DOI: 10.7860/JCDR/2022/55234.16372

Original Article

Paediatrics Section

Effect of Bacterial Diarrhoea on Serum Zinc Levels in Children with Special Reference to Different Bacterial Pathogens: A Cross-sectional Study

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ABSTRACT

Introduction: Diarrhoea significantly leads to morbidity and mortality in under-five children, particularly in developing countries. Reduced serum zinc levels in acute non infectious diarrhoea has an impact on the frequency, severity and duration. However, evidence is still evolving on the status of zinc level in bacterial diarrhoea.

Aim: To assess serum zinc level in bacterial diarrhoea and compare the serum zinc level in children with the different bacterial pathogens.

Materials and Methods: This cross-sectional study was conducted in Department of Paediatrics at Federal Medical Centre, Owerri, Imo State, Nigeria, from August 2015 to February 2016, among 201 children aged 6 to 59 months with diarrhoea. Stool specimens were isolated for bacteria, using conventional culture techniques, while serum zinc levels were determined using atomic absorption spectrometry. Serum zinc level <65 µg/dL was regarded as zinc deficiency. The data collected was analysed using the Statistical Package for Social Sciences (SPSS) version 19.0.

Results: Out of 201, 58 (28.9%) of the children with diarrhoea had bacteria in the stool. The most common organism isolated was *Escherichia coli* (33,16.4%) followed by Salmonella (13, 6.5%). All of the children with positive stool culture, irrespective of the type of microbial agent, had zinc deficiency with significant association between infective diarrhoea and zinc deficiency (χ^2=15.437; p-value=0.004). Children with shigella diarrhoea had the lowest mean serum zinc level, compared to other bacterial agents (33.6±4.4 µg/dL, p-value <0.001).

Conclusion: Bacterial pathogens contribute significantly to the cause of diarrhoea in under-five Nigerian children, and are significantly associated with zinc deficiency. Thus, reinforcing the need for zinc supplementation and food fortification programs within the population, and maybe for longer in children with identified bacterial diarrhoea.

Keywords: Acute diarrhoea, Bacterial pathogen, Children, Dysentery, Persistent diarrhoea, Zinc deficiency

INTRODUCTION

Worldwide, diarrhoeal diseases are reported as the leading cause of mortality among children aged five years and below [1]. Despite being preventable and treatable, diarrhoeal disease is the second leading cause of death in children under-five years old, and is responsible for killing about 525,000 children every year, especially in developing countries where poor sanitation and lack of potable water supply are majorly contributory [1]. In Africa, Asia, and South America, diarrhoea accounts for one in eight deaths of under-five yearly, with an estimated 16% of children's deaths in Nigeria annually [2,3].

Bacterial pathogens account for approximately 38% of mortalities resulting from childhood diarrhoea, contributing to the high burden of the disease [4]. Studies have documented *Escherichia coli* as the most implicated bacterial pathogen associated with infective diarrhoea, with upto 41.4% prevalence in South-eastern Nigeria [5,6]. Other bacterial organisms isolated with variable prevalence depending on region comprise Salmonella spp., Shigella spp., *Campylobacter jejuni* and *Vibrio cholera* [5,6]. Bacterial diarrhoea have been associated with low socio-economic class, malnutrition and a deteriorating environment; and greatly puts children at risk of macronutient and micronutrient malnutrition [7].

Zinc has been recognised as an essential micronutrient in human health [8]. Zinc is important in maintaining the immune system, acts as an antioxidant and also helps in tissue repair growth and reproduction [8]. Furthermore, zinc is a co-factor for the hormone thymulin which promotes T-cell function and proliferation of CD8 cells, thereby, aiding increase in cytokine release [9,10]. Additionally, zinc has been documented to have antibacterial properties hence its proposed effectiveness in children with infective diarrhoea [11,12].

Diarrhoea reduces the net absorption of zinc as a result of rapid intestinal transit and destruction of the absorptive villous mucosa while zinc deficiency reduces absorption of water and electrolytes, as well as, clearance of aetiologic pathogens, thus prolonging the resolution of diarrhoea episodes [13]. These mechanisms are worse in bacterial diarrhoea, where diarrhoea is caused primarily by elaborating enterotoxins (which also requires the organisms to adhere to the surface of the intestinal cell), and by invading the intestinal mucosa, both of which increase intestinal secretion of water, electrolytes and zinc [14]. Documented evidence have shown reduced levels of zinc in acute diarrhoea strengthening the World Health Organisation (WHO) recommendation of zinc supplementation to reduce morbidity and mortality from acute diarrhoea but not much is known about zinc levels in bacterial diarrhoea [15-18].

Hence, the study aimed to assess the serum zinc levels in the children with bacterial diarrhoea, compare the serum zinc level in children with the different bacterial pathogens as well as the different types of diarrhoea.

MATERIALS AND METHODS

This cross-sectional study was conducted in Department of Paediatrics at Federal Medical Centre, Owerri, Imo State, Nigeria, from August 2015 to February 2016, including 201 children aged 6 to 59 months with diarrhoea. Imo State has a population projected to have increased to 5.11 million [19]. Owerri is the capital of Imo State with 401,873 people living in the city. The residents are predominantly farmers and traders Imo State [19]. The Paediatric Department includes the Emergency Paediatric Unit (EPU), the Special Care Baby Unit (SCBU), the Children's Ward and the Children's Outpatient Clinic (CHOP).

Ethical approval for this study was obtained from the Ethics Committee of Federal Medical Centre, Owerri (Ref:017868) and study informed written consent was obtained from the parents/guardian of the enrolled children. Diarrhoea was defined as the passage of three or more loose or liquid stools per day or more frequent passage than is normal for the individual [15].

Inclusion criteria: Children aged 6-59 months with diarrhoea whose parents gave consent were included in the study.

Exclusion criteria: Those who were on zinc tablets within two weeks of the study were excluded from the study.

Sample size calculation: The sample size was calculated using the formula below:

$n=(2\times Z^2\times pq)d^2$

where,

n=minimum sample size,

z=normal standard deviation set at 1.96 which corresponds to the 95% confidence interval,

p=prevalence of zinc deficiency in children with diarrhoea. In this study, the prevalence rate was considered to be 50%, q=1-p,

d = degree of accuracy desired (in this study, an observed difference of 0.10 or more was considered significant at the 0.05 level)

Therefore,

$n=2\ (1.96)\ (1.96)\ (0.50)\ (1-0.50)\ (0.10)^2$

$n=(7.6832\times 0.25)\ 0.01$

n=192 (minimum sample size),

Allowing 10% non response=19,

Hence, final sample size was 211.

Procedure

The sampling method was consecutive and children who met the inclusion criteria were recruited. Subjects were recruited from the EPU, CHOP and the Children's Ward until the calculated sample size was achieved. Two resident doctors were trained by the researcher on collecting data using the questionnaire, collection of samples for serum zinc and stool microscopy/culture before commencement of study. Structured questionnaires were administered by the research team to obtain information including age and gender. Socio-economic class was determined using the method proposed by Oyedeji GA [20].

Sample collection: After obtaining consent and proper disinfecting, 3 mL of venous blood was collected from the antecubital vein of each study participant. The sample was put into a prelabelled sterile anticoagulant free bottle that had been made free of trace elements and later analysed using Varian AA240 Atomic Absorption Spectrophotometer. Serum zinc level of less than 65 µg/dL was regarded as zinc deficiency.

Fresh faecal samples were collected in a prelabelled sterile screw cap bottle. Samples were stored at 4°C until they were analysed. For the culture, faecal samples were emulsified in sterile peptone water and a loopful was inoculated on Xylose Lysine Deoxycholate (XLD), MacConkey (MCA) and Salmonella-Shigella Agar plates. These were incubated overnight at 37°C and read the next day.

STATISTICAL ANALYSIS

The data collected was analysed using the Statistical Package for Social Sciences (SPSS) version 19.0. The mean and standard deviation of quantitative variables were determined while categorical variables were summarised using percentages. Chi-square (χ^2) and Fisher's-exact where necessary were used to assess relationships between the categorical variables. The student's t-test was used to compare the mean of two variables, p-values <0.05 were regarded as significant.

RESULTS

A total of 211 under-five children were recruited into the study, but only 201 samples were analysed. Ten children were excluded because their samples spilled.

Socio-demographic parameters: [Table/Fig-1] shows the socio-demographic distribution of the study population. The mean age of the children was 17.6±11.8 months. Children between the ages of 6-12 months had the highest prevalence of diarrhoea accounting for 92 (45.8%) of cases. Males had the greater proportion (109, 54.2%).

Variables	Frequency (%)
Age group (months)	
6 – 12	92 (45.8)
13 – 24	73 (36.3)
25 – 36	20 (10.0)
37 – 48	9 (4.5)
49 – 59	7 (3.4)
Gender	
Male	109 (54.2)
Female	92 (45.8)
Socio-economic class	
Upper	86 (42.8)
Middle	86 (42.8)
Lower	29 (14.4)

[Table/Fig-1]: Socio-demographic characteristics of the study population (N=201).

Bacterial isolates of the stool of children with diarrhoea: Out of total 201, 58 (28.9%) of the children with diarrhoea had microorganisms in the stool. [Table/Fig-2] shows that the most common organism isolated was *Escherichia coli* in 33 (16.4%) of subjects.

Culture	Number of children (%)
No growth	143 (71.1)
Escherichia coli	33 (16.4)
Salmonella spp.	13 (6.5)
Shigella spp.	8 (4.0)
Coliforms spp.	4 (2.0)
Total	201 (100)

[Table/Fig-2]: Bacterial isolates of the children with diarrhoea.

Prevalence of zinc deficiency according to bacterial isolates: The relationship between the presence of bacterial microorganisms in the stool and zinc deficiency in children with diarrhoeal disease is shown in [Table/Fig-3]. All (100%) of the children with positive stool culture irrespective of the type of microbial agent had zinc deficiency. There was a significant association between infective diarrhoea and zinc deficiency (χ^2=15.437; p-value=0.004).

Stool culture	Zinc deficient (n=169)	Normal, (n=32)	χ^2	p-value
No growth	111 (77.6)	32 (22.4)	15.437	0.004
E. coli	33 (100.0)	0		
Salmonella spp.	13 (100.0)	0		
Shigella spp.	8 (100.0)	0		
Coliforms spp.	4 (100.0)	0		

[Table/Fig-3]: Prevalence of zinc deficiency according to bacterial isolates.
p-value <0.05 considered significant

Relationship between serum zinc level and bacterial isolates in the stool: Children with bacterial isolate in stool had significantly lower serum zinc levels when compared to children without bacterial isolates in their stool (p-value <0.001). Among the children with bacterial isolates in their stool, those with Shigella

had the lowest level of serum zinc followed by those infected with *E. coli* [Table/Fig-4].

Stool culture	Serum zinc level Mean±SD (µg/dL)	p-value
None	52.0±14.7	<0.001
Coliforms spp.	42.6±12.2	
Escherichia coli	36.3±8.3	
Salmonella spp.	34.7±5.1	
Shigella spp.	33.6±4.4	

[Table/Fig-4]: Relationship between serum zinc level and bacterial isolates in the stool.
p-value <0.05 considered significant

DISCUSSION

In the current study, 28.9% of the participants had bacterial diarrhoea. This finding is similar to another study from India that reported 25.6% prevalence [21]. This underscores the need to screen children with diarrhoea for possible bacterial aetiology, given that viral causes of diarrhoea are known to be more common and the use of antibiotics is not recommended as part of the initial management of diarrhoea, except in cases of dysentery [15,21]. Higher prevalence of 73.3%, 41.4% and 61.1% respectively were reported by researchers in Ilorin, North Central Nigeria, Enugu, South East Nigeria and in Nicaragua. This reflects that bacterial pathogen constitute a significant burden to the aetiology of acute diarrhoea, particularly in low-income countries where factors including poverty, malnutrition, lack of potable water, poor sanitation and hygiene promote the spread of theses bacterial infections [6,22-24]. However, the difference in this prevalence may reflect the epidemiological variations of organisms and possibly the impact of the socio-economic levels of the population within these localities.

All children with positive bacterial isolates in stool irrespective of the type of bacteria had zinc deficiency. Also, children with infective diarrhoea had significantly low levels of zinc. These findings have been corroborated by other researchers too [22,26,27]. The finding may be explained by the fact that children with bacterial diarrhoea are more likely to have persistent diarrhoea and dysentery which increases the loss of zinc in stool, and are more likely to have fever with increased metabolic demand for micronutrients including zinc [25]. With existing zinc deficiency, there is increased susceptibility and severity of gastrointestinal infections which distorts the structure and function of the gastrointestinal tract and worsens the risks and complications of diarrhoea [12]. It is therefore important to maintain an optimal level of serum zinc in children with bacterial diarrhoea by supplemention.

Among children with bacterial isolates in their stool, those with Shigella had significantly the lowest level of serum zinc when compared to their counterparts that had Salmonella, *Escherichia coli* and Coliforms isolated from the stools. This agrees with what was observed among under-five children in Iran and Ilorin, Nigeria [22,26]. The fact that Shigella causes more invasive gastrointestinal disease than the other organisms, which leads to more damage to the intestinal mucosa and greater loss of fluid and micronutrients including zinc in the stool may explain this finding [28]. Whether higher doses and longer duration of zinc supplementation should be considered for differing isolated enteropathogens of diarrhoea is a question that further research will answer to explore the beneficial effects from the current strategy of zinc supplementation with respect to isolated enteropathogens of diarrhoea.

Limitation(s)

Serotyping of the types of *Escherichia coli* and other types of coliforms could have contributed to the research but was not done.

CONCLUSION(S)

This study showed that zinc deficiency occurred consistently in all children with diarrhoea caused by all the cultured bacterial pathogens. Consequently, this may indicate the need for longer zinc supplementation in children with identified bacterial diarrhoea. However, given that serum zinc levels were significantly different across the isolated bacteria. Further studies are needed to understand the implication of this finding on zinc supplementation.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jan 31, 2022
- Manual Googling: Feb 24, 2022
- iThenticate Software: Apr 28, 2022 (19%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jan 25, 2022**
Date of Peer Review: **Feb 09, 2022**
Date of Acceptance: **Mar 17, 2022**
Date of Publishing: **May 01, 2022**