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Serum zinc levels of hospitalized children with acute diarrhea differ by the isolated viruses

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ABSTRACT

Background: Acute diarrhea constitutes a major global burden to morbidity and mortality in under-five children. Research has shown that micronutrient zinc plays a pivotal role in childhood diarrhea; however, there are contradictory reports of its therapeutic benefit across the various causative enteropathogens.

Objectives: The aim of the study was to determine the prevalence of viral etiology (rotavirus, adenovirus, and norovirus) and compare the serum zinc levels of children with acute diarrhea.

Methods: A comparative cross-sectional study in which 100 hospitalized children with acute diarrhea aged one–59 months and 100 controls were recruited. Viruses were investigated from stool specimens using the immunochromatographic technique, while serum zinc was determined through the colorimetric method. Data analysis was with SPSS 20 software package.

Results: The prevalence of viruses in the subjects was 62.0% with rotavirus isolated in 30 (30.0%) of the patients, while adenovirus and norovirus were detected in 21 (21.0%) and 11 (11.0%) patients, respectively. Rotavirus was the only virus detected in 2 (2.0%) of the controls. The mean serum zinc level of 65.3 ± 7.4 µg/dl in the subjects was significantly lower than 69.0 ± 6.5µg/dl in the controls ($P < 0.001$). The prevalence of zinc deficiency in the subjects (47.0%) was significantly higher than 32.0% in the controls ($P = 0.030$). The mean serum zinc levels differed significantly among the viruses isolated in the subjects ($P < 0.001$).

Conclusions: Viruses contribute largely to etiology of acute diarrhea in Nigerian under-five children. Zinc deficiency was also highly prevalent in the study population. The serum zinc levels varied significantly across the different viruses isolated.

Keywords: Acute diarrhea, adenovirus, children, Nigeria, norovirus, rotavirus, zinc

Introduction

In 2015, acute diarrhea accounted for 8.6% of the 5.8 million deaths in children less than 5 years globally, constituting a major health challenge in this vulnerable age group.[1] It is the fourth leading cause of morbidity and mortality in under-five children worldwide.[1,2] Developing countries bear the greatest burden of the disease as it contributes 10% each to deaths in Nigeria, India, and Pakistan, which together account for 40% of global under-five mortality.[3,4] Enteric viruses such as rotavirus, adenovirus, and norovirus are the leading causes of viral infective diarrhea together constituting 41% of mortalities due to childhood diarrhea.[1] Of note is rotavirus constituting the highest pathogen-specific diarrhea morbidity and mortality with recent global estimates showing that it accounted for about 30% of deaths due to childhood diarrhea.[5] Furthermore, Nigeria has the second-largest number of estimated rotavirus deaths globally, singularly accounting for 32% of global rotavirus deaths in 2015.[5]

These viruses disrupt intestinal function through enterotoxin production and mucosal damage resulting in net secretion of fluid and electrolytes.[6,7] Diarrhea, with associated symptoms such as vomiting and fever, may lead to rapid clinical deterioration with subsequent dehydration, electrolyte imbalance, and shock.[6,7] Despite the high burden, these viruses contribute to childhood diarrhea, there is a dearth of information regarding their prevalence in developing countries due to non-performance of routine diagnostic tests.[8] This is attributable to factors such as weak health systems and low laboratory capacity making the generation of quality data as well as the institution of control strategies difficult.[8]

Zinc is an important micronutrient with enzymatic, structural and regulatory functions show to be necessary for protein synthesis, immune function, cell growth and differentiation, and intestinal transport of water and electrolytes.[9,10] Zinc deficiency contributes substantially to the morbidity and mortality of young children worldwide as evidenced by its high prevalence and association with diarrhea, pneumonia, and malaria.[11,12] Global estimates show that 17.3% of the population have inadequate zinc intakes, with the highest prevalence in sub-Saharan Africa and South Asia.[12,13] Zinc deficiency has also been shown to be responsible for nearly 4% under-five deaths and 1% of all years lost due to ill health, disability or early death (disability-adjusted life years).[12]

Studies have documented the beneficial effect of zinc supplementation on the duration and severity of diarrhea, thereby reducing mortality.[14-16] This lead to a recommendation by the World Health Organization and United Nations Children's Fund for the use of zinc supplementation for all children with diarrhea.[17] However, some studies have reported that the therapeutic benefit of zinc may not cut across all pathogens causing acute diarrhea.[18-21] Against this background, this study was conducted to determine the prevalence of rotavirus, adenovirus, and norovirus as viral etiology among children with acute diarrhea and compare their serum zinc levels.

Methods

We have previously reported the details of the study design in an earlier publication to determine serum zinc levels and bacterial pathogens in children with acute diarrhea.[22] This comparative cross-sectional study was conducted at the Emergency Paediatric Unit of the University of Ilorin (UITH) and Oke-ose community both located in Ilorin, Kwara State, North Central Nigeria. From between December 2015 and August 2016, eligible children aged between 1 and 59 months were recruited. Ethical approvals were obtained from the University of Ilorin Hospital Ethical Review Board (UITH/CAT/189/18^{A}/905) on December 17, 2014 and renewed on May 27, 2016 (UITH/CAT/189/19^{A}/637). All caregivers of eligible children were informed of the purpose of the study, expected procedures and potential risks and benefits following which written consent were obtained before sample collection.

A total of 100 children with acute diarrhea were recruited. Acute diarrhea was defined as passage of loose stools at least 3 times within a 24 h period lasting fewer than 14 days.[23] An equal number of apparently healthy age- and sex-matched controls were recruited from Oke-ose community where the hospital is located. The exclusion criteria were children with persistent diarrhea (>14 days), severe malnutrition, malaria, sickle cell disease, pneumonia, presence of gastrointestinal anomalies, prior zinc supplementation, and/or blood transfusion in the preceding 3 months, and previously recruited children.

Demographic and clinical details were obtained using a structured questionnaire which was pre-tested before commencement of the study. History of diarrhea was obtained from the caregiver of each subject, and a physical examination of all the study participants including weight and length/height was done. The weights and lengths of children aged <2 years and/or 12 kg were assessed using a bassinet weighing scale (Waymaster, England) and an infantometer, while older children had their weight and height measured using a beam balance weighing scale with a stadiometer attached (Marsdens, England).

The minimum sample size required for this study was determined using the formula: [24]

$$n = \frac{\left[p_1(1 - p_1) + p_2(1 - p_2)\right]}{(p_1 - p_2)^2} \times C_{p,power}$$

Where n is the desired sample size; p_1 is 69.1%;[25] p_2 is 44.0%;[25] and $C_{p,power}$ is 13 which was determined by $P = 0.05$ and 95% power.[24] A purposive sampling was used until the desired sample size was attained.

Fresh stool samples were collected into properly labeled sterile universal bottles and immediately processed or kept in a refrigerator at 4°C until processing (within 6–8 h of collection). The viruses were detected by immunochromatographic technique using rapid enzyme immunoassay kits manufactured by Oxoid Inc., United Kingdom. Fecal samples were diluted to 10% suspension by adding 1 ml of sample diluent from the immunoassay kit. Briefly, two drops (100 µl) each of diluted fecal samples, negative control, and positive control were added to separate microwells. Thereafter, two drops (100 µl) of enzyme conjugate were added to each microwell and incubated at room temperature for 60 min. The contents of the microwells were then poured out and washed with diluted wash buffer. Two drops (100 µl) of color substrate were added to each microwell and incubated at room temperature for 10 min. Results were interpreted according to the manufacturer's instructions.

Blood samples were collected for estimating serum zinc, total protein, albumin, and C-reactive protein. 5 mm of venous blood was drawn from the peripheral veins of all recruited children. The blood samples were then centrifuged and the sera obtained were transferred into acid-washed plain bottles and immediately stored at −20°C until the time of analysis. Serum zinc was analyzed with a Jenway™ spectrophotometer 6300 model (Jenway Limited, Essex, United Kingdom) for measuring optical density at 560 nm, after preparation with the zinc fluid monoreagent (5-Br-PAPS) kit (Centronic GmbH, Wartenberg, Germany), a quantitative colorimetric assay of Zn^{2+}. Children with levels of <65 µg/dl were considered zinc deficient.[26] Furthermore, serum total protein and albumin levels were determined using total protein assay and albumin

assay (Agappe Diagnostics Limited, Kerala, India). C-reactive protein levels were determined using C-reactive protein (CRP) assaykits (Monobind Inc., Lake Forest, CA, USA). This was done to provide an objective laboratory evidence for the presence or otherwise of ongoing inflammation at the time of the recruitment.[26]

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 20.0 software (IBM Corp., New York, USA). The mean and dispersion of quantitative variables and proportion for the qualitative variables were presented. Chi-square test was used to test for significance of the difference between categorical variables. The student *t*-test (t), analysis of variance (ANOVA) test, and the least significant difference (LSD) *post hoc* test were used when appropriate. The level of significance was established at $P < 0.05$.

Results

Demographic characteristics

There were 63 males (63.0%) and 37 females (37.0%) among the subjects, while the controls comprised 60 males (60.0%) and 40 females (40.0%). There was no significant difference between the two groups in terms of age, sex, and height ($P > 0.05$). However, underweight malnutrition shown by mean weight for age z score was significantly higher in the subjects when compared with the controls ($P < 0.001$) [Table 1].

Isolated viruses

Sixty-two (62.0%) of the subjects had viral etiology compared with 2 (2.0%) of the controls. Rotavirus accounted for 30 (30.0%) of the subjects while 21 (21.0%) and 11 (11.0%) of the isolates were adenovirus and norovirus, respectively. The two (2.0%) viruses isolated from the controls were rotavirus. There was a significant difference in the occurrence of all the viruses between the subjects and controls ($P = 0.001$) [Table 2]. Age distribution of infection in the subjects showed that infants had the highest infection rate comprising 50 (80.6%) of the population with rotavirus infection significantly higher than the other viruses ($P = 0.004$) [Table 3].

Serum zinc and other biochemical parameters

The mean total serum zinc levels of 65.3 ± 7.4μg/dl in the children with acute diarrhea were significantly lower than 69.0 ± 6.5μg/dl detected in the control group ($P < 0.001$). The mean serum total protein, albumin, and CRP levels also significantly differed between the subject and control groups ($P < 0.001$) [Table 4]. Zinc deficiency was observed in 47 (47.0%) of the children with acute diarrhea and 32 (32.0%) of the controls ($P = 0.030$). Furthermore, the subjects with low zinc status had significantly lower mean serum zinc levels of 56.9 ± 7.1 μg/dl than the corresponding value of 62.7 ± 6.9 μg/dl in the controls ($P < 0.001$) [Table 5].

Table 1: Demographic characteristics of the study population

Variable	Subject *n*=100 (%)	Control *n*=100 (%)	t/χ^2	*P*-value
Male: Female ratio*	63:37	60:40	0.190	0.663
Mean age in months±SD†	8.7±1.8	8.8±2.0	−0.372	0.712
Mean weight in kg±SD†	7.7±2.0	8.5±2.5	−2.499	0.013
Mean height in cm±SD†	70.5±9.0	72.2±9.1	−1.328	0.186
Mean WAZ±SD†	−1.2±1.1	−0.5±1.2	−4.300	0.001

SD: Standard deviation, kg: Kilogram, cm: Centimeter, WAZ: Weight for age Z-score, *calculated using the Chi-squared test, †calculated using the student's *t*-test

Table 2: Distribution of viral pathogens in the study population

Variable	Subjects (*n*=100)	Prevalence (%)	Controls (*n*=100)	Prevalence (%)	χ^2	*P*-value
Viruses	62	62.0	2	2.0		
Rotavirus*	30	30.0	2	2.0	22.781	0.001
Adenovirus*	21	21.0	0	0.0	21.000	0.001
Norovirus*	11	11.0	0	0.0	9.091	0.001

*Calculated using Chi-square test

Table 3: Age distribution by viral etiology of children with acute diarrhea

Variable	Type of virus				χ^2	*P*-value
	Frequency (*n*)	Rotavirus *n* (%)	Adenovirus *n* (%)	Norovirus *n* (%)		
	Age group (months)					
1–11	50	26 (52.0)	17 (34.0)	7 (14.0)	10.840	0.004
12–23	9	4 (44.5)	3 (33.3)	2 (22.2)	0.250^{Y}	0.882
24–59	3	0 (0.0)	1 (33.3)	2 (66.7)	0.750^{Y}	0.687

Y: Yates corrected Chi-square

Table 4: Serum zinc, total protein, albumin, and CRP levels in the study population

Variable	Subject (*n*=100)	Control (*n*=100)	*t*	*P*-value
Mean zinc±SD in µg/dl†	65.3±7.4	69.0±6.5	−3.757	0.001
Mean total protein±SD in g/l†	53.5±9.4	92.4±11.8	−19.157	0.001
Mean albumin±SD in g/l†	40.6±6.8	45.5±6.1	−5.364	0.001
Mean CRP±SD in mg/dl†	24.6±4.0	2.9±0.8	53.199	0.001

SD: Standard deviation, µg/dl: Microgram per deciliter, g/l: Gram per liter, mg/dl: Milligram per liter, CRP: C-reactive protein, †calculated using student's *t* test

Table 5: Serum zinc levels based on the zinc status of the study population

Variable	Subject	Control	*P*-value	OR (95% CI)
Zinc deficiency‡ *n* (%)*	47 (47.0)	32 (32.0)	0.030	1.884 (1.060−3.349)
Normal zinc status *n* (%)*	53 (53.0)	68 (68.0)		
Zinc deficiency‡ (mean±SD) in µg/dl†	56.9±7.1	62.7±6.9	0.001	NA

OR: Odds ratio, CI: Confidence interval, SD: Standard deviation, µg/dl: Microgram per deciliter, NA: Not applicable, *calculated using Chi-square test, ‡serum zinc levels<65 µg/dl, †calculated using student's *t* test

Relationship between serum zinc and isolated viruses

Among the subjects, those with rotavirus-induced diarrhea had the lowest mean zinc level of 53.1 ± 6.4 µg/dl. A significant difference was observed in the mean serum zinc levels among these children ($P < 0.001$) [Table 6].

Table 6: Serum zinc levels according to viral pathogens of acute diarrhea

Viruses	*n*	Mean serum zinc in µg/dl±SD§	*F*	*P*-value
Rotavirus	30	53.1±6.4[a]	44.702	0.001
Adenovirus	21	66.0±7.7[b]		
Norovirus	11	73.8±6.3[c]		

SD: Standard deviation, §calculated using analysis of variance followed by least significant difference *post hoc* test, The different alphabets (a, b, c) connote a significant difference between the means

Discussion

This study emphasizes the importance of viruses as a cause of childhood diarrhea as demonstrated by the high prevalence (62.0%) reported. This is at variance with other studies that reported lower prevalence between 17.7% and 42.6% in Africa.[27-31] The high prevalence in the present study may be due to the exclusion of subjects with certain co-morbidities such as malaria and severe malnutrition, most of whom will present with associated symptoms such as acute diarrhea.

Rotavirus was the most frequently isolated pathogen which lends further credence to its well-documented role in childhood diarrhea globally. A prevalence of 30.0% was found in this study which is in accordance with other reports from Nigeria,[31,32] Africa,[30,33] and Asia[34] although lower prevalence rates were documented in studies conducted in Nigeria,[27,35] Kenya,[36] and India.[37] On the contrary, other Nigerian studies reported a prevalence of 56.0%.[38,39] The lower prevalence of rotavirus detected in this study may be due to seasonal factors, as the period of sample collection in this study (December–August) excluded some months in the Harmattan season which may have reduced the isolation rate. The documented peak period of rotavirus infection is during the cool Harmattan season and timing of the collection of stool samples during this period would increase the isolation rates. [32,38,40]

The proportion of norovirus in the subjects identified in this study (11.0%) is in keeping with the prevalence of 12.6% reported in a systematic review conducted in 12 African countries.[41] However, the prevalence from this study is higher than the value reported by workers across Nigeria[28,30,42] as well as studies emanating from Cameroon[43] and India.[37] Conversely, higher prevalences of 16.4% in Ghana[44] and 23.1% in China[45] have been reported.

The prevalence of adenovirus detected in the subjects was 21.0%, which is in agreement with 22.3% reported in Northern Nigeria[46] although lower detection rates were found in other studies across Nigeria[27,29,31] and Africa.[30,33] Against the background of relatively higher prevalences of both norovirus and adenovirus in this study when compared to many other African studies, it is noteworthy that these pathogens are becoming important causes of childhood diarrhea in this clime.

This study found a significantly higher occurrence of rotavirus diarrhea among the viral causes of diarrhea in infancy. A plausible explanation for this finding may be the fact that the severity of rotavirus infection is age-dependent with clinically significant disease occurring mostly in infancy.[6,40] Indeed, older children tend to have asymptomatic infections due to immunity acquired through previous exposures.[6,40]

The relatively lower serum zinc levels in children with acute diarrhea as against healthy controls in this study are in accordance with earlier studies from Bangladesh and Iran.[25,47] This substantiates the well-documented interrelationship between serum zinc and diarrhea.[10,48,49] Zinc plays a crucial role in the function of the immune system through

antioxidant, antimicrobial, and anti-inflammatory roles.[50-52] Furthermore, its positive influence on the gut mucosa at the cellular and molecular level enhances gastrointestinal barrier function.[10,49] Zinc deficiency, therefore, increases the vulnerability to, and severity of intestinal infections which then causes damage to the structure and function of the intestinal tract and subsequently diarrhea. In addition, one of the main sources of zinc loss from the human body is through the intestine, and diarrhea potentiates excess intestinal zinc losses.[53] Serum zinc is also reduced during acute infections/inflammation, due to the acute phase reactant-induced redistribution of zinc to the liver, as well as decreased plasma proteins such as albumin which is the main transport protein for zinc.[54]

The high prevalence of zinc deficiency found in the study population as well as the enormity of the differences in serum zinc levels between the children with acute diarrhea and the controls in this study is in accordance with findings in a related study conducted on Bangladeshi children.[25] This reiterates the global burden of zinc deficiency in earlier reports with the highest prevalences documented in Africa and South Asia.[11,12] On the contrary, a report emanating from Iran did not find any zinc deficiency in the healthy controls despite a high prevalence of 62.5% in the subjects.[47]

The difference in population status of zinc as shown by the aforementioned studies may be partly explained by the quality of the local staples in the population where these studies were conducted. The commonly consumed foods in Ilorin where this study was conducted comprise of staple foods such as rice, yam, maize, and cassava, all of which are high in myoinositol hexaphosphate (phytates) which irreversibly binds zinc within the intestinal lumen thereby inhibiting the absorption and utilization of zinc.[12,54,55] Furthermore, animal-rich sources of zinc such as meat, fish, and poultry are consumed in a limited quantity because of cost-related non-affordability of these foodstuff by the local population.[55]

It is of note that this study found a significant difference in mean serum zinc levels among the isolated viruses of acute diarrhea. A similar finding was documented among isolated bacteria pathogens of diarrhea in Iranian children.[47] These findings may imply that the differing effect of zinc supplementation on isolated enteropathogens observed in some earlier reports[19,21] may be partly attributed to the causal effect of these pathogens on baseline zinc levels as well as the function of the influence of supplemented zinc on the ion channels of the gut mucosal cells where these pathogens act to induce diarrhea.[19,56,57] It is therefore suggested that to maximize the beneficial effects of zinc in childhood diarrhea, fine-tuning the current zinc therapy based on isolated pathogen would not be amiss.

The primary limitation of this study was that the isolated viruses were not serotyped.

Conclusion

There is a high burden of viral-induced childhood diarrhea in this clime, necessitating the need for the commencement of routine viral diagnostic tests in a bid to reduce the incessant use of antibiotics for cases of diarrhea that may be of viral origin. The study also observed a high prevalence of zinc deficiency in under-five Nigerian children particularly those with acute diarrhea. Therefore, zinc supplementation and food fortification schemes should be instituted in the population. This study suggests a fine-tuning of the current zinc therapy in acute diarrhea based on the isolated pathogen, although a replication and substantiation of the findings' would be desirable.

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Disclosure of Interest

There are no existing conflicts of interest.

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