SLC39A4 Mutation in Zinc Deficiency Patients

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Objective: *To analyze the clinical presentation and SLC39A4 mutations in zinc deficiency patients.*
Material and Method: *The authors conducted a cross-sectional study on all cases of zinc deficiency treated at Queen Sirikit National Institute of Child Health between January 2004 and December 2012. Demographic data, clinical manifestations, laboratory results, treatment and outcome were analyzed. Genetic, SLC39A4 for acrodermatitis enteropathic (AE), mutation analysis was performed in all cases.*
Results: *There were 15 cases, 10 males and 5 females. The age of onset was between 2 and 10 months (median 3 months). Duration of the disease ranged between 3 days and 17 months (median 2 months). Acral and periorificial dermatitis, diarrhea and alopecia were present in 15 cases (100%), 12 cases (80%) and 8 cases (53%) respectively. The characteristic triad of acral and periorificial dermatitis, diarrhea and alopecia was observed in only 6 patients (40%). Serum zinc level ranged between 10 and 111 mcg/dl (mean 49.69±33.87 mcg/100 ml). Low serum zinc level was observed in 10 cases (67%). All of the patients were treated with zinc sulfate 5 mg/kg/day. All cutaneous lesions and diarrhea had resolved within 7 days of starting therapy. A genetic study of SLC39A4 gene in our 15 patients revealed that 3 patients had homozygous c.1878_1879ins21 (p.G627_T633dup) in exon12. These three patients have to receive lifelong zinc supplementation to prevent recurrence of the disease. The other twelve patients, who did not carry the gene mutation, did not have symptoms after discontinuance of oral zinc therapy. This is the first report of genetically confirmed acrodermatitis enteropathic in Thailand.*
Conclusion: *Acrodermatitis enteropathica is a rare disease, which needs lifelong zinc supplementation. A genetic study of SLC39A4 gene will confirm the diagnosis. Most of patients presenting with characteristic triad of acral and periorificial dermatitis, diarrhea and alopecia in Thailand were acquired zinc deficiency. Early recognition and treatment of the disease will decrease morbidity and mortality.*

Keywords: *Acrodermatitis enteropathica, Breast milk, Congenital zinc deficiency, SLC39A4 gene mutation, Transient neonatal zinc deficiency*

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Zinc is an essential nutrient for humans and is quantitatively the second most important after iron[1]. Zinc is involved in multiple functions and is necessary for optimum growth and development[2]. The functions of zinc have been organized into 3 categories: catalytic, structural, and regulatory. Zinc is an essential component of the catalytic site of hundreds of different metalloenzymes[3] Zinc deficiency causes clinical triad of acral and periorificial dermatitis, diarrhea and alopecia[4]. It can be divided into two forms: congenital (acrodermatitis enteropathica) and acquired form. Causes of acquired zinc deficiency are inadequate intake, malabsorption, excessive loss, increased demand, etc. Acrodermatitis enteropathica (AE; MIM# 201100) is a rare autosomal recessive disorder of zinc deficiency. AE was first identified by Danbolt and Closs in 1942[5]. In 1973, Moynahan and Barbes showed that the disease is caused by a defective zinc uptake in the duodenum and jejunum due to abnormality in the zinc transporting protein[6]. The genetic defect was mapped on chromosomal region 8q24.3 in 2001[7] and in 2002 the defective gene identified as SLC39A4, which encodes the zinc transporter Zip4[8]. The incidence is

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estimated to be 1 in 500,000 children[9]. It is common in sub-Saharan Africa and South East Asia[9,10]. Since the first description, about 30 mutations of SLC39A4 gene have been reported[11]. One of the acquired form of zinc deficiency is transient neonatal zinc deficiency. Transient neonatal zinc deficiency (TNZD; MIM#608118) is an autosomal dominant disorder of maternal heterozygous mutation in the SLC30A2 gene on chromosome 1p36 causing low zinc in breast milk[12]. The heterozygous mutation mothers have normal serum zinc levels but deficient zinc in breast milk. Maternal zinc supplementation could not correct zinc level in breast milk. This condition has similar symptoms to AE but does not require zinc supplementation after weaning from breast milk. The accurate diagnosis for these 2 diseases is important because over supplementation of zinc can cause copper deficiency and immune dysregulation[13]. Therefore, the authors conducted the study of SLC39A4 analysis in zinc deficiency patients to confirm AE and the need to be on zinc supplementation for life.

Material and Method

The authors performed a cross-sectional study on all cases diagnosed with AE treated in Queen Sirikit National Institute of Child Health, between January 2004 and December 2012. Inclusion criteria included patients suspected with zinc deficiency (by some triad symptoms) and responded to treatment with zinc sulfate alone. Exclusion criteria included patients with underlying diseases such as short bowel syndrome, malnutrition or on parenteral nutrition. The authors recorded age, gender, onset, duration of the disease, parental consanguinity, family history of AE, clinical signs suggestive of AE: acral dermatitis, diarrhea and alopecia, treatment and outcome. Serum zinc and alkaline phosphatase levels were also analyzed. After receiving written informed consent, we collected blood samples from all patients for DNA extraction from leukocytes, using the Genomic DNA Mini kit (Geneaid Biotech Ltd., Taiwan). SLC39A4 mutation screening was performed by polymerase chain reaction (PCR)-amplifying every exon and its flanking intronic regions, and by sequencing the products on ABI 3730XL, using ABI Big Dye terminator V3.1 (Applied Biosystems, Foster City, CA, USA). Use of human tissue was approved by the Ethics Committee of Queen Sirikit National Institute of Child Health.

Statistical analysis

Descriptive statistics was used to analyze the data: mean with standard deviation and median were presented for the variables.

Results

There were a total of 15 cases, 10 males and 5 females. They were 2 preterm and 13 term infants. The age of onset was between 2 and 10 months (median 3 months). Duration of the disease ranged between 3 days and 17 months (median 2 months). Eleven patients were exclusively breast-fed infants, the other four patients were formula-fed infants. Family history of AE was found in four patients, who were siblings from two families. The first family included patient 1 and 2 and the second family included patient 5 and 12. Parental consanguinity was found in patient 9, 13 and 14. Length of follow-up ranged from 2 months to 19 years (median 39 months) as shown in Table 1.

The symptoms and laboratory results of each patient were shown in Table 2. Acral and periorificial dermatitis **(**Fig. 1), diarrhea and alopecia were found in 15 cases (100%), 12 cases (80%) and 8 cases (53%), respectively as shown in Table 3. Acral and periorificial dermatitis and diarrhea were the first symptom in 9 cases (60%) and 6 cases (40%) respectively as shown in Table 3. Alopecia was not the first symptom in any patient. The triad of symptoms was observed in only 6 cases (40%) as shown in Table 4. The serum zinc levels ranged between 10 and 111 mcg/dl (mean 49.69$\pm$33.87 mcg/100 ml; normal range: 70-110 mcg/100 ml). Low zinc level was found in 10 cases (67%). Low level of both zinc and alkaline phosphatase was found in 7 cases (47%) as shown in Table 5.

A genetic study of SLC39A4 gene in our 15 patients revealed that three patients (patient 5, 12, 15) from two families had homozygous c.1878_1879 ins 21 (p.G627_T633dup) in exon12.

All patients were treated with zinc sulfate 5 mg/kg/day. All cutaneous lesions and diarrhea were resolved within 7 days of starting therapy. The treatment is well tolerated by all patients without side effects. No growth failure or intellectual delay was observed in our patients. Symptoms did not recur after discontinuation of oral zinc therapy in all twelve patients who did not carry gene mutation. Multiple relapses occurred in three patients with mutation following treatment interruptions. The relapses occurred within 2-4 weeks after cessation of treatment and began with peri-orificial lesions (Fig. 2).

Discussion

In the present study, the authors found that

Table 1. Characteristics of each patients

Patient	Sex	Birth status	Onset (months)	Duration	Feeding	FH of AE	Consanguinity	Length of FU
1	F	Term	2	1 months	Breast milk	Yes	No	2 months
2	M	Term	2	12 months	Breast milk	Yes	No	11 months
3	M	Preterm	2	5 days	Formula milk	No	No	10 months
4	M	Term	2	2 months	Breast milk	No	No	39 months
5*	F	Term	3	19 days	Breast milk	Yes	No	19 years
6	F	Term	3	1 month	Breast milk	No	No	31 months
7	F	Term	3	21 days	Breast milk	No	No	25 months
8	M	Term	3	14 days	Breast milk	No	No	25 months
9	M	Preterm	4	3 months	Formula milk	No	Yes	42 months
10	F	Term	4	2 months	Breast milk	No	No	44 months
11	M	Term	4	3 days	Breast milk	No	No	51 months
12*	M	Term	5	17 months	Breast milk	Yes	No	13 years
13	M	Term	5	5 months	Formula milk	No	Yes	39 months
14	M	Term	6	4 months	Breast milk	No	Yes	16 months
15*	M	Term	10	3 months	Formula milk	No	No	54 months

* Gene mutation was found

Table 2. Symptoms and laboratory results of each patients

Patient	Symptoms			Laboratory results	
	Dermatitis	Alopecia	Diarrhea	Zinc (mcg/dl)[a]	ALP
1	+	+	+	87	L
2	+	+	+	18	L
3	+	-	-	30	L
4	+	-	+	85	L
5*	+	+	+	12.9	L
6	+	-	+	111	N
7	+	-	+	10	L
8	+	+	+	38.5	L
9	+	-	+	24	N
10	+	-	+	61	L
11	+	-	+	81	L
12*	+	+	-	22	L
13	+	-	+	45	N
14	+	+	+	25	N
15*	+	+	-	95	L

* Gene mutation was found; ALP = alkaline phosphatase; N = normal level; L = low level; [a] normal range = 70-110 (mcg/dl)

Table 3. Clinical manifestations and first symptom of the patients

Clinical manifestations	Number (%)	First symptom number (%)
Acral and periorificial dermatitis	15 (100)	9 (60)
Diarrhea	12 (80)	6 (40)
Alopecia	8 (53)	0

Table 4. Combination of clinical manifestations

Clinical manifestations	Number (%)
1. Dermatitis + diarrhea + alopecia	6 (40)
2. Dermatitis + diarrhea	6 (40)
3. Dermatitis + alopecia	2 (13)
4. Dermatitis only	1 (7)

Table 5. Laboratory results

Laboratory tests	Number (%)
1. Low levels of both serum zinc and alkaline phosphatase	7 (47)
2. Low levels of alkaline phosphatase only	4 (26)
3. Low levels of serum zinc only	3 (20)
4. Normal levels of both serum zinc and alkaline phosphatase	1 (7)

acral and periorificial dermatitis was a common manifestation and usually the first symptom of zinc deficiency as shown in Table 3. The typical triad of symptoms may not be seen in all zinc deficiency patients. This study showed only 6 from 15 cases (40%) had the triad of symptoms. Zinc deficiency should be included in the differential diagnosis of patients who present with some and not necessarily the complete triad of zinc deficiency.

Low zinc level was found only in 10 cases (67%). Low levels of serum alkaline phosphatase, a zinc-dependent metalloenzyme, a valuable indicator of zinc deficiency[14] was found in eleven patients (73%). Low levels of both zinc and alkaline phosphatase were found in 7 cases (47%). All except one patient had either low zinc levels and/or serum alkaline phosphatase. All patients were treated with zinc sulfate 5 mg/kg/day. Clinical improvement occurred within 3 days in all patients. Even though most of the patients were treated for presumed milk hypersensitivity reaction with dietary modification, topical corticosteroids or topical antibiotics before coming to our hospital, no improvement was observed in any of them. The authors suggest that clinical symptoms and responsiveness to medication are more important than laboratory data in the diagnosis of zinc deficiency. Waiting for full triad of symptoms and abnormal laboratory (zinc level and serum alkaline phosphatase) may burden the patient more.

The authors found the gene mutation in three patients from two families. These three patients had

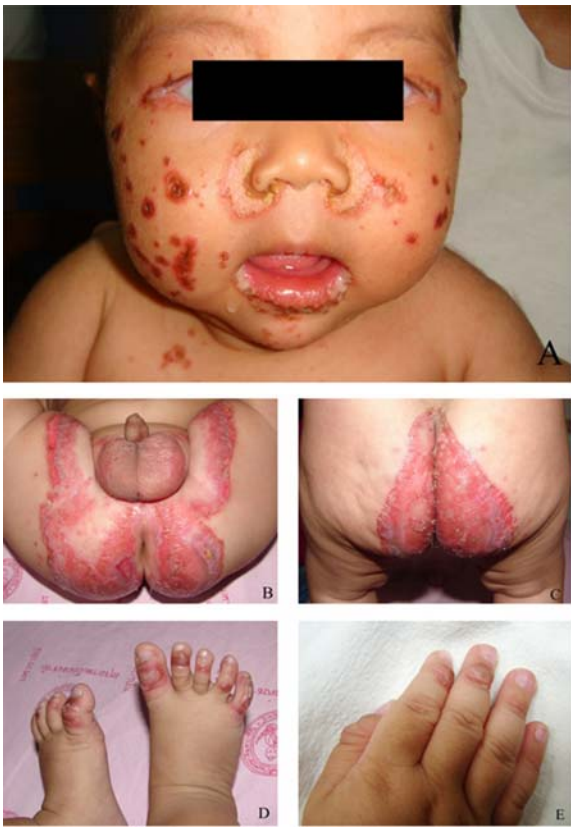

Fig. 1 Erythema, erosions, blisters, and crusts are shown in periorificial areas (A-C) and acral area of the hands and feet (D-E).

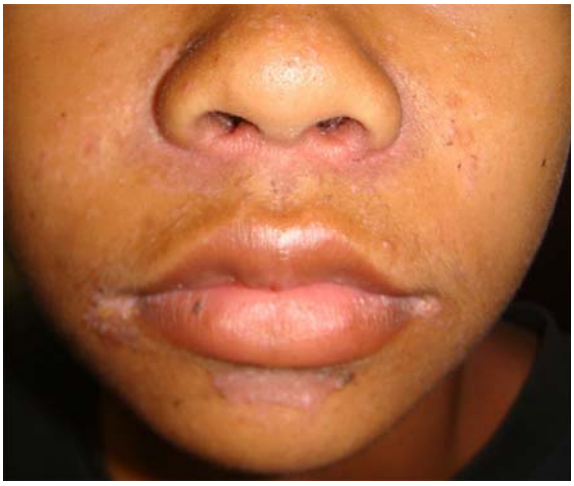

Fig. 2 Relapses in periorificial areas.

clinical symptoms of AE, which responded to zinc supplement, and could not be weaned off from it. The laboratory results along with clinical manifestation

confirmed the diagnosis of AE in these patients. The same mutation was found in all 3 of the patients even though families came from different provinces in different part of Thailand. This may be a common mutation of AE in Thailand. More AE sample analyses are needed to prove our hypothesis.

Symptoms did not recur after discontinuation of oral zinc therapy in all twelve patients, who did not carry gene mutation. Most of them were breast-fed infants. They might have TNZD. Unfortunately, this was a retrospective, cross-sectional study, so zinc levels in both the mother's blood and milk were unavailable. Mutation of SLC30A2 in these mothers should be further studied to determine if these mothers have low milk zinc concentration but normal serum zinc. Information and counseling of TNZD will help these mothers in preventing zinc deficiency in their other children.

Most of the patients presenting with characteristic triad of zinc deficiency in Thailand had acquired zinc deficiency. Mutation analysis of AE helped us to determine which patients needed to be on long-life zinc supplement and prevented over supplementation of zinc supplementation in non-AE patients. To the best of our knowledge, this is the first report, which genetically confirmed AE in Thailand.

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Potential conflicts of interest

None.

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การกลายพันธุ์ของจีน SLC39A4 ในผู้ป่วยที่ขาดสังกะสี

วนิดา ลิ้มพงศานุรักษ์, จุฬาลักษณ์ คุปตานนท์, พอน สิงหามาตร, ศรีสุภลักษณ์ สิงคาลวณิช, ศศินิภา สิริสุทธิสุวรรณ

วัตถุประสงค์: เพื่อวิเคราะห์อาการแสดงและการกลายพันธุ์ของจีน SLC39A4 ในผู้ป่วยที่ขาดสังกะสี

วัสดุและวิธีการ: ได้ทำการศึกษาแบบ cross-sectional study ในผู้ป่วยที่ได้รับการวินิจฉัยว่าขาดสังกะสี ที่มารับการตรวจรักษาที่สถาบันสุขภาพเด็กแห่งชาติมหาราชินี ระยะเวลา 9 ปี ในช่วงระหว่างเดือนมกราคม พ.ศ. 2547 ถึง เดือนธันวาคม พ.ศ. 2555 โดยเก็บรวบรวมข้อมูลอาการทางคลินิก ผลการตรวจทางห้องปฏิบัติการ การรักษา ผลการรักษาและผลข้างเคียงในผู้ป่วยเพื่อนำมาวิเคราะห์ทางสถิติ นอกจากนี้ผู้ป่วยทุกรายจะได้รับตรวจหาการกลายพันธุ์ของจีน SLC39A4

ผลการศึกษา: มีผู้ป่วยทั้งหมด 15 ราย เป็นเพศชาย 10 ราย เพศหญิง 5 ราย อายุที่เริ่มมีอาการระหว่าง 2-10 เดือน (ค่ามัธยฐาน 3 เดือน) ระยะเวลาที่มีอาการระหว่าง 3 วัน ถึง 17 เดือน (ค่ามัธยฐาน 2 เดือน) ผื่น acral และ periorificial dermatitis อาการท้องเสียและอาการผมร่วงพบในผู้ป่วยจำนวน 15 ราย (100%), 12 ราย (80%) และ 8 ราย (53%) ตามลำดับ มีผู้ป่วยจำนวน 6 ราย (40%) เท่านั้นที่มาด้วยลักษณะเฉพาะของโรคครบทั้ง 3 อาการ แสดงระดับสังกะสีในซีรัมอยู่ระหว่าง 10 ถึง 111 mcg/dl (ค่าเฉลี่ย 49.69±33.87 มคก./100 มล.) มีผู้ป่วย 10 ราย (67%) ที่มีระดับสังกะสีในซีรัมต่ำ ผู้ป่วยทุกรายได้รับการรักษาด้วย zinc sulfate 5 มก./กก./วัน หลังการรักษา 7 วันอาการทางผิวหนังและอาการท้องเสียหายเป็นปกติ การตรวจทางพันธุกรรมในผู้ป่วยทั้งหมด 15 ราย ตรวจพบการกลายพันธุ์ของจีน SLC39A4 ในผู้ป่วย 3 ราย ในตำแหน่ง c.1878_1879 ins21 (p.G627_T633dup) ที่ exon12 ผู้ป่วยทั้ง 3 ราย จำเป็นต้องได้รับ zinc sulfate ตลอดชีวิตเพื่อป้องกันการเกิดโรคซ้ำ ผู้ป่วยที่เหลือ 12 ราย ที่ไม่มีการกลายพันธุ์ของจีน SLC39A4 สามารถหยุดการรักษาด้วย zinc sulfate โดยไม่เกิดโรคซ้ำ การศึกษานี้เป็นการศึกษาแรกที่รายงานการกลายพันธุ์ของจีนของผู้ป่วยโรค acrodermatitis enteropathica ในคนไทย

สรุป: โรค acrodermatitis enteropathica เป็นโรคทางพันธุกรรมที่มีการขาดสังกะสีที่พบไม่บ่อย และจำเป็นต้องได้รับการรักษาด้วยสังกะสีตลอดชีวิต การตรวจหาการกลายพันธุ์ของจีน SLC39A4 ช่วยในการวินิจฉัย สาเหตุส่วนใหญ่ของผู้ป่วยคนไทยที่มีอาการแสดงของการขาดสังกะสีเกิดจากสาเหตุอื่นไม่ได้เป็นโรคทางพันธุกรรม การวินิจฉัยและให้การรักษาโรคตั้งแต่เริ่มมีอาการจะช่วยลดผลข้างเคียงและลดอัตราตาย