A PRELIMINARY STUDY OF THIAMINE STATUS IN NORTHEASTERN THAI CHILDREN WITH ACUTE DIARRHEA

Patcharee Boonsiri[1], Roongpet Tangrassameeprasert[1], Charnchai Panthongviriyakul[2] and Puangrat Yongvanit[1]

Departments of [1]Biochemistry, [2]Pediatrics, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

Abstract. This study is a preliminary determination of thiamine status in children with diarrhea and metabolic acidosis admitted to hospital. Children with diarrhea (N = 14; age 2 m-6 yr) were divided into 2 groups according to anion gap type; group 1 (21.4%) with a normal anion gap (5.5 ± 5.2 mmol/l) and group 2 (78.6%) with a wide anion gap (21.2 ± 5.2 mmol/l). Blood was taken on the day of admission to determine thiamine and lactate levels. Sixty-six point seven percent of patients in group 1 had a normal lactate level (1.5 ± 0.8 mmol/l) and 33.3% had a high lactate level (2.2 mmol/l); none had thiamine deficiency (TPPE < 20%). High lactate (3.5 ± 1.4 mmol/l) was found in 54.5% of group 2 and thiamine deficiency was observed in 18.2% of this group. In conclusion, no thiamine deficiency was noted in patients with normal anion gap, but thiamine deficiency was not uncommon in patients with a wide anion gap, regardless of lactic acidosis.

INTRODUCTION

Diarrhea remains an important cause of morbidity and mortality among children in Thailand, with more than one million cases reported in 2002. Even though the diarrhea-related mortality rate has decreased from 1.11 deaths/100,000 population in 1988 to 0.23 deaths/100,000 population in 2002, the diarrhea-related morbidity rate has remained unchanged (1,488 and 1,687 cases/100,000 population in 1993 and 2002, respectively) (Bureau of Epidemiology, 2002). In Northeast Thailand, 35% of children with acute diarrhea admitted to Srinagarind Hospital, Khon Kaen, during the year 1998-2000 had metabolic acidosis with a wide anion gap (Medical Statistic Unit of Srinagarind Hospital). There are many factors that can cause a wide anion gap in diarrheal patients, including tissue hypoperfusion and/or tissue hypoxemia, impaired blood circulation, sepsis resulting in lactic acid production, and starvation with ketone production (Mizock, 1989; Luft, 2001). Another possible cause of a wide anion gap may be lactic acidosis from thiamine deficiency (Oriot ***et al***, 1991; Klein ***et al***, 2004; Thauvin-Robinet ***et al***, 2004). It has been observed that some children with diarrhea and a wide anion gap admitted to Srinagarind Hospital could not be completely treated for metabolic acidosis with rehydration and bicarbonate treatment, but could be treated by the addition of thiamine (personal observation).

Thiamine is a nutrient indispensable to vertebrates. Thiamine pyrophosphate (TPP) plays a role of coenzyme for several enzymes, including pyruvate dehydrogenase complex (PDH), which catalyzes the reaction of pyruvate to acetyl CoA in carbohydrate metabolism (Gubler, 1991). Thiamine deficiency impairs the function of PDH, which leads to accumulation of pyruvate. Excess pyruvate is

Correspondence: Dr Patcharee Boonsiri, Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand.
Tel/Fax: 66 (043) 348386
E-mail: patcha_b@kku.ac.th

then converted to lactate by lactate dehydrogenase (LDH) (Evans, 1985). We investigated children with acute diarrhea and metabolic acidosis admitted to Srinagarind Hospital. The aim of this study was to determine their thiamine status, particularly those who had a wide anion gap with a high lactate level (blood lactate level ≥ 2 mmol/l or 18 mg/dl).

MATERIALS AND METHODS

Subjects

The study group consisted of 14 children (6 boys and 8 girls), age 2-74 months, with acute diarrhea who admitted to Srinagarind Hospital, Khon Kaen University from November 2001 to January 2003. Children with renal failure, heart failure or diabetes were excluded from the experiment.

Blood sample collection

Blood samples of children with diarrhea were collected into heparin and sodium fluoride (NaF) tubes by venipuncture. All blood samples were centrifuged immediately at 3,000 rpm for 10 minutes at 4ºC. The plasma in the NaF tube was aliquoted for lactate analysis. To prevent changes in lactate both during and after the blood was drawn, exercise of the hand or arm immediately before and during the procedure were avoided.

Red blood cells in a heparinized tube were obtained after centrifugation and the buffy coat on top of the packed red blood cells was removed by suction. The packed red blood cells were washed twice with an equal volume of 0.9% sodium chloride (NaCl) and were aliquoted into an Eppendorf tube, followed by adding an equal volume of 0.5% sterox for thiamine determination. All plasma and hemolyzed red blood cell samples were kept at -70ºC until analysis.

Thiamine assessment

Thiamine was assessed by determination of erythrocyte transketolase activity (ETKA), both with and without the addition of thiamine pyrophosphate (TPP) as described by Smeets *et al* (1971). Hemolysate of red blood cells in 0.5% sterox at -70ºC was thawed and diluted with distilled water at a ratio of 1:5 (v/v). One point twenty-five ml and 1.20 ml of 0.1 mol/l Tris-HCl buffer pH 7.6 containing 1.2 mmol/l $MgCl_2$ as cofactor of erythrocyte transketolase, respectively, were added into the activity and stimulation cuvettes. Next, 25 μl of 5 mmol/l NADH was added to both cuvettes. Fifty microliters of 5.07 mmol/l TPP was then added to the reaction mixture in the stimulation cuvettes to a final concentration of 0.19 mmol/l followed by the addition of diluted hemolysate (1:5) with hemoglobin concentrations ranging from 10-50 g/l to the above mixture. The reaction mixtures were then incubated for 15 minutes and 30 minutes at 37ºC, the absorbance of NADH was read at 340 nm against air blank by a spectrophotometer (Milton Roy spectronic 3000 array). The results were expressed as the percent stimulation resulting from the added TPP—*viz.* the thiamine pyrophosphate effect or TPPE. A TPPE > 15% indicated a low thiamine status, though > 20% indicated thiamine deficiency.

Lactate determination

Lactate was measured according to the method of Livesly and Atkinson (1974). The reaction mixture consisted of 2.0 ml of Tris-hydrazine buffer pH 9.6 contained 5 units of LDH and 100 μl of diluted plasma sample (1:5). They were mixed in a glass tube and kept at 4ºC. Two hundred microliters of 15 mmol/l NAD^+ solution was added to the reaction mixture and mixed again. Then, the solution was incubated immediately for 15 minutes at 37ºC. The absorbance at 340 nm was measured. The lactate concentration of each sample was calculated using a lactate standard curve. This study defined a lactate level ≥ 2 mmol/l as high or lactic acidosis according to Fattal-Valevski *et al* (2005).

Serum electrolyte measurement

Serum electrolyte levels in the children

with diarrhea were carried out by an automate machine (Beckman Coulter, Synchron CX7 Clinical System) at the Clinical Chemistry Unit, Srinagarind Hospital. The normal range of electrolyte according to Tietz (1994) is 20.6-28.2 mmol/l.

Ethics

Informed consent was obtained from each subject and the study was approved by the Ethics Committee of the Faculty of Medicine, Khon Kaen University.

Statistical analysis

The numerical data were analyzed for normal distribution by using the Kolmogorov-Smirnov test. Data are presented as mean ± SD and were analyzed by paired or unpaired *t*-tests as appropriate. Data were analyzed using SPSS 10.1 for windows (SPSS, Chicago, Illinois, USA). Statistical significance was accepted at a p-value less than 0.05.

RESULTS

Fourteen children (6 boys and 8 girls, age 2-74 months) who had watery and/or mucous type acute diarrhea were enrolled in this study. They were admitted to Srinagarind Hospital, Khon Kaen, Thailand, because of dehydration and/or convulsions. The clinical setting of these

Table 1
Clinical condition of children with acute diarrhea on day of admission (N=14).

Characteristic	Value
Vital sign[a]	
Body temperature (°C)	38.1 ± 0.7
Febrile (N = 12)	38.3 ± 0.6
Afebrile (N = 2)	37.0
Respiratory rate (times/minute)	37.0 ± 8.6
Blood pressure (systolic/diastolic) (mmHg)	98.6 ± 10.5 / 61.3 ± 7.6
Pulse rate (times/minute)	145.3 ± 22.2
Dehydration status (N)	
Mild (<5% dehydration)	3
Moderate (5-9% dehydration)	10
Severe (>10% dehydration)	1
Type of diarrhea (N)	
Watery	5
Mucous	3
Water + Mucous	6
Electrolytes[a]	
Sodium (mmol/l)	142.8 ± 9.7
Chloride (mmol/l)	114.4 ± 13.4
Bicarbonate (mmol/l)	10.6 ± 4.9
Anion gap[a]	
Normal anion gap (mmol/l) (N = 3)	5.5 ± 5.2
Sodium (mmol/l)	140.3 ± 48.5
Chloride (mmol/l)	124.0 ± 17.1
Bicarbonate (mmol/l)	10.9 ± 6.7
Wide anion gap (mmol/l) (N = 11)	21.2 ± 5.2
Sodium (mmol/l)	143.5 ± 10.3
Chloride (mmol/l)	111.7 ± 11.8
Bicarbonate (mmol/l)	10.6 ± 4.6

[a] Mean ± SD

Table 2
Thiamine status in children with diarrhea and normal or wide anion gap according to their lactate concentration.

	Diarrheal group (N = 14)			
	Group 1 (N = 3, 21.4%) Normal anion gap (5.5 ±.5.2 mmol/l)		Group 2 (N = 11, 78.6%) Wide anion gap (21.2 ±.5.2 mmol/l)	
	Subgroup 1A Normal lactate[b]	Subgroup 1B High lactate[b]	Subgroup 2A Normal lactate[b]	Subgroup 2B High lactate[b]
N (%)	2/3 (66.7%)	1/3 (33.3%)	5/11 (45.5%)	6/11 (54.5%)
Lactate level (mmol/l)[a]	1.5 ± 0.8	2.2	1.9 ± 1.2	3.5 ± 1.4
Thiamine status[c]				
Normal (N/N)	2/2	1/1	4/5	5/6
%TPPE[a]	8.3 ± 3.3	11.8	8.2 ± 5.4	10.9 ± 3.2
Deficiency (N/N)	-	-	1/5	1/6
%TPPE	-	-	19.9	37.8

[a] Mean ± SD; [b] Normal lactate concentration < 2 mmol/l; high lactate concentration ≥ 2 mmol/l
[c]Cut-off point at 20% TPPE

diarrheal children on day of admission is shown in Table 1. Their electrolyte levels exhibited low serum bicarbonate concentrations (10.6 ± 4.9 mmol/l) These subjects were divided into 2 groups according to their anion gap level. The differential diagnosis of metabolic acidosis was calculated as [Anion gap = Na^+ - (HCO_3^- + Cl^-)]. Study group 1 had a normal anion gap (N = 3, 21.4%) and diarrheal group 2 had a wide anion gap (N = 11, 78.6%).

Each of the study groups was divided into 2 subgroups according to their plasma lactate levels (Table 2). All the children in group 1 had a normal thiamine status regardless of their lactate level, whereas 2 of the children in group 2 (18.2%) had thiamine deficiency (one with a normal lactate level in subgroup 2A and the other with a high lactate level in subgroup 2). Five of the six children (83.3%) with a wide anion gap and a high lactate level (3.5 ± 1.4 mmol/l) in subgroup 2B had normal thiamine levels (10.9±3.2% TPPE). One of the six children (16.7%) in this group had thiamine deficiency (37.8% TPPE). Thiamine deficiency was found in 14.3% of all the children with diarrhea.

DISCUSSION

Thiamine deficiency in infants is an extremely rare condition in developed countries (Wyatt *et al*, 1987), but it has been diagnosed in infants and children living in Israel (Fattal-Valevski *et al*, 2005) and Northeast Thailand (Changbumrung *et al*, 1987; Tanphaichitr *et al*, 1990; Limkittikul *et al*, 2000). Thiamine deficiency occurs mainly in breastfed infants of mothers who have inadequate intake of thiamine (World Health Organization, 1999). Raw fermented fish consumption in women living in Northeast Thailand resulted in a reduction of TPPE (Vimokesant *et al*, 1975). This study is a preliminary investigation of thiamine status in northeast Thai children with acute diarrhea. Low levels of serum bicarbonate in these children on the day of admission caused an increased anion gap. In this study, most of the children with diarrhea (N = 11, 78.6%) had a wide anion gap, which indicates an overload of an acid with additional anions. The possible clinical conditions causing this include lactic acidosis, ketoacidosis, amino or organic acidemia and

renal tubular acidosis (Bar *et al*, 2007). Rocktaeschel *et al* (2003) reported the anion gap is a good predictor of hyperlactatemia. Sensitivity of the anion gap increases with increasing hyperlactatemia (Aufricht *et al*, 1992). Three of the children in our study had mild dehydration, 10 had moderate dehydration and 1 had severe dehydration. It has been reported that lactic acidosis is commonly found in children with diarrhea and mild to severe dehydration (Stacpoole, 1993). Direct assay of blood lactate was done to confirm the degree of lactate elevation in our patients. According to Stern (1994), normal venous lactate concentration is ≤ 2.0 mmol/l and extracellular lactate concentration is 1-2 mmol/l. Children with diarrhea and an anion gap higher than 12 mmol/l, were classified as having a wide anion gap according to Stern (1994). In the present study, 50% of patients had lactic acidosis. Park and Gubler (1969) have suggested that plasma levels of lactate are elevated in thiamine deficiency. Thiamine deficiency can impair pyruvate dehydrogenase function. As a result, an accumulation of pyruvate occurs and leads to increased production of lactate (Gubler, 1991).

Many studies have shown thiamine deficiency in Northeast Thailand where Srinagarind Hospital is located (Changbumrung *et al*, 1987; Tanphaichitr *et al*, 1990; Limkittikul *et al*, 2000). We found 2 of 14 children (14.3%) in this study had thiamine deficiency (Table 2). All had a wide anion gap. None of the children in normal anion gap group had thiamine deficiency. Two of the children in group 2 (18.2%) had thiamine deficiency. One of them had a normal lactate level. The difference in lactate level between these 2 patients may be explained by the severity of thiamine deficiency (19.9% *vs* 37.8% TPPE for the child with a normal lactate level and with the high lactate level, respectively). In this study, children with diarrhea and a wide anion gap and thiamine deficiency had no thiamine supplementation. For patients with diarrhea and a wide anion gap acidosis, thiamine deficiency should be considered.

In conclusion, this study showed that in patients with acute diarrhea and normal anion gap acidosis, thiamine deficiency was not a problem. In contrast, thiamine deficiency was noted in patients with acute diarrhea and wide anion gap metabolic acidosis, regardless of lactic acidosis. A large scale study is warranted.

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