and cell border with rounding of the cell cytoplasmic borders,

and often have been compared with 'tear-drop' appearance (3). Epithelial tufts in duodenal biopsies can present variably and has been reported to affect up to 70% of villous surface (3). A duodenal mucosal biopsy is valuable to rule out features of infective pathology, MVID, AIE and CeD in the post-weaning phase. A periodic-acid Schiff stain (PAS) or CD10 immunostain is helpful to visualize the apically expressed layer of microvilli and ruling out a MVID. Absence of prominent villous flattening, diffuse loss of goblet cells, increased IELs, amphophilic epithelial cell cytoplasm, increased apoptotic activity and loss of crypt neuroendocrine cells are helpful features to rule out a possible AIE in such a case. Epithelial tufts are characteristic of CTE and can be identified in small intestine, colon, rectum or even in stomach (2). Duodenal biopsies from older children with CeD can mimic the changes of CTE, however, age, history of disease onset, type of diarrhoea, associated dysmorphic facial features and interstitial keratitis and negative serum anti-tissue transglutaminase (anti-tTG) can rule the former. On electron microscopy examination of intestinal biopsies in CTE disarray of microvilli with increased number of desmosomes (>3 per paracellular junction) have been reported (6, 7). A systematic step-wise approach is hence needed to reach to a definite diagnosis in such a case (6). However, in some cases laboratory work up may fail to establish a diagnosis and high index of clinical suspicion is the key in suspected infants, and repeat biopsies may be needed. Utility of MOC31/ EpCAM immune stain has been emphasized in studies with sensitivity and specificity of 100% for histological confirmation of CTE, in an appropriate clinical and histological context, like the index case (8).

Over the years the molecular pathogenesis of CTE evolved from reports of familial inheritance in members of Maltese ancestry (2, 7, 9), to reports on altered distribution of alpha2/beta1 integrin, epithelial basement membrane matrix proteins and paraseptal abnormalities as reduced and lamellated laminin layer, overexpression of heparan sulfate and increased expression of desmoglein, and abnormality of desmosomes (10). Histologically comparable changes in Elf3-deficient mice also has been demonstrated due to deficiency of transforming growth factor beta type 2 (11). Off late, CODEs as a whole were found to have many monogenic disorders involving IL10, IL10RA, IL10RB, CYBB, CYBA, NCF1, NCF2, NCF4, FOXP3, XIAP7, EPCAM and TTC7A genes (2). Among these, homozygous, compound heterozygous, and rarely heterozygous noncoding/ splicing mutations, missense or nonsense or frameshift mutations of the EPCAM gene, encoding for human epithelial adhesion molecule have been most commonly reported in patients with CTE (12). The EPCAM gene is mapped in chromosome 2p21, encoding a 40 kDa transmembrane glycoprotein consisting of an extracellular domain (encoded by exon 1–6), a single transmembrane domain (encoded by exon 7), and an intracellular domain (encoded by exon 8–9) (12). Most pathogenic variants were identified in exons 1, 2, 3, 5, 6, or introns 4, 5, 6 in CTE. Deletion of exons 1-7 of EPCAM gene were also rarely been reported (12). In the index case homozygous splice-mutation of intron 5 of EPCAM gene was detected. All these genetic changes possibly arrests the enterocyte apoptosis, and deplete the underlying sub-epithelial basement membrane, leading to lack of senescence and formation of tufts (13, 14). Off late, ex-vivo enteroid model has been developed for CTE based on EPCAM gene mutations, further establishing the major role of EPCAM gene in mucosal epithelial homeostasis (14). In a few syndromic CTE cases SPINT2 gene mutation was also demonstrated. SPINT2 is normally present in intestinal mucosa, and SPINT2-/- also results in a CTE-like phenotype (15). SPINT2 mutation mediated indirect depletion of EpCAM protein has also been described (15).

Studies were also undertaken to find a genotypic and phenotypic correlation in CTE patients. Patients with frameshift mutations were found to have more severe disease, requiring TPN and have the highest fatality. While, patients with splice mutation require partial TPN (3-6 times a week), and clinical outcome cannot be reliably predicted in patients with nonsense or missense mutations (13). Hence, pathological analyses of intestinal biopsies not only helps in identifying the anatomic changes in epithelium, genotypic changes can give important prognostic information and guide management.

As described in the index patient, symptomatic improvements were noted with TPN. In a follow-up study on 13 patients of CTE getting home-based TPN, the overall survival demonstrated was 92% (12). In

patients with splice mutation of EPCAM gene long-term or partial TPN may help to alleviate symptoms. Patients often continue to have the diarrhoea with continuous enteral feeding comprising of protein hydrolysate or amino acid based formulas. However, in many patients long term TPN is tolerated and home-based PN is crucial (17). However, home-based care is a problem like the index case, who later died because of sepsis. Some patients may become dependent on TPN, develop intestinal failure and sometime associated end-stage liver cirrhosis (14). As the timing of intestinal transplant is crucial, in patients with severe disease with frameshift mutation of the EPCAM gene, intestinal transplantation can be considered, along with other associated complications as progressive liver disease, loss of vascular access and recurrent sepsis (14, 17). As a clear association with consanguinity has been demonstrated, genetic counseling and antenatal diagnosis in high-risk couples is important.

Conclusion

CTE is a rare anatomical mucosal cause of chronic protracted diarrhoea in infants having poor outcome. However, a multidisciplinary approach, timely clinical suspicion, diligent clinical work up, experienced pathological examination of mucosal biopsies can help to narrow down the differential diagnoses and guide further line of work up. Genotyping in patients with CTE not only helps to confirm the diagnosis, also may predict the clinical outcome and the appropriate treatment needed. Pathologists must pay attention to patients' age and presenting symptoms and perform diligent microscopic examination and work up to rule out causes of congenital diarrhoea with enteropathies.

Conflict of interests

The authors declare that they have no conflict of interest.

References

1. Reifen RM, Cutz E, Griffiths AM, Ngan BY, Sherman PM. Tufting enteropathy: a newly recognized clinicopathological entity associated with refractory diarrhea in infants. J Pediatr Gastroenterol Nutr 1994;18:379-385.

2. Ye Z, Huang Y, Zheng C, Wang Y, Lu J, Wang H, et al. Clinical and genetic spectrum of children with congenital diarrhea and enteropathy in China. Genet Med 2019;21:2224-2230.

3. Sherman PM, Mitchell DJ, Cutz E. Neonatal enteropathies: defining the causes of protracted diarrhea of infancy. J Pediatr Gastroenterol Nutr 2004;38:16-26.

4. Catassi C, Fabiani E, Spagnuolo MI, Barera G, Guarino A. Severe and protracted diarrhea: results of the 3-year SIGEP multicenter survey. J Pediatr Gastroenterol Nutr 1999;29:63-68.

5. Haas K, Martin B, Martín M, Kerner J. Intractable diarrhea in two brothers: late diagnosis of tufting enteropathy in adolescence. Dig Dis Sci 2016;61:381-383.

6. Mantoo MR, Malik R, Das P, Yadav R, Nakra T, Chouhan P. Congenital diarrhea and enteropathies in infants: approach to diagnosis. Indian J Pediatr 2021;88:1135-1138.

7. Patey NA, Scoazec JY, Cuenod-Jabri BA, Canioni DA, Kedinger M, Goulet O, et al. Distribution of cell adhesion molecules in infants with intestinal epithelial dysplasia (tufting enteropathy). Gastroenterology 1997;113:833-843.

8. Ranganathan S, Schmitt LA, Sindhi R. Tufting enteropathy revisited: the utility of MOC31 (EpCAM) immunohistochemistry in diagnosis. Am J Surg Pathol 2014;38:265-272.

9. Reifen RM, Cutz E, Griffiths AM, Ngan BY, Sherman PM. Tufting enteropathy: a newly recognized clinicopathological entity associated with refractory diarrhea in infants. J Pediatr Gastroenterol Nutr 1994;18:379-385.

10. Lachaux A, Bouvier R, Loras-Duclaux I, Chappuis JP, Meneguzzi G, Ortonne JP. Isolated deficient α6β4 integrin expression in the gut associated with intractable diarrhea. J Pediatr Gastroenterol Nutr 1999;29:395-401.

11. Ng AY, Waring P, Ristevski S, Wang C, Wilson T, Pritchard M, et al. Inactivation of the transcription factor Elf3 in mice results in dysmorphogenesis and altered differentiation of intestinal epithelium. Gastroenterology 2002;122:1455-1466.

12. Güvenoğlu M, Şimşek-Kiper PÖ, Koşukcu C, Taskiran EZ, Saltık-Temizel İN, Gucer S, et al. Homozygous missense epithelial cell adhesion molecule variant in a patient with congenital tufting enteropathy and literature review. Pediatr Gastroenterol Hepatol Nutr 2022;25:441-452.

13. Pathak SJ, Mueller JL, Okamoto K, Das B, Hertecant J, Greenhalgh L, et al. EPCAM mutation update: variants associated with congenital tufting enteropathy and lynch syndrome. Hum Mutat 2019;40:142-161.

14. Goulet O, Salomon J, Ruemmele F, de Serres NP, Brousse N. Intestinal epithelial dysplasia (tufting enteropathy). Orphanet J Rare Dis 2007;2:1-6.

15. Das B, Okamoto K, Rabalais J, Kozan PA, Marchelletta RR, McGeough MD, et al. Enteroids expressing a disease-associated mutant of EpCAM are a model for congenital tufting enteropathy. Am J Physiol Gastrointest 2019;317:580-591.

16. Cai C, Chen Y, Chen X, Ji F. Tufting enteropathy: a review of clinical and histological presentation, etiology, management, and outcome. Gastroenterol Res Pract 2020;2020.

17. Ashworth I, Wilson A, Aquilina S, Parascandalo R, Mercieca V, Gerada J, et al. Reversal of intestinal failure in children with tufting enteropathy supported with parenteral nutrition at home. J Pediatr Gastroenterol Nutr 2018;66:967-971.