Serum Zinc Level In Children With Acute Diarrheal Disease

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Abstract

Background: Acute diarrhea remains a leading cause of death among infants and young children in low income countries. Zinc deficiency is associated with an increased risk of gastrointestinal infections, adverse effects on the structure and function of the gastrointestinal tract, and impaired immune function.

Aim of the work: Our aim of work is to determine the role of Zinc as a risk factor in the development of acute diarrhea in children.

Methods: Ninety children with acute diarrhea and eighty age-matched controls were enrolled. All of them were subjected to complete blood picture, stool analysis, serum Zinc level and serum electrolyte (for cases only).

Results: When comparing the mean serum zinc level among cases and controls there was no statistically significant difference found.

There was statistically significant difference between cases and controls as regards to hemoglobin level and TLC.

Stool analysis showed combined infection of ameba and oxyuris infections in 30% of cases in contrast to16.3% of controls .Also amebiasis was found in54.4% of our cases and 37.5% of the control group.

A significant association was found between mean serum zinc level and serum sodium level; high mean serum zinc level was noticed in patients with low serum sodium level. There was no correlation between mean serum zinc level and patient's age, their anthropometric measures, frequency of diarrhea, and time of weaning, hemoglobin level, serum Na level and serum K.

Key Words: Zinc, Diarrhea, Dehydration, Children

INTRODUCTION

Acute diarrhea remains the leading cause of death among infants and young

children in low income countries (**Bryce** *etal*. **,2005**)

Every year more than a million children less than five years of age succumb to the fluid loss and dehydration associated with the majority of diarrhea related deaths (**Mathers** *etal*.,**2007**)

In2008 the WHO formed an estimation of child mortality due to diarrhea in developing countries. In Egypt proportion of diarrhea death in children under five death is 47.7 % in 1980 (**Boschi** *etal*.,**2008**)

The scientific community, over the past four decades, established a consensus on the most effective measures to reduce the incidence, morbidity, and mortality of acute diarrheal disease (**Assis** *etal*.,**2013**).

Exclusive breastfeeding for at least 6 months and supplemented up to 2 years of age has a significant impact in reducing the disease incidence and severity. In the field of biomedicine, the development of a vaccine against rotavirus and universal vaccine coverage are important contributions that have an impact on acute diarrheal disease incidence, by decreasing the severe forms and the number of hospitalizations, thus reducing the risk of death (**UNICEF.,2009**).

Most studies were conducted in poor regions and recruited children at higher risk of developing more severe diarrheal episodes, including persistent diarrhea. At that moment, the recommendation was to use zinc associated with Oral Rehydration Therapy for all children younger than 5 years old (**Lamberti** *etal*.,**2013**).

In 2004, the WHO and UNICEF brought attention to the impact of zinc in reducing the severity of the diarrheal episode and the number of subsequent ADD episodes in children younger than 5 years (**WHO/UNICEF,2004**).

Zinc is the intrinsic metal component or activating cofactor for more than 70 important enzyme systems, including carbonic anhydrase, the alkaline phosphatases, dehydrogenases, and carboxypeptidases. It is involved in the regulation of nucleoproteins and the activity of various inflammatory cells and plays a role in growth, tissue repair and wound healing, carbohydrate tolerance, and synthesis of testicular hormones. The highest concentrations of zinc in the body are found in the liver, pancreas, kidney, bone, muscles and eyes(**Steven** *etal*.,**2015**)

Zinc deficiency is associated with an increased risk of gastrointestinal infections, adverse effects on the structure and function of the gastrointestinal tract, and impaired immune function. Dietary deficiency of zinc is especially common in low-income countries because of a low dietary intake of zinc-rich foods (mainly foods of animal origin) or inadequate absorption caused by its binding to dietary fiber and phytates often found in cereals, nuts and legumes (**Haider** *and Bhutta.*,**2009**).

Acute zinc deficiency causes a decrease in innate and adaptive immunity, chronic deficiency increases the production of inflammatory cytokines, influencing the outcome of a large number of inflammatory diseases(**Bonaventura** *etal*.,**2015**).

Research in children suggests that zinc supplementation (20 mg per day for 10 days

in children older than two months) may play a crucial role in treating and preventing acute diarrhea, particularly in developing countries. Studies demonstrate a decrease in the risk of dehydration, and in the duration and severity of the diarrheal episode by an estimated 20% to 40% **(Bhutta *etal*.,2000).**

AIM OF WORK

Our aim of work is to determine the role of Zinc as a risk factor in the development of acute diarrhea in children.

Patients and methods

This case-control study was conducted in the outpatient clinic of Children's Hospital, Fayoum University, Egypt between February and September 2016. It included 90 children diagnosed as acute diarrhea and 80 children of matching age and sex as a control group.

Inclusion criteria:

1-Patients diagnosed as acute Diarrhea.

2- Age from 6months-6years old.

3- Both sexes.

4- Infective diarrhea either bacterial or viral in origin.

Exclusion criteria:

1 -patients with chronic diarrhea (diarrhea persisted more than 2weeks).

2 -Patients taking zinc therapy or drugs that interfere with zinc absorption.

3 -Patients with chronic debiliting disease and chronic malabsorption disorders.

In our study we considered acute diarrhea, according to WHO definition (i.e. the passage of three or more loose or liquid stools per day or more frequently than is normal for the individual for less than 2 weeks duration).

All patients were subjected to;

1-Full history taking focusing on

  - Duration, frequency, character of diarrhea and repeated episode.

  - Associated symptoms as vomiting, fever and abdominal pain.

  - History of drug intake, change type of artificial milk, history of cow milk intake before attack of diarrhea and recent addition of new foods, history of same condition in another member of family (food poisoning).

   -History of complications of diarrhea as convulsion, oliguria, abdominal distension and nutritional history in the form of type of feeding, time of weaning.

2- Clinical assessment

 -Full general examination with special emphasis on signs of dehydration, growth parameters and abdominal examination.

Laboratory Investigation

- Complete blood picture
- Stool analysis

- Serum Zinc level (for cases and controls)
- Serum electrolyte (for cases only).

After fulfilling the inclusion criteria and taking proxy consent, a sample of 8ml venous blood sample was collected from children diagnosed as acute diarrhea 3ml from healthy children in sterile tubes.

The blood sample was divided into: 5ml in plain tubes for serum collection

3ml in EDTA tubes for CBC

Plain tubes were incubated in 37 C for 15 minutes then centrifuged at 3000 rpm for 20 minutes at room temperature.

Sera were separated and divided into two aliquots; one for Na, K and the other one was stored at- 20 for zinc analysis.

We use AU480 in chemical analysis for Na, K. and Spectrophotometer 5010 for Zinc analysis

Measurement principles

Zinc forms with 2(5-Brom 2-pyridylazo)5-(N-propyl-N sulfo-propylamino).Phenol a red chelating complex

The increase of absorbance was measured and it was proportional to the concentration of total zinc in the sample.

EXPECTED VALUES

Serum /Plasma children		
<4 months	65-137 µg/dl	10-21 µmol/l
4-12months	65-130 µg/dl	10-20 µmol/l
1-5years	65-118 µg/dl	10-18 µmol/l
6-9years	78-105 µg/dl	12-16 µmol/l
10-13 male	78-98 µg/dl	12-15 µmol/l
Female	78-118 µg/dl	12-18 µmol/l
14-19 male	65-118 µg/dl	10-18 µmol/l
Female	59-98 µg/dl	9-15 µmol/l

Statistical Analysis

- Data was collected and coded to facilitate data manipulation and double entered into Microsoft Access and data analysis was performed using SPSS software version 18 in windows 7.

- Simple descriptive analysis in the form of numbers and percentages for qualitative data, and arithmetic means as central tendency measurement, standard deviations as measure of dispersion for quantitative parametric data, and inferential statistic test:

- **For quantitative parametric data :**
  - In-depended **student t-Test** used to compare measures of two independent groups of quantitative data

▪ One way **ANOVA** test in comparing more than two independent groups of quantitative data.

- **For qualitative data**

▪ **Chi square** test to compare two of more than two qualitative groups.

- **Bivariate Pearson correlation test** to test association between variables

• The level $P \leq 0.05$ was considered the cut-off value for significance.

Ethical Consideration:

This study was reviewed by the Faculty of Medicine Research Ethical Committee. The participants were informed by the researcher about the objectives of the study, the examination, investigation that was done. Also the confidentiality of their information was respected and their right not to participate in the study was ensured.

Discussion

Zinc is one of the most important essential trace metals of human nutrition and its deficiency is a world nutritional problem. This work compiles information about the serum level of zinc in diarrhea.

Diarrhea remains the second leading cause of death among children under 5 years in the developing world **(Bryce *etal*., 2006).**

WHO found that the percentage of under-four years deaths due to diarrhea in **Egypt** was 5% from all causes of death in 2015 , prematurity 24% , congenital anomalies 21.1%, lower respiratory tract infection 12.7% , birth trauma10.7% , non-communicable disease 8.5% ,perinatal and nutritional cause 7.3%, injures 5.7% , sepsis and other infection 2.9% , tetanus 0.9% , meningitis and encephalitis 0.5%, pertussis 0.6% and measles 0.2% **(WHO, 2015).**

We studied 90 patients with acute diarrhea; their mean age was 27.2 ± 23.6 months ranged between 6 and 72 months. Females were 52.9% of our patients, 62.2% of the mothers were educated and 37.8% were illiterate. As regards the residence 62.2% were from rural areas. The majority of our patients 86.7% had pipe sewage disposal.

Prevalence of diarrhea by **Egypt Demographic and health survey in 2005, 2008 and 2014,** found that 19.1% of ill children with diarrhea were from rural areas in 2005, 8% in 2008 and in 2014 were 14.9%. Also found that illiterate mothers were 19.5% in 2005, 7.5%in 2008 and increased again to 16.4% in 2014.

In our study, the mean body weight was 8.3± 2.1 kg, the mean height was 65.8±6.3 cm and the mean head circumference was 42.6±1.9cm.

The majority of our cases (61.1%) were between the 10th -90th percentiles for weight, 28.3% were $\leq$ 5 th percentile in head circumference and 42.3% $\leq$5 th percentile for height.

Height-for-age, a measure of nutritional stunting, is the best known and easiest to measure of the adverse outcomes associated with zinc deficiency in populations. Stunting prevalence is expressed as the percentage of children under 5 years of age with height-for-age below the expected range of a reference population (i.e., <2.0 standard deviations with respect to the reference median) (**Brown** *etal*., **2004).**

WHO considers a prevalence of stunting greater than 20% of the population indicates a public health concern. Although zinc deficiency is not the only factor affecting children's growth, assessment of dietary zinc intake and serum zinc levels can be used to confirm the risk of zinc deficiency in these high-risk countries (**Brown** *etal*., **2004).**

The etiology of stunting includes the following: (1) Nutritional (energy, macronutrients, micronutrients and toxic factors) (2) Infection (injury to gastrointestinal mucosa, systemic effects and immune stimulation) and 3) Mother-infant interaction (maternal nutrition and stores at birth, and behavioral interactions (**Nazanin** *etal*., **2013).**

Poverty, poor food choices, lack of availability or decreased accessibility to certain foods, coupled with the lack of knowledge about the importance of food group diversity for the health and growth of young children may limit the inclusion of micronutrient-rich foods in the diets of children. Deficiencies of iron, iodine, zinc, and vitamins A and B-12 are a major concern among children globally (**Krebs, 2007)**.

In our study there was no statistically significant correlation between serum zinc level and anthropometric measures. A study in **Sultan Qaboos University** found that serum zinc was not affected by age, weight, height or head circumference (**Abolfazl** *etal*., **2015).**Also in another study in **Iran**, they found that the mean serum zinc concentrations were significantly lower among those with lengths less than the fifth percentile for age than among those with

lengths greater than the fifth percentile for age **(Conrad *etal*., 2010).**

In addition the study in **Sultan Qaboos University** found that children with acute bloody diarrhea had significantly lower serum zinc levels in comparison with healthy children. Additionally, hypozincaemia was observed in half of the children with acute bloody diarrhea and some of those with acute watery diarrhea while none of the control group had hypozincaemia. This reduction in serum zinc levels may be related to either the excretion of zinc following acute diarrhea or metabolic reactions against the infections (known as acute phase responses) or both **(Abolfazl *etal* ., 2015)**.

In our study there was no statistically significant difference in serum zinc level between cases and controls. Similarly, a study in **Iran** showed a normal range of serum zinc level among cases with acute diarrhea and controls **(Elham *etal*., 2015).**But in **India,** compared to healthy controls, serum zinc level was higher among ill children with diarrhea **(Arora *etal*.,2006).**

In our study we found that there was no statistically significant difference in serum zinc level between males and females**, Abolfazl *etal*.,(2015)** also found no

difference in zinc level between males and females, in contrast a study in **Shahed University Iran ,Chomeili *etal*.,** **(2004)** found in their study that girls had lower serum zinc level than boys.

Also a study in **Turkey** showed that serum zinc levels were not affected by gender and age **(Akgün *etal*., 2007).**

In our study there was no statistically significant difference in serum zinc level and different types of feeding, in contrast another study showed that the children who were fed breast milk had higher serum zinc level than those formula fed **(Chomeili *etal*., 2004)**.

Vomiting was the main presenting symptom associated with diarrhea found in 70 children (77.8%), followed by abdominal pain in 63 (70%) and fever in 58 of them (64.4%). There was no association between serum zinc level and the symptoms and signs of our cases .A Study of the prevalence of zinc deficiency in **Brazil** found that the serum zinc level was not changed by symptoms **(Ferraz *etal*., 2007).**

In our study, 29 of our cases (32.1%) showed signs of dehydration, 26.7% of them had mild dehydration while 5.6% had moderate dehydration .None of our patients

had severe dehydration. There was no statistically significant difference in serum zinc level among patients with and without dehydration level (p-value >0.05). **Akgün etal., (2007)** found that the serum zinc level of their dehydrated patients (28% of their patients)was higher than those with no dehydration.

In our study, serum electrolytes were assayed in children with acute diarrhea. The mean level of serum Na was 137.3± 2.3, ranged between132 and 146 mmol/l, where a high mean serum zinc level was found in children with hyponatremia (P-value <0.05).

The mean serum K was 4.1±0.5 ،however there was no statistically significant difference in serum zinc level and different potassium level (with p-value> 0.05).

The character of diarrhea was watery in 98.8 % and 1.1% had bloody diarrhea. A bacterial cause was suspected in 35.6% of the cases due to the presence of pus cells (5-25/ HPF) in stool analysis of 32 children, associated with high total leucocytic count and neutrophilia. RBCs in stool were present in one patient (1.1%).

We suspected also a viral cause because some of the cases had watery diarrhea and samples were collected during winter time, but specific investigation weren't done.

Parasitic infestation in the form of oxyuris worms was present in 10% of our cases and 10% of the controls.

Parasitic infections are more common in locations where there is unsafe drinking water and poor handling of sewage. Infection with a parasite is uncommon in developed countries but may be seen in children who have recently ingested contaminated water or who have traveled to or lived in developing countries.

It may last for weeks to months. Intestinal protozoan infections by Giardia and Cryptosporidium are common in humans worldwide. Especially important are infections in children, during pregnancy, and among individuals with HIV/AIDS **(Savioli etal., 2006).**

Amebiasis was the most common protozoal infection found in our cases (54.4%) and in (37.5%) of the control group. Surveillance for distribution of all enteric infections diarrheal disease in children aged younger than 5 years seeking medical advice at **Abu Homos and Manshayet Nasser hospitals from 2005-2007 in Cairo**, **Egypt** found that viruses were 48% ,parasites 26%

and bacteria in 27%   **(El-Mohamady** *etal*.**,2006).**

Prevalence and incidence of Entamoeba histolytica infection in **South Africa** and **Egypt** showed that **Egypt** has high rates of asymptomatic infection of ameba detected in the stool (>21%) **(William** *etal*.**, 2006).**

Combined infection with ameba and oxyuris infestations was found in 30% of our cases and 16.3% of control groups, whereas Giuardiasis was present in 3.3% of the cases and 3.5% of the controls.

Recurrent asymptomatic and symptomatic infections by intestinal parasites among young children can have long-term effects on overall growth and development. A study conducted on children residing in **Mexico** City   **(Long** *etal*.**, 2007)** reported that vitamin A and zinc (20mg of elemental zinc as zinc methionine, daily for 1year) reduced G.lamblia incidence, whereas zinc supplementation alone decreased Entamoeba histolytica-associated diarrhea.

A study in **Iran**, found a decrease in serum zinc levels in 50% of Giardia positive group **(Zarebavani** *etal*.**, 2012).**

In our study a high serum zinc level was found in children with normal stool analysis while children with ameabiasis also showed a high mean serum zinc level.

In our study the mean hemoglobin level was 10.5±0.97 (g/dl), the mean total leucocytic count was10.69±2.4 (cells/m³) and the mean platelet count was 320±66 (cells/m³).

There was statistically significant difference between cases and controls as regards to the hemoglobin level and TLC with most of our cases had low hemoglobin level with most of our cases had low hemoglobin level with low mean corpuscular volume and low mean corpuscular hemoglobin(92.2% versus 77.5%), and leukocytosis (51.1% versus11.3%) with P value respectively **0.009 and <0.05**.

On the other hand, there was no statically significant difference in platelet count between cases and controls. Information on anemia levels obtained in **2005** by **Egypt Demographic and Health Survey** showed that the level of anemia among children aged 6-59 months was much higher in 2005 than in 2000 (49% and 30%, respectively), and rural children were more likely to be anemic than urban children (51% and 44%, respectively); and children in rural Upper **Egypt** had the highest anemia levels.

The frequency of anemia in **Egypt** (as developing countries) is much higher than developed countries, a study in **Fayoum** University hospital aimed to investigate the prevalence of anemia in **Al-Fayom** Governorate (**Al Ghwass** *etal* .,2015) and the study in **Cairo** and **El**-**Minofia** found that the frequency of iron deficiency anaemia was 64% among the studied group. This is higher than other Egyptian studies where the prevalence of iron deficiency anaemia was 43% and 55% among studied sample of school children in **Cairo** and **El**-**Minofia**, respectively (**Elalfy** *etal* **in 2012).**

Therefore, it isn't surprising to find a low hemoglobin level with low mean corpuscular volume and low mean corpuscular hemoglobin in 92.2% of our cases and 77.5% of the control group.

Pallor was found in ten children of our cases, iron deficiency anemia was found with reduced serum iron, ferritin and total iron binding capacity.

Their mean serum iron was 54.2±22 ranged from (31-90) with normal range (100-150 micro gm/dl), the mean serum ferritin was 11.1±2.7 ranged from (5-15), with normal range (15-25 nano gm/dl) and the mean TIBC was 512±341 ranged from

(159-250) with normal range (250-450 micro gm/dl).

Iron deficiency anemia is the most common micronutrient deficiency–associated anemia in the world, affecting up to 60% of children globally .In the United States, the prevalence of anemia (defined as low hemoglobin) continues to be high among children from low-income families, with a prevalence of 15% for preschoolers **(Food and Nutrition Board, Institutes of Medicine ,2005**).

A Study in **Turkey** for serum zinc levels in patients with iron deficiency anemia, showed that serum zinc levels were lower in anemic patients

(**Kelkitli** *etal***., 2016).**

Micronutrient deficiencies are caused by inadequate dietary intake, increased losses from the body, and/or increased requirements .Zinc and iron usually occur together in food sources. Foods with a high content of dietary zinc and iron include oysters, beef, turkey ،chicken, fortified cereals, and processed bean
(**Us Department of Agriculture , 20011).**

However, the bioavailability of micronutrients is less than anticipated despite the substantial quantities found in some of these foods (**Avalos** *etal***.,**

2004),certain components of cereals and legumes, such as phytic acid, fiber, and calcium, affect zinc and iron absorption **(Hambidge *etal*., 2008)**

In our study, the mean serum zinc level was91.9 ±18.5 µg/dl in the normal range, also there was no correlation between serum zinc level and frequency of diarrhea, hemoglobin level, sodium level and potassium level.

Many studies have reported contradictory results on the effect of zinc in the treatment of acute diarrhea. A study of 3–59 month old children affected with acute diarrhea in **Bangladesh** indicated that the daily administration of 20 mg of zinc reduced the duration and frequency of diarrhea **(Baqui *etal*., 2002).**

Also another study in **Brazil**, found that the administration of zinc reduced the duration of diarrhea among Brazilian children <5 years old **(Al-Sonboli *etal*.,2003).**

Zinc supplementation enhances serum zinc concentration when given as a treatment for diarrhea and helps children maintain a more adequate zinc status during the convalescent period **(Baqui *etal*., 2006).**

A study by **Bahl *etal*., (2002) in India,** revealed that the administration of zinc

along with ORS reduced the severity of acute diarrhea among children between 6–35 months old In contrast, **Patel *etal*.,(2005)** demonstrated that the administration of zinc did not affect the duration of or rate of complications arising from acute diarrhea among children aged 6–59 months receiving either ORS and zinc or ORS and a placebo. The researchers suggested that the inefficacy of zinc in their study may have been attributable to a low dose of zinc, poor compliance and the failure of the supplements to replenish the zinc loss**.** Other studies have confirmed these results (**Long *etal*., 2006)** .

The main limitation of our study is the limited number of patients and lack of studying the effect of zinc administration in the course of diarrhea.

Conclusion:

Serum zinc level was found normal in children with acute diarrhea as well as the control group and it did not correlate with the frequency of diarrhea, degree of dehydration, hemoglobin level, serum Na level and serum K.

Recommendations:

1-Stunting is an important health problem that needs to be addressed by improving nutrition of infants and children.

2-Health education programs should focus on rural areas to improve their environment.

3-Amoebiasis and parasitic GIT infections are endemic in Egyptian children and should be properly treated.

4- Anemia is prevalent in Egyptian children; we should increase knowledge and accessibility to proper food choices.

5-Improve hygiene to reduce the risk of acute diarrhea by hand washing with soap and water.

6-Zinc is an important micronutrient trace element in the body that is unlikely to be deficient in children with acute diarrhea .However studies with large numbers of patients are needed to elucidate the specific role of zinc in children with diarrhea.

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