

Acrodermatitis Enteropathica: Case Report Analyses of Zinc Metabolism Electron Microscopic Examination and Immune Function

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Acrodermatitis enteropathica (AE) is a rare autosomal recessive disease that results from an unknown defect in zinc metabolism and usually occurs in the first year of life. Zinc is required for the structure and activity of ~300 metalloenzymes. Therefore, every system could be affected functionally or structurally by this defect. An 11-year-old girl presented with rashes around mucocutaneous junctions, periorbital and perianal areas, and on the extremities. These clinical signs and symptoms had started when she was 3.5 months old. She was the third child of healthy parents who are second cousins. One of her brothers had died with similar manifestations when he was 3 years old. In the initial physical examination, growth retardation, partial alopecia, squamous skin eruptions around the body orifices, pustular lesions on her knees and hands, dystrophic changes of the finger and toe nails, and taste dysfunction were prominent findings. Laboratory results revealed: haemoglobin: 12.3 gr/dl; WBC: 7,300/mm³; platelet count : 402,000 /mm³; serum iron : 152 µg/dl; total iron binding capacity : 501 µg/dl; transferrin saturation : 25.1 %; serum cu level: 118 µg/dl (123–187 µg/dl); and alkaline phosphatase :18 IU/L (98–279 U/L). Serum electrolytes, hepatic, and renal function tests were normal. Zinc levels in different body compartments were decreased (plasma Zn: 20 µg/dl; erythrocyte Zn: 5.7µg/ml; hair Zn: 116.6 µg/g) with normal zinc absorption. Zinc 65 absorption and retention test showed initial elevation, but decreased rapidly after the fourth day compared to the controls and her other family members. By electron microscopy, intestinal mucosal biopsy revealed Paneth cell inclusions. Lymphocyte subpopulations and results of neutrophil chemotaxis also showed some abnormalities. Pathological clinical findings and symptoms totally disappeared in a few weeks with oral zinc therapy (ZnSO₄, 2 mg/kg/day). *J. Trace Elem. Exp. Med.* 13:317–325, 2000. © 2000 Wiley-Liss, Inc.

Key words: acrodermatitis; zinc; family members

INTRODUCTION

Acrodermatitis enteropathica (AE) is a rare autosomal recessive disease, which results from an unknown defect in zinc metabolism and usually occurs in the first year

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of life. Zinc is an essential trace element and is required for the structure and activity of >300 metalloenzymes as well as for stabilisation of cell membranes and cellular organelles [1–3]. In addition, it has some functions during DNA synthesis, cell division, and protein synthesis. It is known that zinc-finger proteins are involved in genetic expression of various growth factors and steroid receptors [1,2]. Therefore, all systems could be effected functionally and/or structurally. The most common symptoms are cutaneous eruptions seen in perioral, periorbital, and perianal regions and paronychia, nail dystrophy, diarrhea, growth retardation, and recurrent infections [3–5].

CASE REPORT

An 11-year-old girl presented with rashes around mucocutaneous junctions, periorbital and perianal areas, and on the extremities. These clinical signs and symptoms had started when she was 3.5 months old. She was the third child of healthy, related (second cousins) parents. One of her brothers had died with similar manifestations when he was 3 years old. In the initial physical examination, growth retardation, partial alopecia, squamous skin eruptions around the mouth, eyes, and other body orifices, pustular lesions on her knees and hands, dystrophic changes of the finger and toe nails, and taste dysfunction were the prominent findings (Fig. 1a). Laboratory results revealed: haemoglobin: 12.3 gr/dl; WBC:7,300/mm³; platelet count: 402,000/mm³; serum iron: 152 µg/dl; total iron binding capacity: 501 µg/dl; transferrin saturation: 25.1%; serum cu level: 118 µg/dl (123–187 µg/dl); and alkaline phosphatase: 18 IU/L (98–279 U/L). Serum electrolytes, hepatic, and renal function tests were normal. Zinc levels in different body compartments (plasma, erythrocyte, and hair) were measured with flame atomic absorption spectrophotometer (AAS) as described previously [6,7].

Blood samples from fasting subjects were taken with plastic disposable syringes and stainless steel needles into two demineralized centrifuge tubes, one of which contained heparin for plasma and red blood cells determinations. The tubes were sealed with parafilm to avoid any contamination. The blood samples then were centrifuged and the serum, plasma, and erythrocytes were separated and kept frozen at -20C in plastic tubes until determination. All measurements were performed at least in duplicate. Hair samples of 1–2 cm length, taken from the patient and family members, were clipped with stainless steel scissors from closest to the scalp in the suboccipital region. The hair samples were initially washed with triple distilled water, then with carbon tetrachloride in order to rid them of lipid particles.

After drying, the samples were weighed and then ashed in an oven at 500C for 12 hours until they took the form of white ash. Samples were then dissolved in 5 ml/l normal HCl and transferred to polyethylene tubes. Hair Zn levels were determined using AAS (Perkin Elmer Model 2380) and expressed as microgram per gram. Low levels (plasma Zn: 20 µg/dl, erythrocyte Zn: 5.7µg/ml, hair Zn: 116.6 µg/g) (Fig. 2) were found in our patient with normal zinc and iron absorptions (Fig. 3). Zinc parameters of family members did not differ from those of control groups, except for the erythrocyte zinc of a brother and hair zinc of a sister.

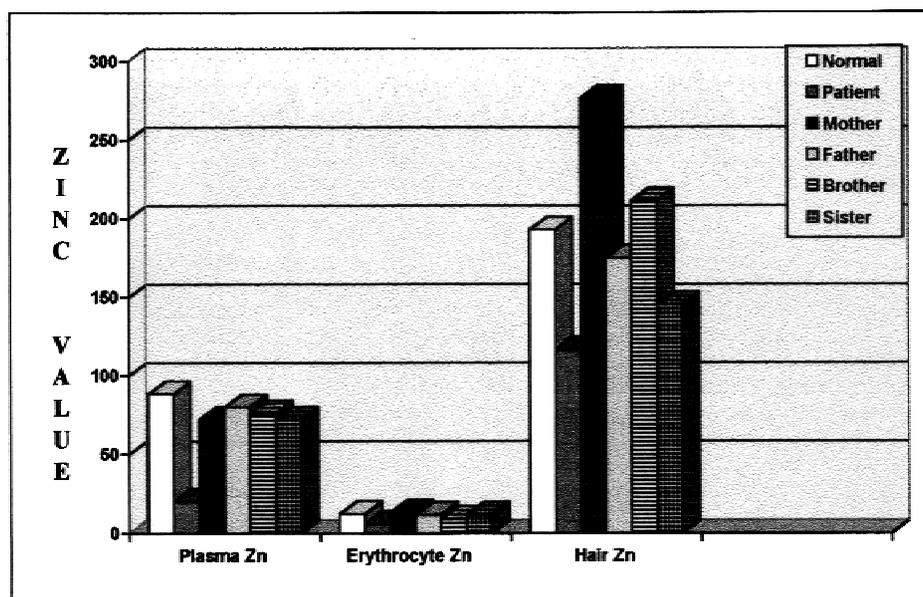
The zinc 65 absorption and retention test was also performed on the patient and all family members. After overnight fasting, 5 µCi (185 Becquerel) Zn⁶⁵(Amersham,



Fig. 1. (a) Patient at time of diagnosis. (b) Patient 2 two months later, after zinc therapy.

UK) was given orally to the patient and all family members. The total amount of zinc in the oral dose was $\sim 1 \mu\text{g}$. After administration of the radioactive zinc, at 1, 2, 3, 4, 7, 9, 15, and 17 days, activity retention was measured by using a whole body counter (Canberra Acuscan 2). Zn^{65} absorption activity was enhanced initially, but decreased rapidly after the fourth day compared to controls and other family members (Fig. 4).

The immunological status of the patient and her family members was evaluated by determination of lymphocyte subpopulations and neutrophil chemotaxis studies. De-



	Plasma Zn µg/dl	Erythrocyte Zn µg/ml	Hair Zn µg/g
Normal	88.5 ± 17.4	12.1 ± 1.8	192.9 ± 12.5
Patient	20	5.70	116.6
Mother	72	13.05	276.7
Father	80	11.55	175.0
Brother	76	8.70	211.3
Sister	72	11.55	145.0

Fig. 2. Zinc levels in patient and family members at time of diagnosis.

creased natural killer cell (NK) count and neutrophil chemotaxis were found in our patient. However, the NK cell count of her mother and neutrophil chemotaxis of her brother also were lower than normal (Table I). Skin and duodenal biopsies were examined by light and electron microscopy. For electron microscopic examination, the skin and duodenal tissue blocks were fixed with 2% glutaraldehyde in 0.1 M Sorensen's phosphate buffer for 2 hours, postfixed with 1% OsO₄ in 0.1 M Sorensen's phosphate buffer for 1 hour, dehydrated with an ethanol series, and embedded in Araldit. Sections 1-µm thick were cut on an LKB pyramitome, stained with toluidine blue borax, and observed on light microscopy. Ultrathin sections were prepared with an LKB ultratome, double stained with uranyl acetate and lead citrate, and observed with a transmission electron microscope [8].

A duodenal biopsy revealed Paneth cell inclusions (Fig. 5). Dilatation of intercellular spaces and ovoid form, opaque epithelial structures in these locations were striking in the skin biopsy by electron microscopy (Fig. 6).

The patient responded well to oral zinc therapy (zinc sulphate heptahydrate form ZnSO₄, 2 mg/kg/day). Her pathological clinical findings and symptoms totally disappeared within a few weeks (Fig. 1b).

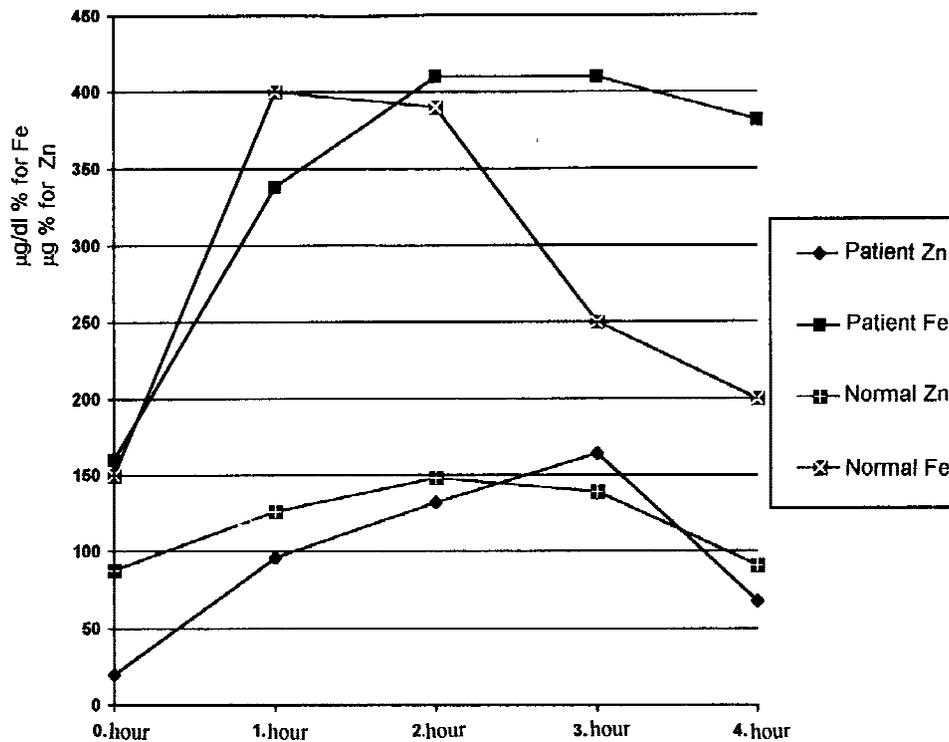


Fig. 3. Iron and zinc absorption tests in patient.

DISCUSSION

Zinc deficiency is caused by low dietary intake, poor intestinal absorption, poor release from the intestine to the circulation, disturbed body Zn distribution, or by lack of zinc at the site of biological action and subsequent loss [4]. Acrodermatitis enteropathica has been described in breast-fed infants related to low zinc levels in the mothers [9–11]. Moynahan [5] reported zinc deficiency in an infant suffering from AE and lactose intolerance. This infant relapsed again and again despite treatment with diodoquin (diiodohydroxyquin). Analyses of the diet revealed a lack of zinc. Complete clinical remission was achieved by oral substitution of 150 mg zinc-sulphate daily [5]. Thus, Moynahan suggested that zinc deficiency was an essential defect in the pathogenesis of that genetic disease. Although the mean serum zinc levels in 94 untreated patients were also low as compared to the normal ranges in another study [4], normal and even elevated levels also may be found [12,13].

Zinc levels in different compartments were measured in our patient and her family members (Fig. 2). Plasma, erythrocyte, and hair zinc levels were low with normal zinc absorption in the patient. The zinc parameters of family members were no different from those of the control groups, except for erythrocyte zinc of a brother and hair zinc of a sister. No evidence of low zinc values was detected in the parents or healthy siblings in the literature [4]. One explanation for this could be low dietary intake of zinc by family members, or at least minor abnormal handling of zinc between dif-

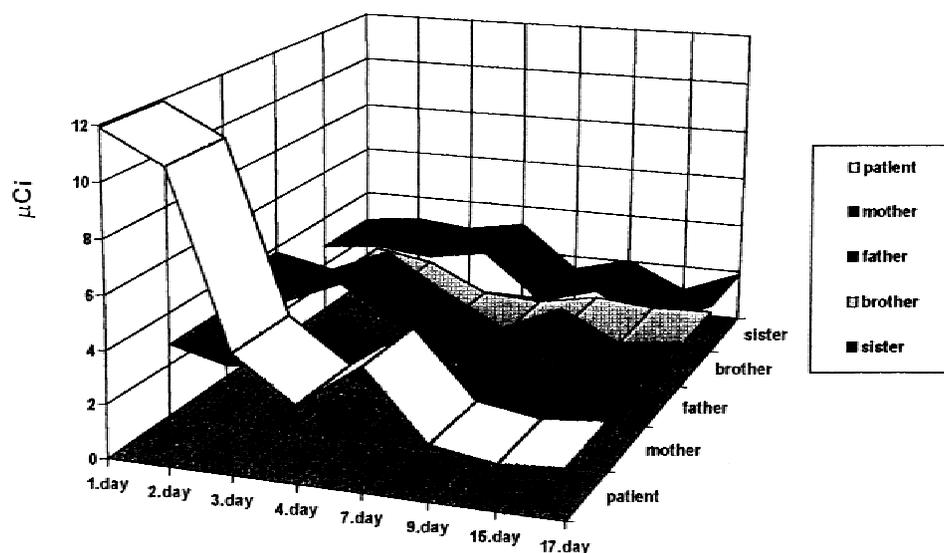


Fig. 4. Zn ⁶⁵ absorption and retention tests.

ferent body compartments. However, Zn⁶⁵ absorption activity was normal for all family members, although our patient had enhanced initial absorption activity and rapid decrease after the fourth day of the test. These results suggest that the low plasma zinc levels were not due to malabsorption, but to rapid elimination. Unfortunately, urine Zn⁶⁵ levels could not be determined because of technical difficulties.

Sandstrom and colleagues [14] studied zinc metabolism with Zn⁶⁵ in a 16-year-old boy with AE. His zinc absorption was found to be within the reference range for healthy subjects. The authors suggested that the primary lesion in AE was a cellular defect in zinc metabolism rather than an impairment of zinc absorption (14).

A variety of immune responses may be associated with zinc deficiency in AE [14–16]. Patients with AE exhibit atrophic thymus, lymphopenia, anergic delayed-type hypersensitivity responses and reduced NK cell activity [15]. Decreased NK cell count and neutrophil chemotaxis were also found in our patient (Table I). In addition, NK cell count of mother and neutrophil chemotaxis of a brother were also lower than the normal ranges. Their peripheral blood total (CD3+) and helper (CD4+) T-cell counts were found to be slightly decreased compared to controls. Decreased Zn levels

TABLE I. Immunologic Parameters in Patient and Family Members

	CD 3+ %	CD 4+ %	CD 8+ %	CD 16+ %	CD 19+ %	CD 56+ %	Neutrophil chemotaxis index
Normal	65.0 ± 3.36	38.2 ± 4.8	23.18 ± 1.43	14.9 ± 1.6	11.72 ± 1.54	10.6 ± 1.2	1.57 ± 0.326
Patient	65.5	42.7	21.7	6.8	13.3	3	1
Mother	53	27	25	7		5	1.7
Brother	48	28	25	14		10	0.9

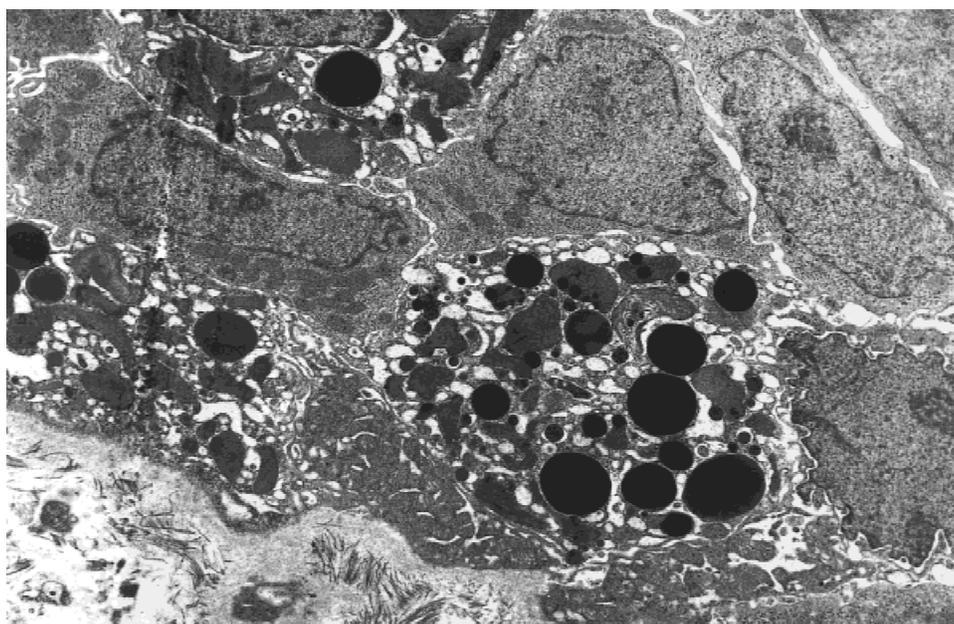


Fig. 5. Duodenal biopsy (electron micrograph. TEM X 3300) Paneth cell before zinc therapy. There is a striking pleomorphism formation of giant granules and inclusion bodies in the Paneth cell.

in different body compartments of those family members might be responsible for their altered immune functions.

The alkaline phosphatase activity in patients with AE is generally low, although normal and even elevated levels have been reported [4]. In our patient, serum alkaline phosphatase was found to be decreased at the time of diagnosis (18U/L). However, after zinc therapy, an increased value (293 U/L) was observed.

Lombeck et al. [17] examined duodenal biopsies from patients with AE by electron microscope. The ultrastructural studies revealed characteristic inclusions in Paneth cells with maximum length of 7 μm . These findings were constant in four different patients. They suggested that the inclusions are not secondary phenomena, but a morphologic manifestation of the primary defect in AE [17]. Ultrastructural pathologic lesions in these Paneth cells disappeared gradually during zinc therapy. Thus the changes in the Paneth cells in AE are the results of, not the cause of, zinc deficiency [3,17]. Abnormal inclusions in the Paneth cells were also observed with electron microscopy of small bowel biopsy in our patients (Fig. 5).

Light and electron microscopy also evaluated skin biopsies from patients with AE. The most characteristic light microscopy features were parakeratosis, absence of the granular layer, and pallor of the upper epidermal cells. Electron microscopy revealed that keratohyalin was low and the upper Malpighian cells were oedematous with vacuoles and large numbers of ribosomes. Large amounts of keratinosome-derived lamellae were found in the intercellular spaces in the keratinisation area, probably related to disturbance of keratinosome metabolism due to Zn deficiency [18]. Dila-

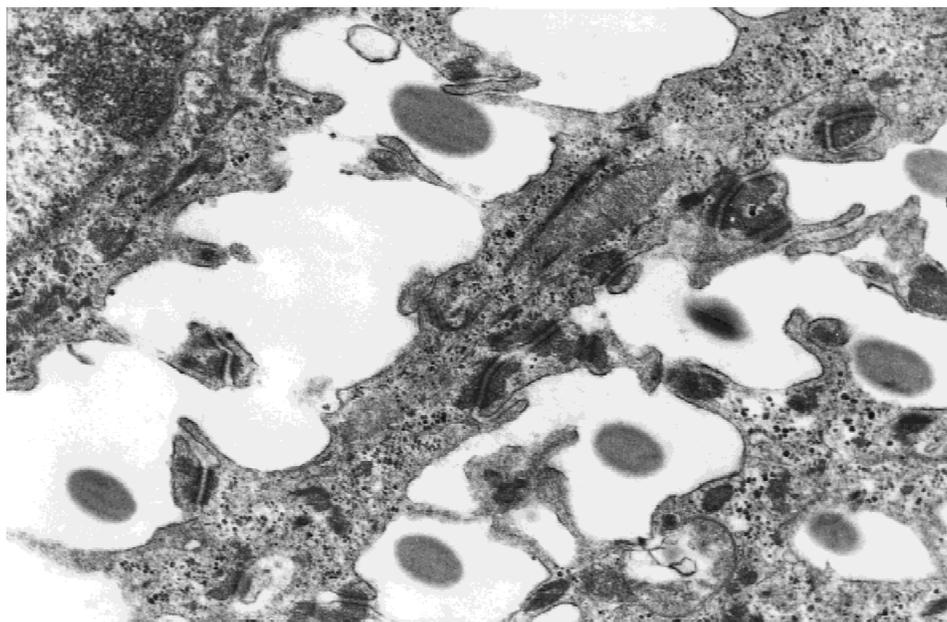


Fig. 6. Skin biopsy (electron micrograph TEM \times 13,000) dilatation of intercellular spaces and ovoid, opaque epithelial structures in these locations.

tation of intercellular spaces and ovoid, amorphous epithelial structures in this localisation were seen in our patient (Fig. 6).

It is known that treatment is effective, safe, and simple by using 3–30 mol Zn per kg/day [19]. Our patient also responded to and tolerated zinc supplementation (Fig. 1b). It became apparent that zinc therapy should be maintained lifelong in patients with AE. Thus they should enjoy normal lives and should have normal life spans. As there is a competitive antagonism between zinc and copper, the higher dosage of zinc supplementation can induce a state of lower copper status and immune dysfunction. Therefore, zinc and copper status should be monitored regularly in patients with acrodermatitis enteropathica to provide a proper dosage of zinc during different physiologic states.

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