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Original Article

Paediatrics Section

Serum Zinc Levels in Thai Children with Acute Diarrhoea

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ABSTRACT

Introduction: Diarrhoea remains a leading cause of morbidity and mortality in children in developing countries. Zinc has been recommended by the WHO for the prophylaxis and treatment of acute diarrhoea. However, data on zinc levels in children remains scarce.

Aim: To assess serum zinc levels in children admitted with acute diarrhoea to the paediatric unit of Srinakharinwirot University Hospital, Thailand.

Materials and Methods: A cross-sectional study was conducted in children admitted to hospital with the diagnosis of acute diarrhoea, between July 2016 and February 2017. Children < 60 months, with watery and/or mucous stool >3 times within previous 24 hours were included. Anthropometric parameters were recorded. Serum electrolytes, Complete Blood Count (CBC) and serum zinc levels were measured. Children with serum zinc level lower than thresholds as recommended by the International Zinc Nutrition Consultation Group criteria and time of collection were defined as zinc deficient. The results were descriptively presented as mean and standard deviation, median and Interquartile Range (IQR), or frequency and percentage. Pearson's chi-square or Fisher-exact test was used to compare proportions between groups, whereas, Student's t-test or Mann-Whitney U-test was used to verify the differences of continuous variables.

Results: Fifty children with acute diarrhoea were included in the study (50% female). The median duration of diarrhoea prior to admission was 24.0 hours (IQR, 12.0-72.0 hours) and the frequency of diarrhoeal episodes in preceding 24 hours was 4 times (IQR, 3-6 times). Mean serum zinc concentration at admission was 69.2±18.5μg/dL. A total of 22 (44%) children had zinc deficiency. There were no significant differences in demographic and clinical characteristics between patients with normal zinc levels and those with zinc deficiency.

Conclusion: There is a high prevalence of low zinc levels in Thai children with acute diarrhoea. More efforts are needed towards improved coverage of zinc supplementation.

Keywords: Gastroenteritis, Minerals, Nutritional deficiency

INTRODUCTION

Diarrhoea remains a leading cause of morbidity and mortality in developing countries, especially in children below 5 years of age [1,2]. Zinc, an essential micronutrient and activating cofactor in more than 300 enzymes [3], is pivotal in many cellular metabolic pathways [4-6]. Interest has focused on the impact of zinc deficiency on the susceptibility to, severity, and clinical outcomes of diarrhoeal diseases. Over the past 2 decades, rigorous randomized, double-blinded, placebo-controlled trials have demonstrated the profound role of zinc supplementation in the treatment [7-9] and prevention [10] of diarrhoeas. Mechanically, zinc may modulate the pathogenesis of diarrhoeal diseases via regulation of intestinal fluid transport [11], epithelial integrity [12] orchestration of mucosal immune responses [13].

Estimates of the global prevalence of zinc deficiency, based on zinc availability in national food supplies and prevalence of stunting indicate a high prevalence in Sub-Saharan Africa and South-East Asia [14]; regions which also bear the highest global burden of diarrhoeal diseases [15]. Aetiologically, Zinc deficiency in developing countries is associated with low intake of zinc rich foods, inadequate zinc absorption from its binding to dietary fibre and phytates often found in cereals, nuts and legumes [16].

Despite the strong recommendation by the World Health Organization of zinc supplementation in both the home and hospital management of acute diarrhoea, it is not routinely practiced in Thailand [17]. Furthermore, there is a paucity of data of zinc levels in children with acute diarrhoea in Thailand. Therefore, this study aimed to assess serum zinc levels in Thai children admitted to the hospital with acute diarrhoea.

MATERIALS AND METHODS

A cross-sectional study was conducted in children who admitted to the Paediatric unit of MSMC Srinakharinwirot University Hospital with the diagnosis of acute diarrhoea, between July 2016 and February 2017. Decisions of admission and general management were accomplished by emergency physicians and attending physicians, respectively. Children aged younger than 60 months who passed abnormal watery and/or mucous stool more than 3 times within previous 24 hours were eligible to the study. In contrast, children who had evidence of systemic infection or neurological disturbances or history of convulsions, or chronic medical conditions due, for instance, to immunodeficiency chronic gastrointestinal conditions were not eligible for the study. Children who met eligibility criteria and their parents were asked to be enrolled to the study. Written informed consent was obtained from parents or legal guardians before enrolment. The study protocol was approved by the human ethic committee of Srinakharinwirot University. Parents and children could withdraw from the study at any point during the study.

After enrolment, demographic characteristics and clinical history were recorded. Weight was measured to the nearest 0.1 kg using an electronic scale. Length was measured in the recumbent position for children less than two years old using an infantometer while height was measured in the standing position for children 2 years and older using a stadiometer to the nearest millimetre. Then, Body Mass Index (BMI) was calculated using this formula:

$$BMI = \text{weight (kg)} / \text{height (or length)} (m^2)$$

During admission procedure, clinical evidence of dehydration was verified in accordance to WHO guidelines [18]. With the use of the 2009 WHO growth standard, children who had

weight less than -2 SD were defined as wasting while children who had length (height) for age less than -2 SD were defined as stunting [19].

Blood samplings were collected and serum sodium, potassium, bicarbonate, Blood Urea Nitrogen (BUN), creatinine and complete blood counts were measured. Serum zinc levels were measured by flam atomic absorption spectrometry. The time of blood drawing and fasting status were recorded. Serum zinc levels equal or higher than 65 µg/dL in the morning sample or 57 µg/dL in the afternoon sample were considered normal [20]. Routinely urine analysis and faecal examination were performed at admission by central laboratory unit. Faecal examination for rotavirus was tested with immunochromatography assay (Rota-strip, Coris Bioconcept, Belgium). A stool culture for the detection of disease-causing bacteria was performed by central laboratory unit. The primary outcome of this pilot project was to determine the prevalence of zinc deficiency in children admitted to hospital with acute diarrhoea. Due to the lack of data specific for Thai children, to calculate the sample size we assumed that the prevalence of zinc deficiency in Thailand would be similar to that reported in India, namely as 42% [21]. With the level of confidence of 95% and a precision of 14%, a sample size of 50 patients was required.

STATISTICAL ANALYSIS

Data were analysed using SPSS version 23.0 statistical package (SPSS, Chicago, IL, USA). Normal distribution of data was assessed using a one-sample Kolmogorov-Smirnov test. Normally distributed variables were descriptively presented as means and standard deviations whereas non-normally distributed variables were descriptively presented as medians and IQR. The Pearson's Chi-square or Fisher-exact test was used, where appropriate, to compare proportions between groups. The Student's t-test and Mann-Whitney U-test were used to verify the differences of the normally distributed and non-normally distributed variables of the two groups, respectively. A p-value of less than 0.05 was considered as statistically.

RESULTS

The study population comprised 50 previously health children diagnosed with acute watery diarrhoea (50% female), with a mean age of 25.8±15.0 months (range 6.0-58.0 months). The patient's demographic and clinical characteristics are presented in [Table/Fig-1]. Clinical assessment revealed wasting in 8 children and stunting in 4 children. The mean serum zinc concentration upon admission of the study population was 69.2 µg/dL while 22 patients (44%) had low serum zinc levels. Mean serum zinc concentration at the time of hospitalization in patients with low serum zinc levels was 53.6±8.5 µg/dL compared to 81.5±14.4 µg/L in patients with normal zinc level (p<0.001). There was no significant difference in prevalence of wasting or stunting between children with normal or low zinc levels (p=0.439 and p=1.000, respectively). The median duration of diarrhoea prior to admission was 24 hours (IQR, 12-72 hours) and the frequency of diarrhoeal episodes was 4 times (IQR 3-6 times). The frequency of associated symptoms was as follows: vomiting in 35 patients (70%), abdominal pain in 10 (20%), fever in 39 (78%), cough in 34 (68%), sore throat in 25 (50%) and headache in 3 (6%). Twenty-one patients (42%) had no or mild dehydration at the time of enrolment and the rest had moderate dehydration.

There were no significant differences in term of age, sex distribution, nutritional status and clinical characteristics including duration and severity of diarrhoea proceeding to hospitalization between groups [Table/Fig-1]. Laboratory findings at time of hospitalization are presented in [Table/Fig-2].

Characteristic	All patients (n = 50)	Normal zinc levels (n = 28)	Low serum zinc level (n = 22)	p-value
Age (months)	25.8 (15.0)	23.9 (13.5)	28.4 (16.7)	0.295
Male (n %)	25 (50)	13 (46.4)	12 (54.5)	0.776
Weight (kg)	12.0 (4.0)	11.5 (3.9)	12.5 (4.2)	0.359
Height (cm)	86.4 (14.9)	84.4 (12.4)	88.8 (17.6)	0.330
Body mass index (kg/m^2)	16.00 (3.24)	16.10 (3.67)	15.87 (2.67)	0.800
Wasting (n %)	8 (16.0)	6 (21.4)	2 (9.1)	0.439
Stunting (n %)	4 (8.0)	2 (7.1)	2 (9.1)	1.000
Duration of diarrhoea before enrolment* (hours)	24.0 (12.0-72.0)	48.0 (12.0-72.0)	24.0 (12.8-30.0)	0.196†
Number of diarrhoeal episodes in preceding 24 hours*	4 (3-6)	4 (3-6)	4 (3-7)	0.960†
Vomiting (n %)	35 (70)	18 (64.3)	17 (77.3)	0.367
Duration of vomiting* (hours)	24.0 (9.0-48.0)	24.0 (24.0-48.0)	13.0 (5.5-36.0)	0.079†
Number of vomiting episodes in preceding 24 hours	2.7 (2.8)	2.5 (2.6)	3.1 (3.2)	0.465
Hydration status (n %)				0.569
Minimal or no dehydration	21 (42)	13 (46.4)	8 (36.4)	
Moderate dehydration	29 (58)	15 (53.6)	14 63.6)	
Body temperature (°C)	38.0 (1.0)	37.9 (0.9)	38.2 (1.0)	0.266
Abdominal pain (n %)	10 (20)	4 (14.3)	6 (27.3)	0.302
Cough (n %)	34 (68.0)	21 (75.0)	13 (59.1)	0.360
Sore throat (n %)	25 (50.0)	15 (53.6)	10 (45.5)	0.776
Headache (n %)	3 (6.0)	0 (0)	3 (13.6)	0.079

[Table/Fig-1]: Demographic and clinical characteristics of the participants present as mean (SD) unless otherwise indicated.
*Presented as median (interquartile range); †Mann-Whitney U test

Characteristic	All patients (n = 50)	Normal zinc levels (n = 28)	Low serum zinc level (n = 22)	p-value
Zinc levels (µg/dL)	69.2 (18.5)	81.5 (14.4)	53.6 (8.5)	<0.001
Haemoglobin (gm/dL)	11.9 (1.4)	11.9 (1.2)	12.0 (1.6)	0.684
Haematocrit (%)	36.3 (4.0)	36.1) (3.2)	36.6 (4.9)	0.688
White blood cell count ($x10^3/mm^3$)	12.2 (4.5)	11.8 (4.3)	12.6 (4.8)	0.534
Neutrophil (%)	60.1 (17.4)	57.6 (17.2)	63.4 (17.5)	0.242
Platelet count ($x10^3/mm^3$)	373.1 (110.4)	385.6 (119.1)	357.1 (98.6)	0.371
Blood urea nitrogen* (mg/dL)	8.5 (5.2-11.4)	8.4 (5.3-11.7)	8.7 (4.3-11.4)	0.939†
Creatinine* (mg/dL)	0.29 (0.26-0.35)	0.28 (0/25-0.33)	0.31 (0.27-0.38)	0.184
Sodium (mmol/L)	136.0 (3.0)	136.5 (2.9)	136.3 (3.0)	0.165
Potassium (mmol/L)	4.0 (0.6)	4.0 (0.7)	4.0 (0.5)	0.673
Chloride (mmol/L)	106.0 (3.7)	106.4 (3.6)	105.5 (3.8)	0.421
Bicarbonate (mmol/L)	17.8 (2.9)	18.3 (3.3)	17.3 (2.3)	0.249
Urine specific gravity*	1.015 (1.005-1.020)	1.015 (1.010-1.020)	1.020 (1.010-1.021)	0.528†
Faecal leukocytes > 10/OIF‡ (n %)	5 (10)	0 (0)	5 (22.7)	0.012
Stool pathogens (n %)				
Bacteria (n %)	7 (14.0)	4 (14.3)	3 (13.6)	1.000
Rotavirus (n %)	3 (6.0)	1 (3.6)	2 (9.1)	0.576

[Table/Fig-2]: Serum zinc levels and other laboratory findings of the participants present as mean (SD) unless otherwise indicated.
*Presented as median (interquartile range); †Mann-Whitney U test; ‡OIF: Oil immersion microscopic field

There were no significant differences in complete blood count, blood urea nitrogen, creatinine and serum electrolytes. Heavy faecal leukocyte (>10/ oil immersion microscopic field) was found in 5 patients and all had low serum zinc levels (p=0.012). Enteric pathogens were detected in 10 patients with no significant difference in prevalence between groups. Salmonella (non-typhoid) was detected by stool culture in 4 and 2 patients with normal and low zinc levels, respectively. One patient with zinc deficiency had shigella infection whereas rotavirus was detected in 3 patients.

DISCUSSION

This cross-sectional study demonstrated that nearly half (44%) of children younger than five years old presenting with acute diarrhoea had low serum zinc levels. There were no differences in terms of nutritional status, age, sex distribution or clinical features between patients with normal or low zinc levels; however, patients who had heavy faecal leukocyte had lower serum zinc levels.

Although, the profound benefits of zinc supplementation in the management of diarrhoea have been established, there are some limitations to the implementation of this treatment strategy for Thai children. Currently, zinc is not routinely used to treat most cases of children with acute diarrhoea in Thailand because of lack of data of zinc status [22] and also concern that high zinc intake may compete for absorption with other nutrients such as iron and calcium. The present study shows that there is a high prevalence of zinc depletion in children with acute diarrhoea. This underscores the vital role of zinc in the maintenance of mucosal immune response to enteric pathogens [23], intestinal epithelial integrity [12] and subsequently, a key determinant of morbidity and mortality of acute and chronic diarrhoeal diseases [24].

Serum zinc concentrationis regarded as the best available biomarker to identify a risk of zinc deficiency in the populations. The joint committees of the World Health Organization (WHO), the United Nations Children's Fund (UNICEF), the International Atomic Energy Agency (IAEA), and the International Zinc Nutrition Consultative Group (IZiNCG) recommend, using of serum zinc concentration as a standard objective and quantitative assessment of the zinc status of a population [25]. The rationale to support of use of serum zinc concentration as a standard biomarker to identify zinc status is that serum zinc reflects dietary zinc intake. Moreover, it responds consistently to zinc supplementation and reference data are available for most age and sex groups. Therefore, the finding in the present study may indicate that children with acute diarrhoea are at risk of zinc deficiency.

Zinc deficiency increases susceptibility to diarrhoea via several mechanisms. A study in mice has shownthat zinc deficiency can induce profound effects on the intestinal micro- and macroscopic morphology such as decreased villous height and crypt depth, infiltration of the lamina propria by activated inflammatory cells as well as and loss of intestinal mucosal integrity [26]. Conversely, diarrhoeal diseases can impact both tissue and serum levels of zinc, via reduced intestinal absorption and increased faecal loss [27]. In the present study, serum zinc levels were measured during the episode of diarrhoea, therefore, a transient effect of the diarrhoea on the serum zinc level cannot be ruled out.

The present study had a robust design, utilized the new guideline for cut-off levels [20] to identify zinc deficiency which accounted for 4 confounding variables includingage, sex, time of day of blood sample collection and fasting status of subjects. Prevalence of zinc deficiency in the present study was relatively higher than that reported from Indian children with acute diarrhoea which the prevalence ranged from 28.4% to 41.7% [21,28,29]. The difference in prevalence may partially explained by the difference in the cut-off level, used to identify zinc deficiency.

There is scarce data of zinc status in Thai children. While the prevalence of low zinc levels in healthy Thaiinfants has been shown, our study is the first, to our knowledge, to demonstrate serum zinc levels in diarrhoeal patients in Thailand. Wasantwisut et al., reported mean serum zinc concentration at the level of 72.3 µg/dL in healthy infants, aged 4-6 months, which does not completely represent the total spectrum of children who suffer from severe diarrhoea [30]. Overall prevalence of zinc deficiency in their study ranged from 33%-50%, depending on the cut-off values, highlighting the high prevalence of zinc deficiency even in apparently children in Thailand.

The present study found that patients who had heavy faecal leukocyte had a tendency towards low serum zinc levels. High faecal leukocyte are associated with inflammatory causes of diarrhoea [31], and chronic inflammatory processes that impair intestinal absorption of zinc, such as diarrhoea, could lead to perturbations in zinc homeostasis [32].

The present study has some strengths and limitations. Notably, this is the first study in to show that high prevalence of zinc deficiency in Thai children with acute diarrhoea. This finding will emphasise the need of further studies to address the role of zinc and zinc supplementation as a standard supplementation in children suffer with acute diarrhoea. Furthermore, the present study used the new standard recommendation of the cut-off levels for diagnosis of zinc deficiency.

LIMITATION

The study has some limitations. Firstly, the population size was small; however, it was sufficient to detect prevalence of low zinc levels in diarrhoeal children which close to expectation of 42% in hypothesis. Secondly, the study did not measure dietary zinc intake of the participants. Zinc absorption is inhibited by dietary phytates or dietary components, thereby affecting zinc bioavailability [33,34]. Therefore, we were unable to estimate the possible impacts of these dietary factors on our measured serum zinc levels. Moreover, all dietary assessment methods have a limitation in usage, high risk of bias and may not be accurate in assessment especially for children [35]. Finally, enteric pathogens were found in few of our study participants. This could be attributed to the limited capacity of our laboratory to detect a wide array of enteric pathogens.

CONCLUSION

Our study sheds light on the prevalence of low serum zinc levels in children presenting with acute diarrhoea in Thailand, utilizing the most recent guidelines in the interpretation of serum zinc assay results. We have shown that a large proportion of children with diarrhoea have low zinc levels, underscoring the urgency for more efforts towards increasing the coverage of zinc supplementation in under-5 children. Further supplement studies of diarrhoeal children are encouraged.

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