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Protracted Diarrhea: Results of the Five-year Survey in a Tertiary Hospital in Korea

The syndrome of protracted diarrhea (PD) includes several diseases with diverse etiologies. This study was conducted to characterize the spectrum of causes, clinical manifestations, and the outcomes of PD. A retrospective analysis of the clinical and pathological findings was performed on 25 patients with diarrhea starting within the first 2 yr of life and a requirement of parenteral nutrition (PN). According to the intestinal histopathology, patients were classified into four groups: immune enteropathy (12 cases), lymphangiectasia (6 cases), epithelial dysplasia (5 cases), and unclassified (2 cases). All patients with epithelial dysplasia had earlier onset of diarrhea and longer duration of PN than those in the other groups. Three patients (12%) had an evidence of a familial condition. Five patients (three with microvillous inclusion disease and two with immune enteropathy) died. Sixteen patients recovered, and three (two with primary lymphangiectasia and one with microvillous inclusion disease) still had diarrhea. One patient underwent intestinal transplantation for tufting enteropathy. In conclusion, infants with PD should be referred to specialized centers where advanced diagnostic and therapeutic facilities are available, because histological analysis is critical for the diagnosis of PD, and PN or intestinal transplantation is the only therapeutic option in a subset of cases.

Key Words : *Diarrhea; Autoimmune Enteropathy; Microvilli; Epithelial Dysplasia; Lymphangiectasis; Intestine, Small; Mucosal Histology*

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INTRODUCTION

"Intractable diarrhea of infancy" was first described by Avery et al. (1) as a non-infectious diarrhea lasting for more than 2 weeks, with onset before 3 months of age, with consequent malabsorption and failure to thrive. The definition and outcome of intractable diarrhea have changed considerably during the last two decades because of a better understanding of the pathology of the small bowel mucosa and major improvements in nutritional management. The term "intractable diarrhea of infancy" embraces a heterogeneous syndrome with a diverse etiology. By "protracted diarrhea (PD) in early childhood," we allude to children whose diarrhea start within the first 2 yr of life, becomes life threatening, and leads to dependence on parenteral nutrition (PN) for at least two weeks (2). Several chronic enteropathies that may be responsible for intractable diarrhea have been described. These include congenital microvillous atrophy or microvillous inclusion disease (3), tufting enteropathy (4), and other ultrastructural abnormalities of the enterocyte (5), and autoimmune enteropathy (6). Despite recent advances, PD still represents a daunting problem for the pediatric gastroenterologist. It is also becoming a major problem because of its high impact on health care resources.

An attempt to classify intractable diarrhea according to villous atrophy made on the basis of immunohistological and clinicopathologic criteria (7-9). In this study we made a modified application of these classifications and characterized the spectrum of causes and clinical manifestations of this diarrheal syndrome in the patients admitted to a tertiary hospital in Korea.

MATERIALS AND METHODS

Patients

Thirty-six cases recruited in this study included all patients who had been admitted to the Department of Pediatrics, Samsung Medical Center during a 5-yr period (March 1, 1996 through Feb 28, 2001). Of these 36 cases, 25 fulfilled the following criteria; age less than 2-yr at the onset of diarrhea, diarrhea lasting more than 2 weeks, unresponsive to hypoallergenic milk, and need for PN to provide at least 50% of the daily energy intake for a minimum of 2 weeks' duration. Children with anatomic abnormalities of

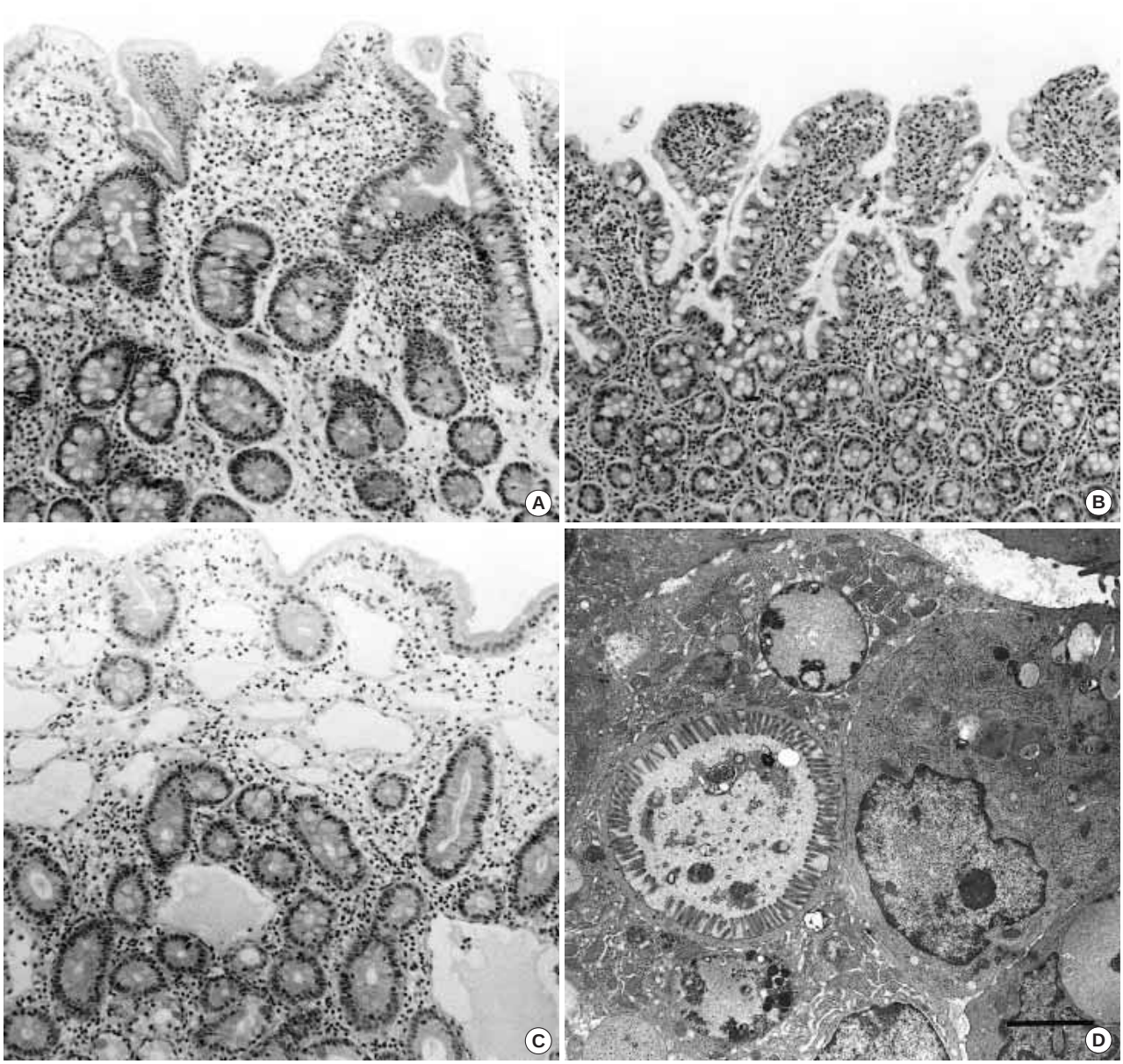

Fig. 1. (A) The histology of immune enteropathy shows moderate villous atrophy with crypt hyperplasia and increased cellularity of the lamina propria and an increased number of intraepithelial lymphocytes and eosinophils (H&E, ×100). (B) The histology of epithelial dysplasia shows normal intestinal villi with abnormal epithelium showing focal disorganization and crowding of surface enterocytes forming tufts (H&E, ×100). (C) The histology of lymphangiectasia shows dilated lymphatics in the intestinal mucosa (H&E, ×100). (D) Electron micrograph of microvillous inclusion disease shows the surface epithelium containing a membrane bound inclusion lined by microvilli (×2,500, Bar represents 4 μm).

the gut, congenital or acquired immunodeficiency syndromes, or congenital carbohydrate malabsorption (e.g., glucose-galactose malabsorption) were excluded from the study.

Methods

The clinical data were collected in each case and included family history, time of symptom onset (Fig. 1), treatment, and outcome. The results of the following investigations were obtained in each case: routine blood tests, fecal-reducing substances, occult blood, α1-antitrypsin stool excretion, stool cultures (for Salmonella, Shigella, *Campylobacter jejuni*, and *Yersinia enterocolitica*), and virus analysis (Rotavirus).

All patients were given duodenoscopy for a small intestinal biopsy for histology and electron microscopy. One to seven biopsies were performed in each patient. Light and electron microscopic analysis included the degree of villous atrophy (mild, moderate, or severe); crypt appearance (hyper-

plasia or normal); epithelial cell appearance (an epithelial cell tuft was defined as a focal crowding and disorganization of surface enterocytes); the mononuclear cellularity of the lamina propria; the density of intraepithelial lymphocytes; an increased number of eosinophils either in the epithelium or in the lamina propria; and appearance of microvilli and the presence of microvillous inclusions. According to the histologic findings, cases were grouped as follows, with a modification of the methods used in the previous studies (7-9); immune enteropathy group, epithelial dysplasia group, lymphangiectasia group, and unclassified group. Immune enteropathy was defined as moderate to severe villous atrophy with significant crypt hyperplasia (8, 9) and an increased number of eosinophils either in the epithelium or in the lamina propria. Epithelial dysplasia was defined as normal to moderate villous atrophy with dysplastic epithelium such as congenital microvillous atrophy, dystrophy, microvillous inclusion, or tufting enteropathy (4, 5, 10, 11). Lymphangiectasia was defined as lymphangiectatic intestinal histologic findings accompanied with protein losing enteropathy without any proven causes (Fig. 1).

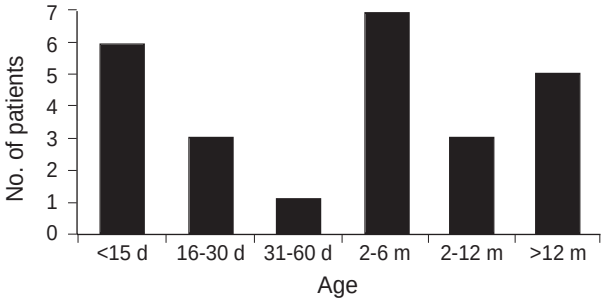

Fig. 2. Age of onset of protracted diarrhea in 25 patients.

RESULTS

The clinical features and outcomes of 25 children enrolled in the study are shown in Table 1. There were 12 boys (48 %) and 13 girls (52%) whose mean age of onset was 7.2± 7.7 months (range: 1 day-2 yr). The distribution of diarrhea onset age is shown in Fig. 2. Twelve cases belonged to the immune enteropathy group and six patients were classified into the lymphangiectasia group. The epithelial dysplasia group included five patients: four patients with microvillous inclusion disease and one with tufting enteropathy. Two cases remained unclassified and showed normal to mild villous atrophy without significant dysplastic epithelium.

Diarrhea started between 1 day and 24 months of age (median=89 days). All five cases included in the epithelial

Table 1. Clinical features and outcomes observed in 25 patients with protracted diarrhea

Patients	Age (day)	Sex	Group	Diagnosis	TPN duration (day)	Villous atrophy (grade)	Outcome
1	1	F	ED	MVID	28	1	expired
2	1	M	ED	MVID	33	1	expired
3	4	M	ED	MVID	152	2	persistent diarrhea
4	1	F	ED	MVID	61	2	expired
5	4	F	ED	Tufting enteropathy	360	1	IT
6	22	F	IE		15	2	recovered
7	120	F	IE		17	2	recovered
8	360	F	IE		16	2	recovered
9	457	F	IE		15	2	recovered
10	358	M	IE		18	2	recovered
11	60	F	IE		30	2	recovered
12	62	F	IE		34	2	recovered
13	90	M	IE		27	3	recovered
14	25	M	IE		124	3	recovered
15	89	F	IE		118	3	expired
16	65	M	IE		148	2	recovered
17	85	M	IE		150	3	expired
18	295	F	LE	Lymphangiectasia	28	0	recovered
19	700	M	LE	Lymphangiectasia	27	0	recovered
20	400	F	LE	Lymphangiectasia	63	0	recovered
21	720	M	LE	Lymphangiectasia	88	0	recovered
22	21	M	LE	Lymphangiectasia	152	0	persistent diarrhea
23	725	F	LE	Lymphangiectasia	151	0	persistent diarrhea
24	7	M	UC		90	0	recovered
25	180	M	UC		149	1	recovered

TPN: total parenteral nutrition, ED: epithelial dysplasia group, IE: immune enteropathy group, LE: lymphangiectasia group, UC: unclassified, MVID: microvillous inclusion disease, IT: intestinal transplantation.

dysplasia group showed diarrhea starting during the first week of life. However, the median age of diarrhea onset was 87 days (between 25 and 457 days) in the immune enteropathy group and 550 days (between 25 and 725 days) in the lymphangiectasia group. Eighty percent of patients showed an onset of diarrhea before one year of age. The onset time of unclassified two cases were 7 days and 180 days each. The median duration of PN in the epithelial dysplasia group was 61 days (between 28 and 360 days). Except two patients who were still alive at the end of this study, the reasons for the discontinuation of PN were deaths. The median durations of PN in the immune enteropathy group and lymphangiectasia group were 29 days (between 15 and 150 days) and 63 days (between 27 and 152 days), respectively. The unclassified two patients received PN for 90 days and 149 days each.

Information concerning siblings was provided in all families; in three families (two families with microvillous inclusion disease and one family with immune enteropathy), there was a sibling died of intractable diarrhea within the first months of his/her life. Therefore, evidence of familial condition was present in 3 (12%) of 25 families.

Treatments included: Total PN (100%), cholestyramine (76%), and steroids (40%), alone or in combination. Twenty patients survived. Among them, 16 (80%) recovered and 3 (15%) still had diarrhea at the end of this study. These three patients consisted of two patients with primary lymphangiectasia and one patient with microvillous inclusion disease. One baby underwent intestinal transplantation for tufting enteropathy after one year of total PN treatment. The remaining five patients who died were three patients with microvillous inclusion disease and two patients with immune enteropathy.

DISCUSSION

The spectrum of PD has changed greatly over the past few decades (8, 12-15). The increased number of some enteropathies, such as microvillous inclusion disease and tufting enteropathy are certainly related to the wider application of sophisticated diagnostic investigations, such as transmission electron microscopic study of the small bowel biopsy specimen and identification of ultrastructural abnormalities of intestinal epithelial cells. An attempt to classify intractable diarrhea according to villous atrophy was made on the basis of immunohistochemical and clinicopathologic criteria (7-9). In a multicenter European study (8), the PD was divided into two groups (group I and group II), where group I represented the prominent mucosal atrophy and group II showed minimal mucosal atrophy with dysplasia. In the present study, we referred to group I as 'immune enteropathy group' because it has been suggested that the specific histological findings (in terms of villous atrophy, crypt hyperplasia, and cellular infiltration of the lamina propria) be associated with a T-cell activation mechanism (8, 16-20). Group II was referred to as 'epithelial dysplasia group' by adding microvillous inclusion disease, microvillous dysplasia, and microvillous atrophy to this group, because this represented histological dysplasia and familial conditions seen in group II in European study (3, 15, 21-24). In a recent study, 29% of children affected by intractable diarrhea with persistent villous atrophy (immune enteropathy group) fulfilled the criteria of autoimmune enteropathy (8). In 1982, Walker-Smith et al. (6) first described circulating autoantibodies against the gut epithelium in an 18-month-old boy with chronic diarrhea, failure to thrive, and small intestinal enteropathy. Since then, several additional cases of autoimmune enteropathy have been reported (25-28). Although we could not finely make the diagnosis of autoimmune enteropathy because of the lack of diagnostic modalities including gut epithelial cell autoantibodies (6, 25, 28-32), at least some of the patients in this study might have had autoimmune enteropathy because all cases in the epithelial dysplasia group were unresponsive to elemental milk, dependent on PN, and had considerable villous atrophy. Epithelial dysplasia group included patients with severe intractable diarrhea with normal to minimal villous atrophy with epithelial dysplasia. These cases presented during the neonatal period (median onset 5 days) with severe watery diarrhea. A considerable proportion (2 of 5 families) of the patients had affected siblings who had died within the first month of life with severe diarrhea of unknown origin. Abnormalities were localized mainly in the epithelium and showed a similar appearance with those described as "tufting enteropathy" by Reifen et al. (4). In some infants with tufting enteropathy, abnormal laminin and heparin sulfate proteoglycan deposition on basement membrane has been reported, i.e., faint and irregular laminin deposition at the epithelial-lamina propria interface, while heparin sulfate proteoglycan appeared widened and lamellar (5). Basement membrane molecules are involved in epithelial-mesenchymal cell interactions, which are instrumental in intestinal development and differentiation (33, 34). The patient with tufting enteropathy reported here is the first case in Korea. Finally the patient had to undergo intestinal transplantation after treatment with PN for over one year.

Microvillous atrophy/inclusion disease can be recognized by performing periodic acid-Schiff stain and electron microscopy (3, 10). Four cases of microvillous inclusion disease included in the epithelial dysplasia group have already been published (35, 36). Microvillus inclusion disease is different from other diseases causing congenital diarrhea in which the ultrastructure of the enterocyte is normal (3, 15, 21-24). Currently, there is no curative therapy available, and afflicted patients require lifelong total PN or intestinal transplantation (37-39). In this study two patients who had protracted diarrhea could not be classified because they showed normal to minimal villous atrophy without prominent dysplasia. The clinical and histological findings of these two patients

were almost similar with those seen in five patients reported in the European multicenter study (8). We speculate that some unknown genetic ultrastructural or biohistological abnormalities may induce PD in this unclassified group of patients.

Protracted diarrhea is reported to be a syndrome with a poor prognosis, with a mortality rate ranging from 5% to 47% (1, 2, 8, 12, 13). This was also true in our study, in which 5 (20%) of 25 patients with PD died during the follow-up. The poorest prognosis was related to congenital ultrastructural abnormalities of the enterocytes that were found in three out of five patients who died. For these cases the only possible treatment at present is long-term PN or small bowel transplantation. The patient with tufting enteropathy included in the epithelial dysplasia group in this study underwent intestinal transplantation.

In summary, the patients in this study presented as clearly two different histological groups: one group is assumed to be related to immune mechanism and the other to some kinds of epithelial anomalies. Interestingly, the less prominent the villous atrophy is the more possible the epithelial anomaly is like as tufting enteropahty or microvillous inclusion disease. Therefore, if an infant with PD shows minimal villous changes in duodenal biopsies, an in-depth histological review and electron microscopy are mandatory.

This study showed that PD is a rare but daunting problem in Korea. Advanced diagnostic facilities including histological analysis are important for the diagnosis of the underlying diseases. For this reason, we believe that infants with PD should be promptly referred to specialized centers where an in-depth diagnostic and therapeutic evaluations are possible.

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