Microvillus Inclusion Disease in Two Korean Infants

We report two cases of microvillus inclusion disease and these are the first cases in Korea. The two babies (one baby had a sibling who died of diarrhea in the neonatal period) had excreted their stools up to 200 ml/kg per day since several days after birth. Workup's included extensive infectious, immunologic, hormonal and rheumatologic studies, all of which were negative or normal. Diagnosis rested on the ultrastructural finding of intracytoplasmic inclusions that contained intact microvilli on electron microscopy. We tried somatostatin analogue (octreotide, 4 μg/kg/day), cholestyramine (up to 4g t.i.d.), steroid (prednisone, 2 mg/kg/day) and intravenous epidermal growth factor (100 ng/kg/hr for 2 weeks), but there was mild improvement with cholestyramine (decrease stool volume) and epidermal growth factor (increase the number of microvilli per cell) but no improvement was noted with the other treatments. Although it is a rare disorder and the prognosis of microvillus inclusion disease is poor, it must be considered if an infant has chronic secretory diarrhea. *(JKMS 1997; 12: 452~6)*

Key Words : *Microvilli; Microvillus inclusion disease; Diarrhea, Infantile; Epidermal growth factor-urogastrone*

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INTRODUCTION

Microvillus inclusion disease, also known as congenital microvillus atrophy, was first described by Davidson et al. (1) in 1978 and is a disorder which presents itself from birth with severe intractable secretory diarrhea (2~5). Familial cases have been reported and occur in a pattern consistent with autosomal recessive inheritance. The pathognomonic features of microvillus inclusion disease are hypoplastic villus atrophy with electron microscopic findings of intracellular brush border inclusions. Diagnosis is often delayed because of difficulty in obtaining a small intestine biopsy specimen in the neonatal period. As there is no effective treatment, accurate diagnosis is important for genetic and prognostic reasons. In this paper we report two cases of microvillus inclusion disease in two Korean infants, who presented with intractable diarrhea from birth and were the first cases in Korea.

CASE REPORT

Case 1 : A currently 3 month-old girl was born by normal vaginal delivery at 36 weeks following a uneventful pregnancy with Apgar scores of 4 and 7 at 1 min and 5 min respectively. The birth weight was 2.7 kg. She has a male sibling who died of abdominal distention, feeding intolerance and diarrhea in the neonatal period. She was found to be stained heavily with meconium and chest roentgenogram showed meconium aspitation pneumonia. The baby passed bloody stool and couldn't tolerate to be fed with iso-osmolar glucose solutions. Mechanical ventilation and intravenous hyperalimentation was instituted to compensate for respiratory insufficiency and a stool output ranging from 150-200 g/kg/day. Transfer to Samsung Medical Center was arranged at 10 days of age after weaning of ventilation. On the morning of 11 days of age she was found in acute respiratory distress, with severe metabolic acidosis, and renal failure (creatinine rose up to 2.9 mg/dl). At that time she had diarrhea more than 150 ml/kg/day and weight of 2.3 kg. A septic workup revealed no evidence of infections and full metabolic screen was unremarkable except hypertyrosinemia. Other workup's included extensive immunologic, hormonal studies and study for autoimmnue enteropathy, all of which were negative or normal. Abdominal roentgenograms demonstrated an ileus. Two duodenal and rectal biopsies were taken at 22 days and at 2 months of age. Liver function tests remained normal until she was 2-months old, when the total bilirubin rose to 2.0 mg/dl (direct 1.6 mg/dl) and which steadily increased up to 4.6 mg/dl. Trials of somatostatin analog (octreotide, 4 ug/kg/day) had no effect. Cyclic total parenteral nutrition was started at 2 months of her age because of cholestasis and preparing the home total parenteral nutrition.

Case 2 : The infant was born via spontaneous vaginal

delivery at 39 weeks gestation after an uncomplicated pregnancy. Birth weight was 3 kg. He passed normal meconium stools and was bottle fed well initially. At 3 days after birth, frequent, loose stools were documented. The diarrhea persisted after discharge, while he was at home. At 7 days of his age he was severely dehydrated (weight, 2.5 kg) and was admitted to the hospital where he was born. Septic workup was performed, antibiotic therapy was begun, and bowel rest started. At 15 days of age, refeeding with one fourth strength soy based formula led to grossly bloody stool and abdominal distention, thrombocytopenia and increased cryoreactive protein. At 4 months of age, the baby was transferred to Samsung Medical Center, where he received antibiotic therapy and total parenteral nutrition after he received a deep venous catheter. An exhaustive workup of the secretary diarrhea was normal for each of the followings; infectious workup (including negative Rotazyme, ova plus parasite study, stool culture, stools for *Cryptosporidium* and *Clostridium difficile* toxin, negative titers for Cytomegalovirus, Herpes), quantititive immunoglobulins, complement levels, sedimentation rate, thyroid, kidney and liver function studies, barium enema, abdominal ultrasonogram. A D-xylose test was abnormal, with serum D xylose of 15 mg/dl at 60 min (normal; >25 mg/dl). At 5 month of his age, abdominal distention and vomiting developed due to an inguinal hernia and he underwent herniorraphy. Echocardiogram revealed moderate to large atrial septal defect and it was repaired at 8 months due to poor weight gain, high basal metabolic rates (over 150 kcal/day) and tachypnea. At 8 months old pulmonary edema and pleural effusion developed. Right peripheral pulmonary vein stenosis was suspected on echocardiogram and was considered as congenital malformation. Trials of cholestyramin caused partially reduced stool volume (from 150 g/kg of body weight/day to 50 g/kg of body weight/day) but somatostatin analog (octreotide, 4 ug/kg/day) and steroid (2 mg/kg/day for 3 weeks) was not effective. Epidermal growth factor (100 ng/kg/hr for 2 weeks) increase the population of the microvilli but had no effect on villus atrophy. Liver function tests remained normal until he was 6 months old, when the total bilirubin rose to 1.5 and which steadily increased to 9.7 mg/dl (direct 8 mg/dl). After starting cyclic total parenteral nutrition, the bilirubin remained under 8 mg/dl. Alanine aminotransferase (ALT) was 172 U/L (normal; below 40 IU/L) and aspartate aminotransferase (AST) was 149 IU/L (normal; below 40 IU/L).

PATHOLOGIC FINDINGS

Upper and lower gastrointesinal tract endoscopy was performed using an Olympus N30. Four pieces of biopsy specimen were obtained at the distal portion of second duodenum. Two of them were for light microscopy the other two were for electron microscopy. The other two specimens were taken at rectum for light microscopy.

Tissues from duodenum and rectum prepared for both routine hematoxylin and eosins were fixed in 10% buffered formalin, processed paraffin embedding, and sectioned.

Electron microscopic examination was performed on duodenal specimens. The tissues were fixed in 4% paraformaldehyde / 0.5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in graded alcohol's, and embedded in Epon-Ar-aldite. One micrometer-thick sections were stained with toluidine blue / basic fuchsin. Ultrasections were double stained with 2% uranyl acetate followed by 0.3% lead citrate. They were examined and photographed.

In case 1, the duodenal biopsies were characterized by moderate degree of villus atrophy. Some mitoses and degenerating epithelial cells were seen in the crypt and there was a mild increase in the number of mononuclear inflammatory cells within the lamina propria (Fig. 1). Mild chronic inflammation was seen in sections of rectum. Well-formed microvillus inclusions were documented by ultrastructural analysis. Microvillus inclusions were found in the enterocytes. They were membrane bound bodies containing microvilli and surrounded by a terminal web zone replete with well developed microfillamentous core rootlets (Fig. 2). Surface epithelical cells showed shortened and infrequent microvilli, increased polyribosomes, increased secondary lysosomes and dilatation of RERs. In case 2, severe villus atrophy with mild

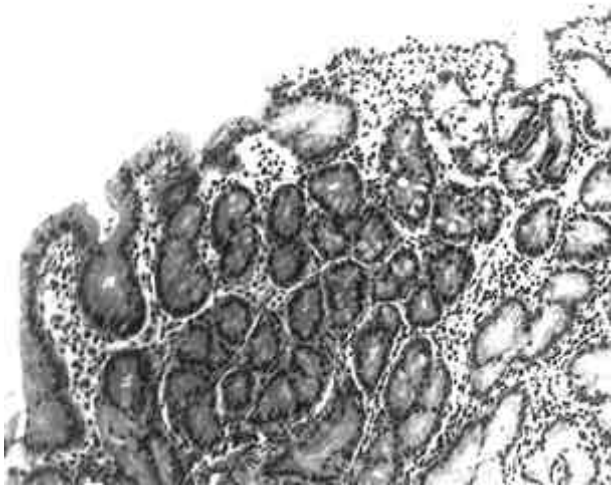

Fig. 1. Duodenal mucosa showing severe villous atrophy and hyperplasia of crypt (case 1) (Hematoxylin and Eosin).

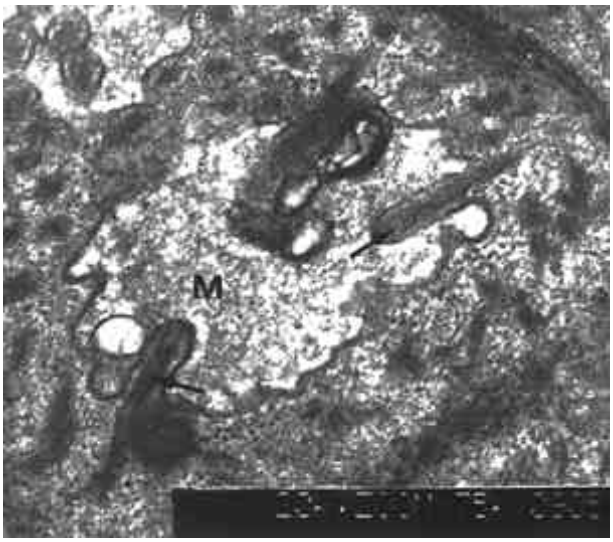

Fig. 2. Microvillus inclusions (M) in surface epitelium. Note microvillus and terminal web (arrows) (case 2) (×40,000).

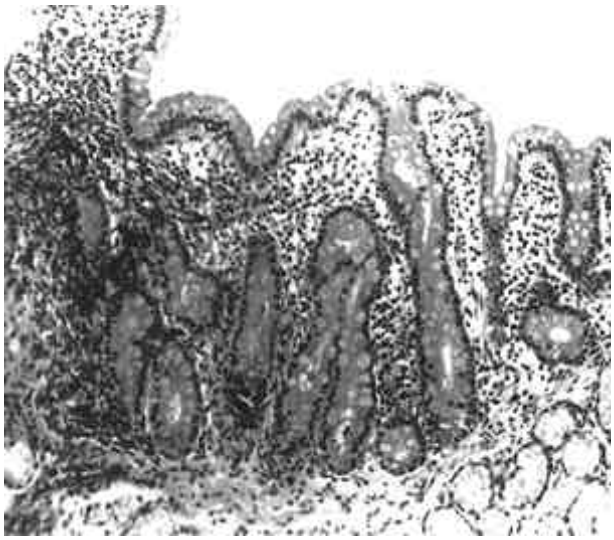

Fig. 3. Duodenal mucosa showing villous atrophy and elongation of crypts (case 2) (Hematoxylin and Eosin).

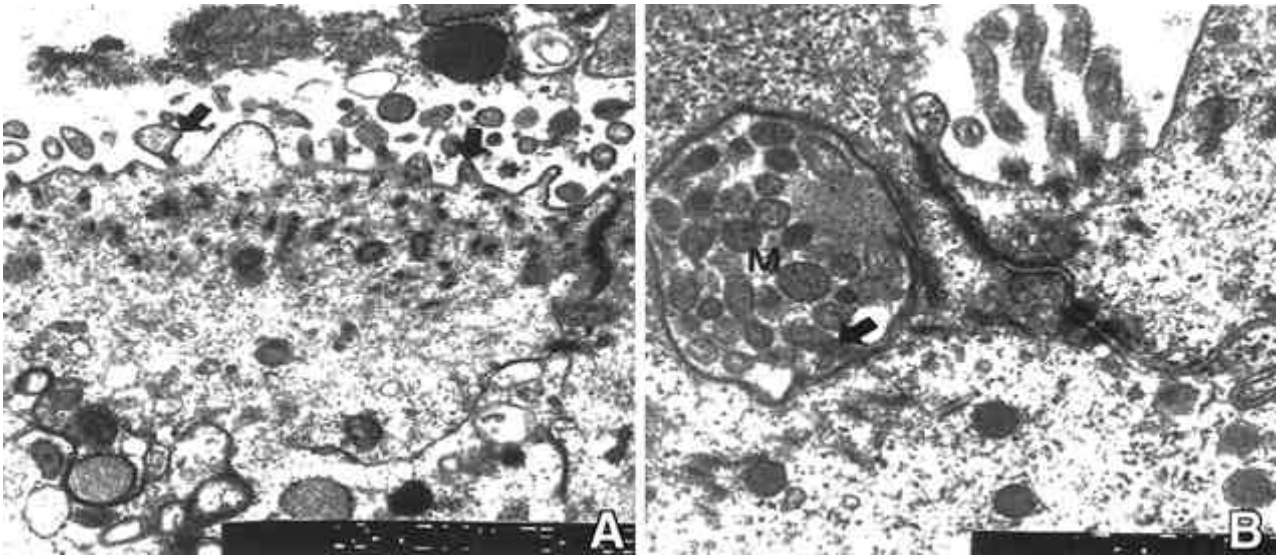

Fig. 4. A : Surface epithelial cells showing abnormal microvilli (arrow) including shortening and infrequent microvilli (case 2) (×20,000)
B : Microvillus inclusion (M) showing membrane bound body containing microvilli (arrow) (case 2) (×34,000).

crypt hyperplasia or increased numbers of inflammatory cell in lamina propria (Fig. 3). Rectal biopsy specimen showed normal but mild mononuclear cell infiltration. Ultrastructural studies of the duodenal mucosa demonstrated marked abnormalities of the surface epithelial cells including cytolysis and shortening, and loss of surface microvilli in a patchy distribution (Fig. 4A). The most striking ultrastructural finding was the presence of numerous intracytoplasmic inclusions in the enterocytes and goblet cells (Fig. 4B).

DISCUSSION

Intractable diarrhea of infancy may result from a variety of known disorders including infections, inflammatory conditions, enzymatic defects, hormonal or metabolic diseases, and anatomical abnormalities (6). Nonetheless, the etiology of many cases remains obscure.

In 1978, Davidson et al. (1) reported a series of five infants with a familial enteropathy characterized clinically by persistent severe diarrhea and pathologically by small

intestinal villus atrophy, crypt hypoplasia, normal or decreased numbers of inflammatory cells in the lamina propria, and disruption of the enterocyte brush border. In one of their patients, ultrastructural study disclosed either an absence of or a shortening and disarray of small intestinal surface microvilli and the presence of unusual intracytoplasmic inclusions within surface enterocytes, which were lined by brush border microvilli. In our cases the crypt cell showed hyperplasia, which findings were not a typical features of microvillus inclusion disease but were considered the secondary changes of chronic mucosal injury.

The term of 'microvillus inclusion disease' was suggested by Cutz et al. (2) in that the microvillus inclusion disease is more specific and emphasizes the typical ultrastructural feature of this condition.

Microvillus inclusion disease is different from other causes of congenital diarrhea in which the ultrastructure of the enterocyte is normal (3, 6~9). The functional implications of microvillus inclusion diseases are an inability to absorb even simple nutrients, as well as profuse diarrhea resulting from a combination of decreased absorption and increased secretion into the bowel lumen (1). In our report we present two additional cases of microvillus inclusion disease, which are the first reported in Korea. In one case of ours we suspected the family history of microvillus inclusion. Cutz et al. (2) have suggested that microvillus inclusion disease is probably inherited as an autosomal recessive disorder.

The prognosis is poor and most of the reported patients have died within the first 18 months of life (7). The European Society for Pediatrics Gastroenterology and Nutrition held a workshop on microvillus inclusion disease in 1987. They surveyed the American and European pediatric gastroenterolgy experiences and reported 31 cases of microvillus inclusion disease; of the 29 cases in which the outcome was known, 10 patients were still alive. The oldest child was 6.5 years of age (7).

Currently, there is no curative therapy available, and afflicted patients require lifelong total parenteral nutrition or intestinal transplantation or combined bowel-liver transplantation (10~12).

Epidermal growth factor stimulates epithelial cell proliferation in several tissues, including gastrointestinal mucosa (13). Some authors reported total three cases suggesting that polypeptide urogastrone epidermal growth factor stimulates proliferation and differentiation of mucosal enterocytes in infants with microvillus inclusion disease (14~15). We observed the increase of the population of microvillus after 2 week's treatment of epidermal growth factor in patient 2. Further studies will be needed to evaluate the mechanism and the effectiveness of epidermal growth factor in infants with microvillus inclusion disease.

In the recognition and diagnosis of this disease, clinical suspicion is most important. In the case of an infant with intractable secretary diarrhea present from birth or shortly thereafter, it would be prudent for a pediatrician to consult the baby to pediatric gastroenterologist to obtain a small intestinal biopsy because definitive diagnosis of microvillus inclusion disease rests on the ultrastructural findings. The alert pathologist can suspect this disorder in the appropriate clinical setting by recognizing the light microscopic features of small intestinal villus atrophy without crypt hyperplasia or increased inflammation. PAS staining demonstrates a discontinuous brush border and highlights the presence of apical cytoplasmic vacuoles that correspond to the microvillus inclusions seen on electron microscopy.

Although it is a rare disorder, it must be considered if an infant has chronic secretory diarrhea.

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