A CASE-CONTROL STUDY OF THE PREVALENCE OF HYPOZINCAEMIA AMONG UNDER-FIVE CHILDREN WITH ACUTE DIARRHOEA IN ILESA, NIGERIA.

A dissertation submitted in part fulfillment of the requirements for the award of the Fellowship of the National Postgraduate Medical College of Nigeria in the Faculty of Paediatrics.

By

Dr ABOLURIN Olufunmilola Olubisi

M.B,Ch.B. (O.A.U, Ile-Ife) 2007

November, 2015
DECLARATION

It is hereby declared that this work is original, and has neither been presented to any other College for a Fellowship, nor has it been published or submitted elsewhere for publication.

Signature/ Date…………………………………

Dr. Olufunmilola Olubisi Abolurin
ATTESTATION

We hereby attest that this study was carried out by Dr. O.O. Abolurin of the Department of Paediatrics, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, and that the dissertation was written under our supervision.

SUPERVISOR:

Name: Professor O.A. Oyelami

Status: Professor of Paediatrics/ Consultant Paediatrician

Department of Paediatrics and Child Health,

Obafemi Awolowo University, Ile-Ife.

Signature: ______________________

Date: ______________________

SECOND SUPERVISOR:

Name: Dr. S.B.A. Oseni

Status: Senior Lecturer/ Consultant Paediatrician

Department of Paediatrics and Child Health,

Obafemi Awolowo University, Ile-Ife.

Signature: ______________________

Date: ______________________
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DEDICATION

This work is dedicated to God, the source of my strength and ability, and to my wonderful companions, Adebayo Abolurin and Temiloluwa Abolurin who have always been a source of encouragement to me.
ACKNOWLEDGEMENTS

I hereby acknowledge with profound gratitude the dedicated efforts of my supervisors, Professor O.A. Oyelami and Dr. S.B.A. Oseni and I am grateful to them for their excellent supervision, guidance and useful contributions towards the success of this project.

I appreciate the useful suggestions and input of all the other Consultants in the Department, particularly Professor J.A. Owa, Professor O.O. Adeodu, Dr. S.A. Adegoke, Dr B.P. Kuti and Dr T.A. Aladekomo, who also made efforts to ensure the success of the study. I am grateful to all my fellow resident doctors as well as the house officers in the unit, who were very supportive and were always ready to provide assistance towards the success of the project.

I also wish to express profound gratitude to Professor A.O. Ogundaini, the former Director of the Central Science Laboratory, for his help in providing the necessary materials required for sample preparation and analysis, and for always making himself available when needed. I am also immensely grateful to Professor C.A. Obafemi, the present Director, for his support and the fatherly attention received from him. The Laboratory Scientists at the Central Science Laboratory are greatly appreciated for their help and assistance during sample analysis. I also appreciate the staff of the Chemical Pathology Laboratory at the Wesley Guild Hospital, Ilesa for their cooperation during sample separation and storage. I appreciate Dr T.O. Ojo of the Department of Community Health for his useful suggestions during data analysis.

I am grateful to my family for their encouragement and support. I appreciate all the children and their parents who participated in the study for their understanding and cooperation. God bless you all.
LIST OF ABBREVIATIONS

➢ AAS - Atomic absorption spectrophotometry
➢ CEW - Children’s Emergency Ward
➢ CI - Confidence interval
➢ DAMA - Discharged against medical advice
➢ DNA - Deoxyribonucleic acid
➢ FET – Fisher’s exact test
➢ HCL - Hydrochloric acid
➢ HIV - Human immunodeficiency virus
➢ IITA - International Institute of Tropical Agriculture
➢ IZiNCG - International Zinc Nutrition Consultative Group
➢ LR – Likelihood ratio
➢ MCS – Microscopy, culture and sensitivity
➢ OAUTHC - Obafemi Awolowo University Teaching Hospitals Complex
➢ OR - Odds ratio
➢ ORS - Oral rehydration salt
➢ RNA - Ribonucleic acid
➢ SD - Standard deviation
➢ SPSS - Statistical Programme for Social Sciences
➢ UFWC - Under-five Welfare Clinic
➢ UNICEF - United Nations Children’s Fund
➢ WGH - Wesley Guild Hospital
➢ WHO - World Health Organization
DEFINITION OF TERMS USED IN THE STUDY

1. *Diarrhoea*: Diarrhoea is the passage of unusually loose or watery stools, at least three times in a 24 hour period.¹

2. *Acute diarrhoea*: Diarrhoea that lasts several hours or days, but with duration less than 14 days.¹

3. *Acute watery diarrhoea*: Acute diarrhoea with absence of blood in the stools.¹

4. *Dysentery*: Diarrhoea with visible blood in the stool.¹

5. *Fever*: Elevation in body temperature with axillary temperature greater than 37.5°C.²

6. *Hypozincaemia*: Serum zinc level less than 65 µg/dl.³

7. *Hyperzincaemia*: Serum zinc level equal to or greater than 160 µg/dl.⁴

8. *Severity of dehydration*: Classification of dehydration into mild, moderate or severe based on assessment of clinical signs of dehydration.⁵

9. *Severe undernutrition*: Value of z-score below -3 on a WHO weight-for-age, height-for-age or weight-for-height growth chart, or presence of oedema.⁶
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SUMMARY

Diarrhoea is still a significant contributor to childhood morbidity and mortality in developing countries. Zinc deficiency increases the susceptibility of children to diarrhoea, and increases the severity and duration of diarrhoeal illnesses, thereby increasing the risk of mortality. Thus the World Health Organization recommends zinc supplementation as an adjunct treatment for all diarrhoea episodes in children, since it has been proven to reduce diarrhoea morbidity and mortality. However, there have been only a few studies which investigated the relationship between zinc deficiency and diarrhoea among Nigerian children. The aim of the present study was to determine the relationship between serum zinc levels and the type and severity of diarrhoeal diseases in children.

This study was a prospective case-control study conducted at the Under-five Welfare Clinic (UFWC) and the Children’s Emergency Ward (CEW) of the Wesley Guild Hospital (WGH), Ilesa between 15th October 2013 and 10th September 2014. Two hundred and fifty under-five children with diarrhoea were recruited consecutively and were matched with 250 Controls, who were apparently healthy children without diarrhoea. History of the nature and duration of diarrhoea, and of the socio-economic background were obtained and all the patients were examined. Blood samples were collected for zinc assay before the commencement of treatment, including zinc supplements. Serum zinc assay was repeated after completion of zinc supplements. Serum zinc levels were determined using atomic absorption spectrometry (AAS). Other necessary investigations such as serum electrolytes and urea were carried out when indicated.

Male to female ratio was 1.4: 1 and a majority (86.4%) of the children were below two years of age. The mean ± SD serum zinc level of the Subjects was significantly lower than that of the Controls (78.8 ± 35.6 µg/dl versus 107.3 ± 46.8 µg/dl; t = -7.66, p <0.001). The prevalence
of hypozincaemia was significantly higher in the Subjects [30.4% versus 12.4% in the Controls; OR (95% CI) = 3.09 (1.94, 4.90); \(\chi^2 = 24.08, p < 0.001\)], while the prevalence of hyperzincaemia in the Controls was significantly higher than that in the Subjects [12.4% versus 2.8%; OR (95% CI) = 4.91 (2.12, 11.37); \(\chi^2 = 24.08, p < 0.001\)]. Among the Subjects, dysentery was associated with a lower mean ± SD serum zinc level (65.1 ± 25.0 µg/dl versus 80.5 ± 36.3 µg/dl; \(t = -2.13, p = 0.034\)) and a higher prevalence of hypozincaemia [48.1% versus 28.3%; OR (95% CI) = 2.36 (1.06 – 5.23); \(\chi^2 = 4.51, p = 0.034\)]. There was no statistically significant difference in the mean ± SD serum zinc level (84.5 ± 39.7 µg/dl versus 76.7 ± 33.7 µg/dl; \(t = -1.56, p = 0.120\)) or the prevalence of hypozincaemia (26.5% versus 31.9%; \(\chi^2 = 0.68, p = 0.409\)) between Subjects with signs of dehydration and those with no signs of dehydration. The differences between the distribution of zinc levels in relation to the degree of dehydration were not statistically significant (LR = 7.29, \(p = 0.121\)). There was a higher prevalence of hypozincaemia among Subjects from low socio-economic class, compared with those from high socio-economic class [33.8% versus 15.2%; OR (95% CI) = 2.85 (1.22, 6.64); \(\chi^2 = 6.14, p = 0.013\)]. The prevalence of hypozincaemia was also higher among Subjects who had never been breastfed, compared with those that were breastfed [100% versus 29.6%; OR (95% CI) = 16.62 (1.26, 219.45); FET \(p = 0.027\)]. The difference in the prevalence of hypozincaemia between Subjects that were underweight (15/45; 33.3%) and those who were not (61/205; 29.8%) was not statistically significant (\(\chi^2 = 0.22, p = 0.637\)).

Among the Controls, the prevalence of hypozincaemia was significantly higher among those who were underweight [33.3% versus 10.5%; OR (95% CI) = 4.27 (1.57, 11.62); \(\chi^2 = 9.25, p = 0.002\)]. The difference in the prevalence of hypozincaemia between Controls from low and high socio-economic status families was not statistically significant (11.4% versus 14.5%; \(\chi^2 = 0.48, p = 0.486\)). Furthermore, the prevalence of hypozincaemia among Controls that were
breastfeeding was not significantly different from those who had stopped breastfeeding (10.8% versus 14.4%; $\chi^2 = 0.75, p = 0.388$). There was a significant rise in the mean serum zinc levels of both Subjects and Controls following zinc supplementation ($p < 0.001$). All three children that died had a combination of dehydration, electrolyte derangement(s) and hypozincaemia.

It is concluded that hypozincaemia is associated with diarrhoea among under-five children in this environment. This is particularly important among children with dysentery, those from low socio-economic class and those who were not breastfed. Therefore, zinc supplementation should be part of the treatment of childhood diarrhoea, particularly dysentery and diarrhoea in children from low socio-economic class families.
INTRODUCTION

Diarrhoea is the passage of unusually loose or watery stools, at least three times in a 24 hour period.\(^1\) Diarrhoeal disease represents a major public health problem, accounting for almost 10 percent of under-five deaths, with an estimated 0.6 million deaths per year globally.\(^7\) The overall incidence of diarrhoea is about 3.2 episodes per child year.\(^8\)

Diarrhoea is commonly associated with childhood malnutrition, with its frequency and severity increasing as the severity of malnutrition increases.\(^1,\,9\) Furthermore, micronutrient deficiencies are common among malnourished children who may therefore suffer from various nutrient-specific deficiency disorders, including zinc deficiency.\(^10\)

Zinc is an essential micronutrient that is important for growth and development as well as immune function. It strongly influences the immune system, affecting both non-specific and specific immunity. Persons with zinc deficiency therefore experience increased susceptibility to a variety of pathogens.\(^11\)-\(^13\) Zinc is also involved in numerous aspects of cellular metabolism including the catalytic activity of several enzymes. In addition, it plays a role in protein synthesis and deoxyribonucleic acid (DNA) synthesis as well as in cell division.\(^13\)-\(^15\)

Zinc supplementation has been reported to reduce the incidence, severity and duration of diarrhoea in children and has been proven to reduce diarrhoea morbidity and mortality.\(^16\)-\(^19\) Thus, zinc supplementation is currently recommended by WHO as an adjunctive treatment for all diarrhoea episodes in children.\(^20\) However, only a few reports are available on the relationship between zinc deficiency and diarrhoea among Nigerian children. This study was carried out to determine the relationship between serum zinc levels and the type and severity of acute diarrhoea in Nigerian children. The results could guide implementation of the WHO recommendations in Nigeria.
LITERATURE REVIEW

AETIOLOGY AND COURSE OF DIARRHOEA IN CHILDREN

Diarrhoea remains a significant contributor to global childhood morbidity and mortality as it is a leading cause of illness and death among children in developing countries.\(^1\) Most cases of diarrhoea resolve within the first week of the illness.\(^8\) However, about three to 20 percent of all diarrhoeal episodes in children under the age of five years persist for 14 or more days and are referred to as persistent diarrhoea.\(^8\) Such persistent cases account for a large percentage of diarrhoea-related deaths.\(^8\) Diarrhoea with visible blood in the stools is referred to as dysentery\(^1,\)\(^21\) and occurs in about 10 percent of diarrhoeal episodes in under-five children.\(^21\)

Rotavirus infection is the most common identifiable viral cause of acute gastroenteritis in all children, and accounts for at least 35 percent of severe and potentially fatal watery diarrhoea episodes.\(^8\) Other common causes of acute diarrhoea in developing countries include *Escherichia coli*, *Salmonella* spp. and parasitic agents such as *Giardia lamblia* and *Cryptosporidium*.\(^1,\)\(^8\) *Shigella* spp. and *Campylobacter jejuni* are more important as causative agents of dysentery but can also cause acute non-bloody diarrhoea.\(^1,\)\(^21\) *Shigella* is responsible for 50 percent or more of all episodes of dysentery in young children, and a much higher proportion of episodes that are clinically severe.\(^21\)

ZINC NUTRITION AND METABOLISM

Zinc is a trace element that is essential for normal growth and development. The main source of zinc in the body is dietary, and good sources of zinc include meat, fish, eggs, dairy products and seafoods.\(^22,\)\(^23\) Cereals and legumes are also good sources of zinc, but they usually contain high levels of phytate, which reduces the amount of zinc available for
Phytates bind zinc and inhibit its absorption, resulting in lower bioavailability of zinc from such food items. Polished rice, starchy roots/ tubers, fruits and vegetables are not good sources of zinc. Breast milk usually provides sufficient zinc for the first four to six months of life. However, as lactation progresses, the physiological decline in breast milk zinc concentration is notable.

The dietary reference intake of zinc for children is between two and eight milligram per day, depending on the age of each child. Although dietary inadequacy is the key factor in the aetiology of zinc deficiency, excessive losses and impaired utilization of zinc in disease conditions such as diarrhoea are also very important. Achieving dietary adequacy of zinc is difficult amongst poor populations because zinc-rich foods, such as meat and fish, are usually expensive and unaffordable to many households, who therefore rely mainly on cheaper, plant-based diets. These are not only poor in zinc content, but may also hinder the bioavailability of zinc by inhibiting its absorption and utilization.

Dietary zinc is absorbed in the duodenal and jejunal regions of the gastrointestinal tract. Zinc competes with other metals such as iron and copper for absorption in the gastrointestinal tract. About 50-60 percent of zinc in the plasma is loosely bound to albumin, while most of the remainder is tightly bound to macroglobulins. Hypoalbuminaemia therefore impairs the absorption and transport of zinc in the body. Zinc is also present in red blood cells, where it is bound to metalloproteins, particularly carbonic anhydrase, which is an intracellular enzyme. Zinc is an integral component of over 100 metalloenzymes, including carbonic anhydrase, alkaline phosphatase, carboxypeptidase and several dehydrogenases. Presence of zinc is essential for the catalytic functions of such enzymes. Zinc is also a component of some hormones such as testosterone, prolactin and somatomedin.
EFFECTS OF ZINC DEFICIENCY

The effects of zinc deficiency include increased susceptibility to infections, growth retardation, delay in sexual and skeletal maturation, development of orificial and acral dermatitis, poor appetite, altered taste and smell, alopecia, delayed wound healing, and appearance of behavioural changes.\textsuperscript{11, 22, 23}

Zinc deficiency results in impaired immunity, which may increase the risk of infections.\textsuperscript{13} This is because zinc affects multiple aspects of the immune system, from the barrier of the skin to gene regulation within lymphocytes and it is crucial for normal development and function of cells mediating nonspecific immunity such as neutrophils and natural killer cells.\textsuperscript{13, 31} It also affects the development of acquired immunity by influencing the growth and certain functions of T-lymphocytes, B-lymphocytes and macrophages.\textsuperscript{13, 31, 32} The effects of zinc on these key immunologic mediators are due to its roles in basic cellular functions such as DNA replication, RNA transcription, cell division, and cell activation.\textsuperscript{13, 32} Zinc also functions as an antioxidant and can stabilize membranes.\textsuperscript{13}

Zinc deficiency contributes substantially to the morbidity and mortality of young children throughout the world.\textsuperscript{23} It is particularly common in developing countries, because the commonly consumed staple foods have low zinc contents and are rich in phytates.\textsuperscript{22-24} Children with low blood zinc levels have been found to be at risk of increased diarrhoeal\textsuperscript{33-35} and respiratory morbidities\textsuperscript{33, 36} as well as increased risk of developing complications of malaria.\textsuperscript{37} Zinc deficiency also increases the risk of mortality from diarrhoea, pneumonia and malaria by 13-21 percent.\textsuperscript{8} It adversely affects the course of diarrhoeal illnesses in children, resulting in increased severity and prolongation of the duration of the illness.\textsuperscript{33} Low serum zinc levels have also been found to be more prevalent in patients with sepsis,\textsuperscript{38} sickle cell
anaemia\textsuperscript{39} and human immunodeficiency virus (HIV) infection,\textsuperscript{40} when compared with the general population.

**ZINC DEFICIENCY AND MALNUTRITION**

Zinc deficiency is often associated with malnutrition and other micronutrient deficiencies, such as copper and iron deficiency.\textsuperscript{10, 22, 41, 42} Zinc levels have been found to be lower in malnourished children when compared with well-nourished children.\textsuperscript{10, 41-44} Atinmo \textit{et al}\textsuperscript{41} in Ibadan reported significantly lower mean plasma zinc levels in children with undernutrition, marasmus, kwashiorkor and marasmic kwashiorkor when compared with controls who did not have any form of malnutrition. A similar trend was also observed by Ugwuja \textit{et al},\textsuperscript{42} Ahmed \textit{et al}\textsuperscript{43} and Guatem \textit{et al}.	extsuperscript{44} Among malnourished children, zinc levels have also been reported to be lower in kwashiorkor than in marasmus.\textsuperscript{41, 43-45} Blood albumin levels were also lower in malnourished children than in the controls in these studies.\textsuperscript{41, 42}

Clinical and experimental findings have reinforced the link between zinc deficiency, malnutrition and diarrhoeal disease.\textsuperscript{46} Diarrhoea is both a cause and an effect of malnutrition, as diarrhoea prevents catch-up growth, and malnutrition increases diarrhoea frequency and duration.\textsuperscript{1} Furthermore, both malnutrition and diarrhoea serve as major risk factors for zinc deficiency,\textsuperscript{9, 46} while zinc deficiency itself can manifest as diarrhoea.\textsuperscript{11, 22}

**ZINC DEFICIENCY AND DIARRHOEA**

A vicious cycle operates between diarrhoea and zinc deficiency such that diarrhoea reduces net absorption of zinc and other nutrients due to rapid intestinal transit and deterioration of the absorptive mucosa.\textsuperscript{46} On the other hand, zinc deficiency impairs absorption of water and electrolytes as well as clearance of aetiologic pathogens, thus delaying the termination of normally self-limiting diarrhoea episodes.\textsuperscript{46} Greater severity and
longer duration of diarrhoea have been reported in children with low plasma zinc levels, when compared with non-hypozincaemic children.\textsuperscript{33, 47} The exact pathophysiologic mechanism that links diarrhoea with zinc deficiency is yet to be elucidated.\textsuperscript{48} However, in addition to causing impairments in immune function, zinc deficiency has been shown to have direct effects on the gastro-intestinal tract. These effects include villous atrophy, decreased brush border disaccharidase activity, and impaired intestinal transport.\textsuperscript{48}

It is also noteworthy that diarrhoea is a cardinal feature of the clinical syndrome of acrodermatitis enteropathica, a congenital disorder of zinc deficiency; and diarrhoea in such children responds quickly to zinc supplementation.\textsuperscript{49} This further supports the effects of zinc deficiency on the gastro-intestinal tract to cause or worsen diarrhoeal illnesses.

Serum zinc levels have been reported to be lower in children with diarrhoea than in those without diarrhoea. In a case-control study carried out by Okolo \textit{et al} \textsuperscript{34} in Jos, Nigeria, infants with diarrhoea were found to have significantly lower serum zinc levels and significantly higher stool zinc than those without diarrhoea. Rani \textit{et al} \textsuperscript{35} also found lower serum zinc levels in children with diarrhoea.

**INTERVENTION STRATEGIES TO REDUCE ZINC DEFICIENCY**

Considering the public health importance of zinc deficiency, and keeping in mind that zinc deficiency, along with its attendant problems is preventable, the development of effective programmes to reduce zinc deficiency is highly beneficial. Current intervention strategies focus on zinc supplementation, fortification of locally acceptable foods, and dietary modification to consume greater amounts of animal products and fewer fiber and phytates.\textsuperscript{24} Zinc fortification is considered a potentially useful strategy for the control of zinc deficiency, but the success of such intervention programs depends on the population’s access to and
consumption of zinc-fortified foods as well as adequate absorption of zinc from these foods. Cereals are the most common class of foods that are being fortified with zinc.

MANAGEMENT OF DIARRHOEA

Conventionally, diarrhoea is managed by rehydrating the patient according to the degree of dehydration, either by oral rehydration salt (ORS) solution or intravenous fluids (when indicated). ORS solution is absorbed in the small intestine even during copious diarrhoea, thus replacing the water and electrolytes lost in the faeces. ORS has been found to be effective in treating dehydration from acute diarrhoea in over 90% of cases and its use has significantly reduced the morbidity and mortality associated with diarrhoeal diseases in children.

Although, in general, the standard WHO ORS is adequate, lower osmolarity oral rehydration fluids can be more effective in reducing stool output, and are now being recommended by WHO. The standard WHO ORS solution contains 90 mmol/L of sodium and 111 mmol/L of glucose, with a total osmolarity of 311 mmol/L. On the other hand, the reduced osmolarity ORS solution contains 75 mmol/L of sodium and 75 mmol/L of glucose, with a total osmolarity of 245 mmol/L. Reduced osmolarity ORS significantly reduces stool output, vomiting and the need for intravenous infusion when compared to treatment with standard ORS for children with diarrhoea.

Home-made salt–sugar solution (SSS) is also effective for prevention or treatment of dehydration in children with diarrhoea, but is no longer popularly used. SSS can be prepared at home by adding one level teaspoon of salt and 10 level teaspoons of sugar to about 600 mls of clean water. The preparation of SSS requires correct measurements of these components. Unfortunately, mothers frequently have difficulty in remembering the recipe or
in preparing SSS correctly and this may result in solutions that are hyperosmolar and unsafe for children. The absence of potassium and citrate in SSS is a disadvantage, when compared with ORS. As a result of these limitations, SSS is no longer generally recommended by WHO. Routine use of antibiotics in the treatment of diarrhoea is strongly discouraged, with antibiotics only indicated in specific cases such as dysentery and cholera.\textsuperscript{1,20,54}

Zinc has been recommended for the treatment of diarrhoea by the World Health Organization (WHO) and United Nations Children’s Fund (UNICEF) since year 2004, \textsuperscript{20} yet access to this essential treatment remains limited.\textsuperscript{55,56} It is recommended that zinc supplementation be given routinely to children with diarrhoea at a dosage of 20 mg per day for children older than six months and 10 mg per day for those younger than six months for a duration of 10-14 days.\textsuperscript{1,20} This has however not been in practice in many countries, because suitable zinc supplements are not readily available.\textsuperscript{55}

Other factors that have been associated with diarrhoea include lack of breastfeeding, vitamin A deficiency and measles.\textsuperscript{8} Vitamin A supplementation has been found to reduce the incidence and severity of diarrhoea in children.\textsuperscript{57} Likewise, breastfed children are at much lower risk of having diarrhoeal illnesses because breastmilk is rich in immunoglobulins, lactoferrin and vitamin A, which are all protective against diarrhoea. Breastfeeding also protects against diarrhoea-specific morbidity and mortality throughout the first two years of life\textsuperscript{58-61} because of its protective immunological properties, and its protective value against dehydration, which is an important cause of diarrhoeal deaths.\textsuperscript{1} WHO recommends that infants should be exclusively breastfed during the first six months of life and that breastfeeding be continued until at least two years of age.\textsuperscript{1} Continued breastfeeding during diarrhoeal illnesses is also strongly encouraged.\textsuperscript{1}
ZINC SUPPLEMENTATION DURING DIARRHOEA

Zinc supplementation reduces the incidence and severity of diarrhoea, pneumonia, and malaria in children.8, 22, 62 The use of zinc for the treatment of diarrhoea has been estimated to reduce diarrhoea mortality by up to 23 percent 56 via reduction in stool output and duration of diarrhoea in both acute and persistent diarrhoea.16-18, 63, 64 Although the exact mechanism by which zinc supplementation reduces diarrhoea is not fully understood,16, 65 zinc treatment in patients with diarrhoea brings about improved water and electrolyte absorption by the intestine, quicker regeneration of gut epithelium, increased levels of brush border enzymes, and enhanced immune response resulting in increased clearance of pathogens from the intestine.48 When given for the treatment of diarrhoea, zinc supplementation not only reduces the severity of the initial episode, but may also prevent future diarrhoeal episodes in the two to three months following supplementation.56, 66

In a randomized placebo-controlled study63 carried out in Jos University Teaching Hospital, Nigeria, zinc supplementation was found to significantly reduce the average number of watery stools and the duration of diarrhoea as well as the recurrence of diarrhoea within the following three months. Similarly, a descriptive retrospective study67 carried out at the University of Port Harcourt Teaching Hospital, Nigeria showed a reduction in subsequent diarrhoeal episodes following zinc supplementation during treatment of childhood diarrhoea compared to treatment without zinc. Several studies17-19, 68-70 in other countries have also established the reduction in duration and severity of diarrhoeal episodes following zinc supplementation. In contrast, a randomized double-blind controlled study71 carried out in Lagos, Nigeria did not demonstrate significant reduction in duration of diarrhoea or daily stool frequency with zinc supplementation. The difference between this study and the earlier
cited ones could be as a result of exclusion of children with protein-energy malnutrition as well as the small sample size used in the study.

Zinc supplements contain several forms of zinc, including zinc gluconate, zinc sulphate, zinc acetate, zinc citrate and zinc oxide.\textsuperscript{72, 73} The WHO recommends the use of the water-soluble zinc compounds which include zinc sulphate, zinc acetate and zinc gluconate for use in the management of diarrhoea.\textsuperscript{74} Zinc sulphate is the least expensive of these and is therefore the most commonly used.\textsuperscript{73, 75} The percentage of elemental zinc varies by form. Zinc sulphate contains 23 percent elemental zinc, while zinc acetate and zinc gluconate contain 30 percent and 14 percent elemental zinc respectively.\textsuperscript{72, 73} Differences exist among these forms of zinc in absorption, bioavailability and tolerability. Zinc gluconate is better absorbed and has a higher bioavailability than zinc sulphate, but is however much more expensive than zinc sulphate.\textsuperscript{73} These zinc salts are considered to be equally effective since no difference in efficacy has been shown.\textsuperscript{75} Zinc salts have an unsavoury metallic taste which is usually masked by adding suitable flavours to the product during its manufacture.\textsuperscript{75}

Research has shown that serum zinc concentrations increase consistently when individuals consume zinc supplements,\textsuperscript{3, 68, 76, 77} regardless of their initial serum zinc concentration.\textsuperscript{76} However, an individual’s serum zinc concentration does not reliably predict that person’s response to zinc supplementation.\textsuperscript{76} There have been no reports of severe side effects from any form of zinc supplementation for the management of diarrhoea.\textsuperscript{75, 78, 79} The only reported side effect of zinc supplementation has been vomiting probably due to the metallic aftertaste of oral zinc supplements.\textsuperscript{16, 70, 75}
ZINC TOXICITY

Excessive intake of zinc can lead to toxicity.\textsuperscript{11, 23} Zinc toxicity can occur in both acute and chronic forms.\textsuperscript{11, 23} Acute toxicity may occur when large doses of zinc supplements in excess of one gram are consumed.\textsuperscript{23} Manifestations of acute toxicity include nausea, vomiting, diarrhoea, lethargy and abdominal pain.\textsuperscript{11, 22, 23} Chronic toxicity can occur when greater than 50 mg/day of zinc is consumed over several weeks,\textsuperscript{11, 23} and this may manifest as anaemia, neutropenia and central nervous system disturbances predominantly due to lowering of copper levels.\textsuperscript{11, 23, 80, 81}

ASSESSMENT OF ZINC STATUS

Although the importance of zinc for human health is widely recognized, estimating the burden of diseases associated with its deficiency has been difficult due to inadequate tools with which to measure zinc deficiency.\textsuperscript{23, 24} There is no generally accepted index of zinc status that is completely specific and sensitive.\textsuperscript{23, 24} In response to the lack of readily available biomarkers, Brown \textit{et al}\textsuperscript{23} developed a method to estimate the prevalence of zinc deficiency in a population by assessing the bioavailability of zinc in the local diet. Using this approach, Caulfield and Black\textsuperscript{82} estimated the global prevalence of zinc deficiency to be 31 percent. The prevalence ranged from four to 73 percent across sub-regions, with higher prevalence in Central and Southern African regions, as well as South-east Asia.

Serum or plasma zinc levels are the most reliable and commonly used indices to assess zinc deficiency in human studies,\textsuperscript{3, 23, 24} but this method has several limitations due primarily to variations in zinc homeostasis.\textsuperscript{3, 24} Serum zinc levels are reliable only when the blood samples are not haemolysed or contaminated by exogenous sources of zinc.\textsuperscript{3, 24} In addition, values are affected by diurnal variation of zinc levels as well as fasting and meal
consumption.\textsuperscript{3, 24} Values may also be spuriously low when there is presence of infection or inflammation.\textsuperscript{3, 24}

The current suggested lower cut-off value for the assessment of normal serum zinc concentration is 65 µg/ dl for children below the age of 10 years for morning non-fasting blood samples,\textsuperscript{3, 83} while 159 µg/ dl is considered as the upper limit.\textsuperscript{4} Children with serum zinc concentrations lower than 65 µg/ dl are therefore considered to have hypozincaemia, while those with equal to or higher than 160 µg/ dl are considered to have hyperzincaemia.

The results of a 2001-2003 National Food Consumption and Nutrition Survey\textsuperscript{84} in Nigeria showed that 20% of under-fives were zinc deficient. The prevalence of zinc deficiency was higher in rural than in urban areas, highest in the moist savannah zone, intermediate in the dry savannah and lowest in the humid forest zone. The 21 percent prevalence of zinc deficiency reported by Akeredolu and Oguntola\textsuperscript{85} amongst primary school children aged 5-13 years in Lagos was similar to the national average obtained from the National Food Consumption and Nutrition Survey.\textsuperscript{84} On the other hand, the 41.5 percent prevalence reported by Onyemaobi and Onimawo\textsuperscript{86} from under-fives in Imo State was considerably higher, though still within the ranges reported by Caulfield \textit{et al}\textsuperscript{82} and the National nutrition survey.\textsuperscript{84} Differences in methods of analysis, zinc content of soils, choices of foods, as well as the health, socio-economic and nutritional status of the Subjects in these studies may contribute to this disparity.

Studies have also been carried out on serum zinc levels of breastfeeding infants. Airede\textsuperscript{27} reported a significant decline in serum zinc levels over the first eight weeks of life, followed by a gradual rise until the age of 16 weeks to reach values close to birth levels. Thereafter, the values declined again until 20 weeks, and then remained steady until 24 weeks. Despite
these fluctuations in serum zinc levels, breastmilk is usually an adequate source of zinc for infants in the first six months of life.\textsuperscript{25, 26}

Serum zinc concentrations fluctuate by as much as 20 percent during a 24-hour period, largely due to the effects of food ingestion.\textsuperscript{87} Following a meal, there is an immediate initial increase within the first 30 minutes, after which the concentration declines progressively for the next four hours and then rises until food is eaten again.\textsuperscript{87, 88} During the night, the concentration of serum zinc increases progressively, so that the highest levels of the day are generally seen in the early morning.\textsuperscript{87, 89} During acute infections and inflammation, serum zinc concentrations are reduced, probably due to the redistribution of zinc from the plasma to the liver.\textsuperscript{13} Stress can also reduce serum zinc levels because prolonged physical and psychological stress produces changes characteristic of an acute-phase response.\textsuperscript{90} On the other hand, haemolysis can result in extremely high serum zinc levels because the concentration of intracellular zinc is considerably greater than that in serum.\textsuperscript{88}

Zinc concentration is usually higher in serum, when compared with plasma, because zinc can be released from erythrocytes and platelets while awaiting clotting of the sample for serum separation, unlike plasma separation which can be done immediately after collection. A standardized clotting time of 30–40 minutes is recommended before separation of serum.\textsuperscript{88} On the other hand, anticoagulants such as heparin which are required for the separation of plasma can interfere with zinc levels.\textsuperscript{88} Some analysts prefer serum to plasma, as precipitates that form in plasma samples can be problematic due to clogging of the aspirator in atomic absorption spectrometry. Using serum also avoids the possibility of contamination by anticoagulants, where this is a concern. During sample separation for harvesting of serum, centrifugation procedures should be adequate (at 2000 - 3000 revolutions/ min for 10-15 minutes) to remove all blood cells which can interfere with results because of their high
After sample collection, the serum or plasma samples obtained should be properly stored if not analyzed immediately. Generally, refrigeration of plasma or serum samples at 4°C is acceptable for short term storage (i.e. two – three weeks) prior to analysis, but samples should be kept frozen at -25°C or lower for longer storage periods.

Flame atomic absorption spectrometry (FAAS) is the most commonly used analytical method for the measurement of zinc concentrations in serum samples. This method is preferred because it is specific, sensitive and less expensive. It is considered to be the simplest and most practical technique that is suitable for use in lower-income countries and capable of producing accurate results. Colorimetric methods for measuring serum zinc are now obsolete. Other methods include graphite furnace atomic absorption spectrometry (GFAAS), inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma atomic emission spectrometry (ICP-AES), and instrumental neutron activation analysis (NAA).

CONCLUSION

Zinc is an essential micronutrient whose deficiency increases the risk and severity of infections and contributes significantly to overall childhood morbidity and mortality. Zinc supplementation helps in reducing the burden and severity of diarrhoea in children. Serum zinc estimation is the most common method used to assess zinc deficiency. In the current study, flame atomic absorption spectrometry was the method used for analysis of serum zinc because of its high sensitivity and specificity as well as its relative affordability.

JUSTIFICATION FOR THE STUDY
The relationship between zinc deficiency and diarrhoea is not fully understood, and there have been only few studies geared towards investigating such interaction among Nigerian children. To the best knowledge of this researcher, only one study has been carried out on zinc deficiency in children in Osun State, Nigeria and the study analyzed the serum zinc levels of children with severe malaria. Thus, no study has been carried out on serum zinc levels during diarrhoeal diseases in children in Osun State. The serum zinc status of children with diarrhoea in this environment, therefore, remains uncertain and the results of studies carried out in other parts of the country may not be generalizable to children in Ilesa, Osun State. This is because staple foods as well as their zinc contents vary from one community to another, and the zinc content of soils and the plants grown in them also varies among different communities.

The present study was therefore carried out to provide information about the relationships between zinc deficiency and aspects of acute diarrhoea in our community. This could help to raise awareness on the need for provision of zinc supplements for children in Nigeria and thereby help to reduce the morbidity and mortality associated with diarrhoea. This will, in turn, support the drive towards achieving Millenium Development Goal 4, which is aimed at reducing overall childhood mortality rate.

The research question in the study was “What is the relationship between hypozincaemia and the presence, type and severity of acute diarrhoea among under-five children in Ilesa?” while the null hypothesis tested was that “there is no significant relationship between hypozincaemia and the presence, type and severity of acute diarrhoea among under-five children in Ilesa”.

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AIM AND OBJECTIVES OF THE STUDY

The aim of this study was to determine the relationships between serum zinc levels and the presence, type and severity of acute diarrhoea among under-five children in Ilesa.

Specific objectives:
1) To determine the serum zinc levels of under-five children with acute diarrhoea.
2) To determine the prevalence of hypozincaemia among under-five children with acute diarrhoea.
3) To determine the relationship between serum zinc levels and type of diarrhoea.
4) To determine the relationship between serum zinc levels and severity of dehydration.
5) To determine the response of serum zinc levels to zinc supplementation in children with acute diarrhoea.
PATIENTS AND METHODS

STUDY LOCATION

The study was conducted at the Under-five Welfare Clinic (UFWC) and the Children’s Emergency Ward (CEW) of the Wesley Guild Hospital (WGH), Ilesa. The WGH, Ilesa, is one of the units of the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife. OAUTHC serves the health needs of the urban and rural communities in Osun, Ondo and Ekiti States in South-West Nigeria. It is a major referral health facility providing both general and specialist paediatric care for these communities.

The UFWC has five consultation points and attends to an average of 60 patients per day. It provides immunization, nutritional rehabilitation as well as general paediatric care. It is manned by a consultant, a senior registrar, two registrars, and nurses. The CEW has seven beds and admits an average of 90 patients per month. It has two consulting rooms and a procedure room for treatment. It is manned by a team of clinicians comprising a consultant, a senior registrar, two registrars and three house officers. The nursing staff run shifts to provide 24-hour services for the care of patients.

The Diarrhoea Treatment Unit (DTU), which is meant for the care of patients with diarrhoeal illnesses, is adjacent to the CEW. Patients with diarrhoeal illnesses who present at the CEW and the UFWC without features of severe dehydration are referred to this unit for oral rehydration services. Those requiring intravenous rehydration are managed in the CEW. The DTU receives an average of three patients per day.

STUDY DESIGN

This study was a prospective case-control study.
STUDY POPULATION

Reference population: All children presenting at the UFWC and the CEW of the WGH, Ilesa during the study period.

Study population: The Subjects included all consecutive children aged six months to 59 months with acute diarrhoea who satisfied the inclusion criteria. The Controls were children matched for age and sex without diarrhoea, who were apparently healthy. They included children who presented for immunization, routine blood tests like haemoglobin genotype, and those for minor surgical procedures like herniotomy.

Inclusion criteria for the Subjects:

1. Age six months to 59 months.
2. Duration of diarrhoea less than 14 days.

Exclusion criteria for the Subjects:

1. Children whose parents/ guardian refused to give consent.
2. Age less than six months: Infants less than six months were excluded because such children, who are mostly on exclusive breastfeeding, usually pass loose, pasty stools frequently, thus making the definition of diarrhoea in that age group difficult.\(^1\)
3. Persistent diarrhoea\(^8\): Children with persistent diarrhoea were excluded because the mechanism for zinc deficiency in persistent diarrhoea may be different from that of acute diarrhoea since persistent diarrhoea is frequently associated with malnutrition and serious non-intestinal infections.\(^1\)
4. Recent ingestion of zinc supplements or zinc-containing drugs (such as MIM, a multivitamins and minerals preparation) in the previous three months: A period of three
months was chosen because the rate of elimination of absorbed zinc in the body is about one percent per day.$^{92}$ In addition, the effects of zinc supplementation in children have been found to last for two to three months.$^{19}$

5. Severe undernutrition: z-score below -3 on a WHO weight-for-age, height-for-age or weight-for-height growth chart, or presence of oedema.$^{6}$ The exclusion of such children from the study was necessary because severe undernutrition on its own is frequently associated with micro-nutrient deficiency, including zinc.$^{22}, 41, 42$ The children may also have low albumin levels, which can result in falsely low zinc levels.$^{41}, 42$

6. Children being managed for, or who have features to suggest, other known causes of zinc deficiency such as sepsis,$^{38}$ sickle cell anaemia$^{39}$ and HIV infection.$^{40}$

7. Children previously recruited as either Subjects or Controls during an earlier period of the study.

**Inclusion criteria for the Controls:** Apparently healthy children aged six months to 59 months, matched for age and sex with the Subjects. After recruiting each Subject, a Control of the same age and sex was sought for and recruited to ensure proper matching.

**Exclusion criteria for the Controls:**

1. Children whose parents/ guardian refused to give consent.

2. History of recent ingestion of zinc supplements or zinc-containing drugs in the previous three months.

3. Severe undernutrition: z-score below -3 on a WHO weight-for-age, height-for-age or weight-for-height growth chart, or presence of oedema.$^{6}$

4. History of diarrhoeal illness within the previous six weeks: A period of six weeks was chosen to ensure complete recovery from the diarrhoeal illness since it has been
reported that repair of the crypt and villi structure of the intestinal wall after injury normally takes about four weeks.\textsuperscript{93}

5. Children previously recruited as Subjects during an earlier period of the study.

**SAMPLE SIZE**

The minimum number (n) of children required for the study was calculated using the formula: \textsuperscript{94}

\[ n = z^2 \cdot p \cdot (1-p) \div d^2. \]

Where:

‘z’ is the critical value and is equal to 1.96 for a 95\% confidence interval.

‘p’ is the estimated prevalence of zinc deficiency among under-five children, which was taken as 20\%.\textsuperscript{84}

‘d’ is the absolute sampling error that can be tolerated. In this study, it was fixed at 5\%.

Therefore, the minimum, sample size (n) = 1.96\(^2\) \times 0.20 \times (1 - 0.20) \div 0.05\(^2\)

\[ = 246 \]

Thus, the estimated sample size needed to be 95\% confident that the estimate of the population prevalence of zinc deficiency is within 5\% of the percentage frequency was 246. This was approximated to 250. Therefore, 250 subjects and 250 controls were recruited for the study. Any Subject or Control who had incomplete data (such as blood samples that were not analyzed due to obvious haemolysis, sample insufficiency or loss) during the course of the study was consecutively replaced by a new study participant in order to ensure that data/sample collection was complete.
ETHICAL CONSIDERATIONS

Ethical clearance was obtained for the study from the Ethics and Research Committee of the OAUTHC. The certificate is attached as Appendix I. For each child recruited, a written informed consent (Appendix II) was obtained from the parent(s) or the accompanying guardian. Children who were found to have hypozincaemia were given zinc supplements and their caregivers were counseled on the need for zinc-rich diets, while those with hyperzincaemia were counseled to avoid zinc supplements and excessive intake of zinc-rich foods.

DATA COLLECTION

The recruitment of Subjects and Controls was carried out by the Researcher, who administered the data proforma to the parent(s)/guardian(s), and examined the patients. Blood sample collection was also done by the Researcher, as well as management of the patients in conjunction with other resident doctors under the supervision of the consultants.

History and Physical Examination

All children aged six months to 59 months who presented at the UFWC or CEW of the WGH, Ilesa with acute diarrhoea who met the inclusion criteria were recruited into the study. The history obtained from each Subject was recorded in a proforma (Appendix III). The social class of each child was assessed using the method described by Oyedeji95 (Appendix IV), which is based on the occupation and educational qualifications of the parents. Social classes I and II were further classified as high socio-economic status, while social classes III, IV and V were classified as low socio-economic status.95 Acute diarrhoea was classified into acute non-bloody diarrhoea and dysentery, defined as the presence of visible blood in the stool.1 The duration of diarrhoea was classified into three groups as 1-3 days, 4-6 days and 7-13 days, while the frequency of passage of stools was classified as 3-4 episodes/day, 5-6 episodes/day and ≥7
episodes/day. The duration of diarrhoea and frequency of passage of stools were classified based on convenience for data analysis, considering the relative frequencies of the individual numbers and grouping was done to avoid very small frequencies in any of the categories.

The findings on physical examination, including anthropometric measurements (weight, height/ length, mid-arm circumference), axillary temperature and assessment for signs of dehydration, were also recorded in the proforma (Appendix III). Weight was measured to the nearest 0.05 kilogram (kg) using a Wayster infant weighing scale designed for children weighing between 0–13 kg and a ZT-160 Health Scale which is designed for older children who could stand. The ZT-160 Health Scale can measure weights between zero and 160 kg, and also has a stadiometer that can measure heights between 70 centimetres (cm) and 190 cm. This stadiometer was used to measure the heights of children who could stand to the nearest 0.1 cm. The length of infants and other children that were too weak to stand was measured to the nearest 0.1 cm using an inelastic tape measure. Shoes, clothings and diapers were removed during weight measurements.

Mid-upper arm circumference (MUAC) was measured on the left arm with an inelastic tape at the mid-point between the acromium process and the olecranon process. Removal of clothings on the arm and proper positioning of the patient with the arm hanging straight down were ensured before taking the MUAC. Axillary temperature was measured for each child using a mercury-in-glass thermometer inserted in the patient’s axilla for two minutes, and the result was recorded in degrees celcius (°C). Fever was taken as axillary temperature greater than 37.5°C.²

The WHO child growth standards in form of z-scores⁶ (Appendix V) were used to classify the nutritional status of the children as normal (when weight-for-age, height-for-age and weight-for-height z-scores are ≥ -2), underweight (weight-for-age z-score < -2), wasting (weight-
for-height z-score < -2), stunting (height-for-age z-score < -2), overweight (weight-for-height z-score > +2 but ≤ +3) or obese (weight-for-height z-score > +3).

The degree of dehydration was estimated using the guide to clinical assessment of dehydration\textsuperscript{5} (Appendix VI), and dehydration, when present, was classified as mild, moderate or severe.\textsuperscript{5}

**Laboratory investigations**

*Collection of blood samples:* Two and a half milliliters (2.5 ml) of blood was collected from each patient by peripheral venous puncture before treatment, which included zinc therapy, was commenced. The site for venepuncture was cleaned with methylated spirit before blood collection in order to avoid contamination. The blood sample was placed inside a plain specimen bottle and allowed to clot for 30 minutes in a cool place. Longer clotting periods were avoided in order to prevent haemolysis.

Thereafter, the serum was separated by centrifugation at 3000 revolutions per minute for 10 minutes using a clinical macro-centrifuge, and subsequently transferred into another plain bottle. The serum was refrigerated at 4\textdegree C after separation until analysis, which was done within two weeks. Venepunctures as well as serum separation and storage were done majorly by the Researcher, assisted by specific resident doctors and laboratory staff who had been tutored on the study protocol.

*Serum zinc assay:* The serum samples were transported with ice-packs to the Central Science Laboratory, Obafemi Awolowo University, Ile-Ife, about 30 minutes drive from the WGH, for analysis. Zinc assay was done by atomic absorption spectrophotometry (AAS).

The serum samples were prepared for assay by dilution with 2N hydrochloric acid (HCL) solution, followed by centrifugation.\textsuperscript{96} Four point five mls of 2N HCL was added to 0.5 mls of serum to produce a dilution ratio of 1: 10. After dilution, the sample was allowed to stand for 24
hours to form a precipitate, and then centrifuged at 3000 revolutions per minute for 10 minutes, using a clinical macro-centrifuge. Thereafter, the clear supernatant was separated from the precipitate into another bottle. The supernatant was fed by aspiration into the spectrophotometer for the assay of zinc concentration. The concentration of zinc was displayed on the monitor of the spectrophotometer in milligram per litre (mg/ L) and recorded on the data processor. Three readings, and their mean, were displayed on the monitor for each sample analysed. The actual serum concentration of zinc was calculated by multiplying the mean value by the dilution factor of 10, and converted to µg/dl by multiplying with 100.97

All sample dilutions were done by the Researcher, using the 2N HCL solution provided by the laboratory. The Researcher also participated in the assay process done with the AAS machine, together with the laboratory scientists. The AAS machine used was AAnalyzer 400 model by PerkinElmer. A picture of the machine, and a brief explanation of its principle of operation are attached as Appendix VII.

The reference range of serum zinc considered as normal in the study was 65 to 159 µg/dl.3,4 Values less than 65 µg/dl were considered low (hypozincemia) as recommended by the IZiNCG,3 while values ≥160 µg/dl were considered high (hyperzincemia).4

Other Investigations: Other laboratory investigations were carried out as indicated for individual patients, particularly those admitted. These included serum electrolytes, urea and creatinine, blood film for malaria parasites and random blood sugar, which were done routinely for Subjects who required admission, but not for those treated as out-patients. Stool microscopy, culture and sensitivity (MCS) was done for those with dysentery. Haemoglobin genotype and HIV screening were also done to exclude sickle cell anaemia and HIV infection in the Subjects who were admitted, and in others who had history or clinical features to suggest those conditions. Blood
culture was done to exclude sepsis in very-ill looking and lethargic patients, particularly those with tachycardia, tachypnoea and respiratory distress.

Hypokalaemia was defined as serum potassium less than 3.0 mmol/L, hyponatraemia as serum sodium less than 130.0 mmol/L, metabolic acidosis as serum bicarbonate less than 22 mmol/L and azotaemia as serum urea greater than 6.4 mmol/L and/or serum creatinine greater than 62 µmol/L. Hypoglycaemia was defined as random blood sugar less than 40 mg/dL.

**Treatment**

Following clinical assessment, oral rehydration therapy, using the standard WHO ORS, was instituted at the DTU as appropriate for patients with no evidence of dehydration and those with mild or moderate dehydration, while children with severe dehydration were admitted into the CEW and given intravenous fluids. The care-givers were also taught how to prepare and administer ORS solution. Zinc supplements were prescribed for all the Subjects, including those treated as out-patients, in the form of dispersible oral zinc sulphate tablets at a dose of 20 mg of elemental zinc daily for 10 days. The zinc supplements were given free to some of the patients whose parents/guardian could not afford it at that time. In addition, antibiotics were prescribed for children with dysentery according to the unit protocol. The antibiotic prescribed routinely was oral cotrimoxazole, while the ill patients who were admitted were given ciprofloxacin. Antimalarials were prescribed for all Subjects with fever or a history of fever in the preceding five days, as recommended by WHO. Electrolyte derangements were corrected when observed in patients on admission.

Those managed as out-patients were requested to come for follow-up assessment (level of hydration, frequency and consistency of stools, fever, vomiting) one or two days later. Furthermore, all the patients, including those admitted and subsequently discharged, were
requested to come back for repeat zinc assay on the day following completion of zinc tablets (day 11). As soon as their results were obtained, the Controls who had hypozincaemia were also invited for treatment with zinc supplements.

All the care-givers were counseled on the prevention of diarrhoea. In addition, the care-givers of those children with hypozincaemia were counseled on the need for zinc-rich diets, while those with hyperzincaemia were counseled to avoid zinc supplements and excessive intake of zinc-rich foods. The outcome of each of the admitted cases was documented as discharged, discharged against medical advice (DAMA) or death. The patients were considered fit for discharge when stools were no longer watery or frequent, normal feeding had been established and associated features such as fever, vomiting, dehydration and weakness were no longer present.

DATA ANALYSIS

Data analysis was done using the Statistical Programme for Social Sciences (SPSS) version 16.0\textsuperscript{100} and computer programmes for epidemiologists (WINPEPI).\textsuperscript{101} Means ± standard deviations (SD) were computed for continuous variables, while proportions were calculated for discrete variables. Means were compared using the independent samples t-test (t) or ANOVA (F), while proportions were compared using the Pearson chi-square test ($\chi^2$). The likelihood-ratio (LR) test or Fisher’s exact test (FET) was applied to chi-square analysis as applicable in tables where over 20% of the cells had expected counts less than five.\textsuperscript{101} Post-treatment serum zinc levels were compared with the base-line values using the paired samples t-test. The relationship between continuous data was assessed using Pearson correlation (r). Logistic regression analysis (B) was done to determine factors independently associated with hypozincaemia. Probability values (p) <0.05 at 95% confidence interval (CI) were accepted as statistically significant.
RESULTS

During the 11-month period of the study (between 15th October 2013 and 10th September 2014), a total of 1,137 children were admitted into the CEW, while a total of 9,244 patients were attended to at the UFWC. The latter excluded children who came for routine immunizations. Diarrhoeal diseases were responsible for 426 (4.6%) of out-patient consultations at the UFWC, and for 89 (7.8%) of admissions into the CEW. Of the 250 Subjects recruited for the study, 205
(82.0%) were recruited from the UFWC and 45 (18.0%) from the CEW. The 250 children who were recruited as Controls were all from the UFWC.

SOCIO-DEMOGRAPHIC CHARACTERISTICS OF THE SUBJECTS AND CONTROLS

**Age:** The ages of the 250 Subjects ranged from six to 56 months with mean and standard deviation (SD) of 16.6 ± 9.4 months. The ages of the Controls ranged from six to 59 months with a mean ± SD of 16.6 ± 9.3 months. The difference in the mean ages was not statistically significant (t = 0.038, p = 0.970). Of the Subjects, 101 (40.4%) were aged 6-12 months, 115 (46.0%) were 13-24 months, 23 (9.2%) 25-36 months, 8 (3.2%) 37-48 months and 3 (1.2%) 49-59 months. For the Controls, the corresponding frequencies were 98 (38.2%), 114 (45.6%), 26 (10.4%), 8 (3.2%) and 4 (1.6%), respectively. The difference in age distribution between the Subjects and Controls was not statistically significant (χ² = 0.38, df = 4, p = 0.984).

**Sex:** Of the 250 Subjects, 147 (58.8%) were males and 103 (41.2%) were females. The male: female ratio was 1.4: 1. One hundred and forty three (57.2%) of the Controls were males, and 107 (42.8%) were females. The male: female ratio was 1.3: 1. The difference in sex distribution between the Subjects and the Controls was not statistically significant (χ² = 0.13, p = 0.717).

**Socio-Economic Status:** Forty six (18.4%) of the Subjects belonged to the higher social classes (I and II), and 204 (81.6%) to the lower classes (III, IV and V). Among the Controls, 83 (33.2%) belonged to the higher classes and 167 (66.8%) to the lower classes. The difference in distribution of socio-economic status between the Subjects and the Controls was statistically significant (χ² = 14.30, p <0.001).
SYMPTOMS AT PRESENTATION: The mean ± SD duration of diarrhoea at presentation was 3.4 ± 2.5 days and the range one to 13 days. The duration was ≤3 days in 173 (69.2%) of the Subjects, 4-6 days in 53 (21.2%) and ≥7 days in 24 (9.6%). The number of episodes of watery stool per day ranged from three to 12 with a mean ± SD of 4.9 ± 2.2. One hundred and forty (56.0%) had 3-4 episodes per day, 69 (27.6%) 5-6 and 41 (16.4%) ≥7 episodes daily. Twenty seven (10.8%) Subjects had dysentery. A history of fever was present in 182 (72.8%), while 151 (60.4%) had one or more episodes of vomiting. Reduction in urinary output was reported in 17 (6.8%).

IMMUNIZATION HISTORY: Table I shows the immunization status of the Subjects and Controls. The difference in immunization status between the Subjects and Controls was statistically significant (p = 0.003). The proportion of children that were appropriately immunized was significantly higher [92.4% versus 86.4%; OR (95% CI) = 1.91 (1.06, 3.45); \( \chi^2 = 4.75, p = 0.029 \)] among the Controls, while those that had no immunization were significantly fewer [0.0% versus 2.8%; OR (95% CI) = 15.43 (0.88, 270.11); \( \chi^2 = 7.10, p = 0.008 \)] among the Controls. Among the Subjects, 34 (16.7%) of 204 children from the low socio-economic classes versus zero of 46 children from the high socio-economic classes had no or incomplete immunization [OR (95% CI) = 18.82 (1.17, 303.61); FET p = 0.001]. The corresponding frequencies among the Controls were 18 (10.8%) of 167 versus 1 (1.2%) of 83 [OR (95% CI) = 9.91 (1.31, 74.65); FET p = 0.005].

BREASTFEEDING HISTORY: The mean ± SD duration of exclusive breastfeeding was 4.9 ± 1.6 months among the 247 Subjects who were breastfed and 5.3 ± 1.4 months among the 250 Controls. The difference in the duration of exclusive breastfeeding was statistically significant (t
The proportion of Subjects who had exclusive breastfeeding for six months was significantly lower than the Controls [57.2% versus 72.0%; OR (95% CI) = 1.92 (1.33, 2.79); \(\chi^2 = 12.0, p = 0.001\)]. The overall mean ± SD duration of breastfeeding in those Subjects who had been weaned (n = 112) was 14.9 ± 2.9 months, with a range of 10 to 24 months. The mean among the Controls was 14.4 ± 2.7 months with a range of six to 24 months. The difference in total duration of breastfeeding was not statistically significant (t = 1.28, p = 0.201). Table II shows the breastfeeding status of the Subjects and Controls. The difference in breastfeeding status between the subjects and the controls was not statistically significant (LR = 4.22, df = 2, p = 0.121).

**Growth and nutritional status:** Table III illustrates the growth and nutritional status of the Subjects and Controls. The proportion of underweight children was higher among the Subjects (18.0%) than among the Controls (8.4%) and the difference was statistically significant [OR (95% CI) = 2.39 (1.38, 4.15); \(\chi^2 = 10.05, p = 0.002\)]. Similarly, the proportions of children with stunting [OR (95% CI) = 2.17 (1.20, 3.93); \(\chi^2 = 6.73, p = 0.009\)] and wasting [OR (95% CI) = 2.11 (1.23, 3.61); \(\chi^2 = 7.60, p = 0.006\)] were also significantly higher among the Subjects.

Table I: Immunization status of the Subjects and Controls.

<table>
<thead>
<tr>
<th>Immunization status</th>
<th>Subjects n (%)</th>
<th>Controls n (%)</th>
<th>Statistical comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appropriate for age</td>
<td>*216 (86.4)</td>
<td>*231 (92.4)</td>
<td>*(\chi^2 = 4.75, df = 1, p = 0.029)</td>
</tr>
<tr>
<td>Incomplete</td>
<td>(\Phi 27 (10.8))</td>
<td>(\Phi 19 (7.6))</td>
<td>(\Phi \chi^2 = 1.53, df = 1, p = 0.216)</td>
</tr>
<tr>
<td>None</td>
<td>(@ 7 (2.8))</td>
<td>(@ 0 (0.0))</td>
<td>(@ FET p = 0.015)</td>
</tr>
<tr>
<td>Total</td>
<td>250 (100.0)</td>
<td>250 (100.0)</td>
<td></td>
</tr>
</tbody>
</table>

LR = 11.61, df = 2, \(p = 0.003\)
### Table II: Breastfeeding status of the Subjects and Controls.

<table>
<thead>
<tr>
<th>Breastfeeding status</th>
<th>Subjects n (%)</th>
<th>Controls n (%)</th>
<th>Statistical comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Still breastfeeding</td>
<td>*135 (54.0)</td>
<td>*139 (55.6)</td>
<td>*χ² = 0.13, df = 1, p = 0.719</td>
</tr>
<tr>
<td>No longer breastfeeding</td>
<td>Ф112 (44.8)</td>
<td>Ф111 (44.4)</td>
<td>Фχ² = 0.01, df = 1, p = 0.928</td>
</tr>
<tr>
<td>Not breastfed at all</td>
<td>@3 (1.2)</td>
<td>@0 (0.0)</td>
<td>@ FET p = 0.248</td>
</tr>
<tr>
<td>Total</td>
<td>250 (100.0)</td>
<td>250 (100.0)</td>
<td></td>
</tr>
</tbody>
</table>

LR = 4.22, df = 2, p = 0.121

### Table III: Growth and nutritional status of the Subjects and Controls.

<table>
<thead>
<tr>
<th>Nutritional status</th>
<th>Subjects n (%)</th>
<th>Controls n (%)</th>
<th>Statistical comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal weight</td>
<td>*199 (79.6)</td>
<td>*224 (89.6)</td>
<td>*χ² = 9.59, df = 1, p = 0.002</td>
</tr>
<tr>
<td>Underweight</td>
<td>Ф45 (18.0)</td>
<td>Ф21 (8.4)</td>
<td>Фχ² = 10.05, df = 1, p = 0.002</td>
</tr>
<tr>
<td>Overweight</td>
<td>@5 (2.0)</td>
<td>@5 (2.0)</td>
<td>@χ² = 0.00, df = 1, p = 1.000</td>
</tr>
<tr>
<td>Obese</td>
<td>@1 (0.4)</td>
<td>@0 (0.0)</td>
<td>@ FET p = 1.000</td>
</tr>
<tr>
<td>Total</td>
<td>250 (100.0)</td>
<td>250 (100.0)</td>
<td></td>
</tr>
</tbody>
</table>
**Clinical signs in the Subjects at presentation:** Ninety-one (36.4%) of the Subjects were febrile at presentation while nine (3.6%) had subnormal temperatures and 150 (60.0%) had normal temperatures. One hundred and eighty-two (72.8%) had no evidence of dehydration while 36 (14.4%) had mild, 20 (8.0%) moderate, and 12 (4.8%) severe dehydration.

*Relationship between diarrhoeal characteristics and clinical profile of the Subjects:* Table IV shows the relationships between the severity of dehydration and diarrhoeal characteristics. Eight (29.6%) of the Subjects with dysentery versus 60 (26.9%) of those with acute watery diarrhoea had signs of dehydration ($\chi^2 = 0.09$, $p = 0.764$). There was no statistically
significant difference between those with dysentery and those with acute watery diarrhoea in relation to the severity of dehydration (LR = 3.82, df = 2, p = 0.148). Twenty two (15.7%), 26 (37.7%) and 20 (48.8%) of the Subjects who passed 3 - 4, 5 - 6 and ≥7 episodes of watery stool/day, respectively, had signs of dehydration. The differences were statistically significant (χ² = 22.80, df = 2, p <0.001). However, there was no statistically significant difference in the frequency of passage of stools in relation to the severity of dehydration (LR = 6.77, df = 4, p = 0.149).

Forty-seven (27.2%), 16 (30.2%) and 5 (20.8%) of the Subjects whose diarrhoea had lasted 1 - 3, 4 - 6 and 7 - 13 days, respectively, had signs of dehydration (χ² = 0.73, df = 2, p = 0.694). There was no statistically significant difference in the duration of diarrhoea in relation to the severity of dehydration (LR = 6.53, df = 4, p = 0.163). Forty five (24.7%) Subjects with a history of fever versus 23 (33.8%) of those without a history of fever had signs of dehydration (χ² = 2.07, df = 1, p = 0.150). Twenty six (28.6%) of those who were febrile on examination versus 42 (26.4%) of the afebrile Subjects had signs of dehydration (χ² = 0.14, p = 0.712).

| Table IV: Relationships between the degree of dehydration and diarrhoeal characteristics. |
|-----------------------------------------------|---------------------|---------------------|---------------------|
| Diarrhoeal characteristic | Degree of dehydration | Total | Statistical comparison |
| | Mild dehydration | Moderate dehydration | Severe dehydration | n (%) | n (%) | n (%) | |
| Type of diarrhoea | | | | 8 (11.8) | 60 (88.2) | |
| Acute dysenteric | 6 (16.7) | 2 (10.0) | 0 (0.0) | LR = 3.82 df = 2 p = 0.148 |
| Acute watery | 30 (83.3) | 18 (90.0) | 12 (100.0) | |
| Total | 36 (100.0) | 20 (100.0) | 12 (100.0) | 68 (100.0) |

Stool frequency (episodes/day)
<table>
<thead>
<tr>
<th>Duration of diarrhoea (days)</th>
<th>1-3</th>
<th>4-6</th>
<th>7-13</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 – 4</td>
<td>16 (44.4)</td>
<td>3 (15.0)</td>
<td>3 (25.0)</td>
<td>22 (32.4)</td>
</tr>
<tr>
<td>5 – 6</td>
<td>11 (30.6)</td>
<td>11 (55.0)</td>
<td>4 (33.3)</td>
<td>26 (38.2)</td>
</tr>
<tr>
<td>≥ 7</td>
<td>9 (25.0)</td>
<td>6 (30.0)</td>
<td>5 (41.7)</td>
<td>20 (29.4)</td>
</tr>
<tr>
<td>Total</td>
<td>36 (100.0)</td>
<td>20 (100.0)</td>
<td>12 (100.0)</td>
<td>68 (100.0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>History of fever</th>
<th>Present</th>
<th>Absent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>25 (69.4)</td>
<td>11 (30.6)</td>
<td>36 (100.0)</td>
</tr>
<tr>
<td>Absent</td>
<td>14 (70.0)</td>
<td>6 (30.0)</td>
<td>20 (100.0)</td>
</tr>
<tr>
<td>Total</td>
<td>39 (68.3)</td>
<td>17 (57.1)</td>
<td>56 (96.4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fever on examination</th>
<th>Febrile</th>
<th>Afebrile</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Febrile</td>
<td>12 (33.3)</td>
<td>24 (66.7)</td>
<td>36 (100.0)</td>
</tr>
<tr>
<td>Afebrile</td>
<td>8 (40.0)</td>
<td>12 (60.0)</td>
<td>20 (100.0)</td>
</tr>
<tr>
<td>Total</td>
<td>20 (53.3)</td>
<td>36 (61.8)</td>
<td>56 (100.0)</td>
</tr>
</tbody>
</table>

There was no statistically significant difference between the presence or absence of fever in relation to the severity of dehydration as shown in Table IV.

All 27 (100%) Subjects with dysentery versus 155 (69.5%) of those with acute watery diarrhoea had a history of fever. The difference was statistically significant \[ \text{OR (95\% CI)} = 1.44 (1.32, 1.57); \chi^2 = 11.31, p = 0.001 \]. On the other hand, nine (33.3%) with dysentery versus 82 (36.8%) with acute watery diarrhoea were febrile on examination \[ \chi^2 = 0.12, p = 0.726 \].

**Admission and outcome:** Forty-five (18.0%) Subjects were admitted. These included eight (4.4%) of the 182 who had no evidence of dehydration and 37 (54.4%) of the 68 with
dehydration [OR (95% CI) = 25.96 (11.09, 60.79); \( \chi^2 = 83.90, p <0.001 \)]. Among those with dehydration, all the 12 (100.0%) with severe dehydration, 12 (60.0%) of the 20 with moderate dehydration, and 13 (36.1%) of the 36 with mild dehydration were admitted. The relationship between the degree of dehydration and prevalence of admission was statistically significant (\( \chi^2 = 15.17, df = 2, p = 0.001 \)). The reasons for admission among those who did not have severe dehydration included persistent vomiting (n = 28), febrile convulsions (n = 3), prostration (n = 4) and parental anxiety (n = 2). The four patients who had prostration also had persistent vomiting. A history of fever was present in 31 (68.9%) of those admitted, but only 20 (44.4) of them were febrile at admission. The difference was statistically significant (\( \chi^2 = 5.48, df = 1, p = 0.019 \)).

The duration of admission ranged from one to five days with a mean of 2.9 ± 1.2 days. Of those admitted, 40 (88.9%) were discharged, two (4.4%) discharged against medical advice (DAMA), and three (6.7%) died. The parents of the two patients that DAMA claimed they had no funds for further hospital stay. Both were admitted because of persistent vomiting and left one day after admission, when vomiting had subsided.

Fifteen (33.3%) of the 45 patients that were admitted had electrolyte derangements and/or azotaemia. Six of the 15 had only one abnormality, and nine more than one abnormality. Of these 15, hypokalaemia occurred in nine, metabolic acidosis in four, hyponatraemia in four, and azotaemia in nine. None of the Subjects had hypoglycaemia.

Of the 18 Subjects with dysentery who had stool MCS done, only 8 (44.4%) stool samples were found to be blood stained. No ova, cyst or trophozoite of any parasite was identified in any of the stool samples. Apart from the growth of Escherichia coli in three of the samples, no other organism was isolated. Trophozoites of P. falciparum were identified in the blood films of 37 (82.2%) of the admitted patients, while 8 (17.8%) had no malaria parasites.
Ten of those who had malaria parasites had no history of fever. Eight of the Subjects who were admitted had blood culture done, and none had bacterial growth.

Five (33.3%) of the 15 Subjects with electrolyte derangements versus 11 (36.7%) of the 30 without electrolyte derangements had hypozincaemia. The difference was not statistically significant ($\chi^2 = 0.05$, $p = 0.826$). All three children that died had electrolyte derangements and hypozincaemia. Two of them were also severely dehydrated on admission while the third had moderate dehydration. The only child with uraemic encephalopathy, who was unconscious and had severe dehydration, was one of the three that died. The child’s serum urea was 43.6 mmol/L, while serum creatinine was 346.0 µmol/L.

The difference in the prevalence of dehydration between the Subjects that died (100%) and those that survived (65/247, 26.3%) was statistically significant [OR (95% CI) = 19.50 (1.48, 257.81); FET $p = 0.019$]. Among the 45 Subjects who were admitted, the prevalence of electrolyte derangements among those that died was significantly higher than that of those who survived [100% versus 28.6%; OR (95% CI) = 17.08 (1.21, 241.12); FET $p = 0.032$].

**SERUM ZINC LEVELS AND PREVALENCE OF HYPOZINCAEMIA AMONG THE SUBJECTS AND CONTROLS**

The mean ± SD and range of serum zinc in the Subjects and Controls are shown in Table V, while the distribution of zinc levels is shown in Table VI. The difference between the means was highly significant [difference (95% CI) = -28.5 (-35.8, -21.2); $t = -7.66$, $p < 0.001$]. The difference in distribution of zinc levels was also highly significant ($\chi^2 = 35.33$, df = 2, $p < 0.001$). The prevalence of hypozincaemia was significantly higher in the Subjects [OR (95% CI) = 3.09 (1.94, 4.90); $\chi^2 = 24.08$, $p < 0.001$], while the prevalence of hyperzincaemia was significantly higher in the Controls [OR (95% CI) = 4.91 (2.12, 11.37); $\chi^2 = 16.41$, $p < 0.001$].


RELATIONSHIPS BETWEEN SERUM ZINC LEVELS AND CLINICAL PROFILE OF THE SUBJECTS AND CONTROLS

Relationship between serum zinc levels and type of diarrhoea:

Subjects with dysentery had a significantly lower mean ± SD serum zinc level of 65.1 ± 25.0 µg/dl compared with a mean of 80.5 ± 36.3 µg/dl among those without visible blood in their stools. The difference between the means was statistically significant [difference (95% CI) = -15.4 (-29.5, -1.2); t = -2.13, p = 0.034]. Hypozincaemia was also more prevalent among those with dysentery [OR (95% CI) = 2.36 (1.06, 5.23); χ² = 4.51, p = 0.034]. The overall difference in distribution of serum zinc levels between the two groups was however not statistically significant (χ² = 5.00; p = 0.082) as shown in Table VII. None of the Subjects with dysentery versus 7 (3.1%) of those with acute watery diarrhoea had hyperzincaemia (FET p = 1.000).

Relationship between serum zinc levels and hydration status:

The mean ± SD serum zinc level among the Subjects with signs of dehydration was 84.5 ± 39.7 µg/dl, while it was 76.7 ± 33.7 µg/dl among those who had no signs. The difference was not statistically significant (t = -1.56, p = 0.120). The mean serum zinc level was 81.5 ± 37.9 µg/dl in Subjects with mild dehydration, 98.8 ± 43.9 µg/dl in those with moderate dehydration and 69.9 ± 32.9 µg/dl in those with severe dehydration. The differences were not statistically significant (ANOVA F = 2.29, p = 0.109).

The prevalence of hypozincaemia was 18/68 (26.5%) in Subjects with signs of dehydration and 58/182 (31.9%) in those with no signs (χ² = 0.68, p = 0.409), while the respective prevalences of hyperzincaemia were 4.4% and 2.2% (FET p = 0.394). The
prevalences of hypo- and hyperzincaemia in relation to the degree of dehydration are shown in Table VIII. There was no statistically significant difference between the distribution of zinc levels in relation to the degree of dehydration (LR = 7.29, df = 4, p = 0.121).

Relationship between the distribution of serum zinc levels and duration of diarrhoea, and frequency of passage of stools:

The prevalence of hypozincaemia among Subjects with duration of diarrhoea ≤3 days (n = 173) was 32.9%, compared with 26.4% among those with duration of 4 - 6 days (n = 53) and 20.8% among those with duration ≥7 days (n = 24). The differences were not statistically significant ($\chi^2 = 1.97; \text{df} = 2; p = 0.374$). Similarly, the differences in distribution of zinc levels in relation to the duration of diarrhoea were not statistically significant (LR = 4.22; df = 4; p = 0.377).

<table>
<thead>
<tr>
<th>Serum zinc</th>
<th>Subjects (n = 250)</th>
<th>Controls (n = 250)</th>
<th>Statistical comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD (µg/dl)</td>
<td>78.8 ± 35.6</td>
<td>107.3 ± 46.8</td>
<td>t = - 7.66; p &lt;0.001</td>
</tr>
<tr>
<td>Range (µg/dl)</td>
<td>8.0 - 246.0</td>
<td>16.0 – 313.0</td>
<td></td>
</tr>
</tbody>
</table>
Table VI: Distribution of serum zinc levels in the Subjects and Controls.

<table>
<thead>
<tr>
<th>Serum zinc</th>
<th>Subjects no (%)</th>
<th>Controls no (%)</th>
<th>Statistical comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypozincaemia</td>
<td>$\Phi 76 (30.4)$</td>
<td>$\Phi 31 (12.4)$</td>
<td>$\Phi \chi^2 = 24.08$, df $= 1$, $p &lt; 0.001$</td>
</tr>
<tr>
<td>Normozincaemia</td>
<td>$\Theta 167 (66.8)$</td>
<td>$\Theta 188 (75.2)$</td>
<td>$\Theta \chi^2 = 4.28$, df $= 1$, $p = 0.038$</td>
</tr>
<tr>
<td>Hyperzincaemia</td>
<td>$@ 7 (2.8)$</td>
<td>$@ 31 (12.4)$</td>
<td>$@ \chi^2 = 16.41$, df $= 1$, $p &lt; 0.001$</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>250 (100.0)</strong></td>
<td><strong>250 (100.0)</strong></td>
<td></td>
</tr>
</tbody>
</table>

$\chi^2 = 35.33$, df $= 2$, $p < 0.001$

---

Table VII: Relationship between the type of diarrhoea and distribution of serum zinc levels.

<table>
<thead>
<tr>
<th>Type of diarrhoea</th>
<th>Hypozincaemia n (%)</th>
<th>Normozincaemia n (%)</th>
<th>Hyperzincaemia n (%)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute dysenteric</td>
<td>*13 (48.1)</td>
<td>14 (51.9)</td>
<td>0 (0.0)</td>
<td>27 (100.0)</td>
</tr>
<tr>
<td>Acute watery</td>
<td>*63 (28.3)</td>
<td>153 (68.6)</td>
<td>7 (3.1)</td>
<td>223 (100.0)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>76 (30.4)</strong></td>
<td><strong>167 (66.8)</strong></td>
<td><strong>7 (2.8)</strong></td>
<td><strong>250 (100.0)</strong></td>
</tr>
</tbody>
</table>

$\chi^2 = 5.00$, df $= 2$, $p = 0.082$

* $\chi^2 = 4.51$, df $= 1$, $p = 0.034$
Table VIII: Distribution of serum zinc levels in relation to the degree of dehydration.

<table>
<thead>
<tr>
<th>Degree of dehydration</th>
<th>Hypozincaemia n (%)</th>
<th>Normozincaemia n (%)</th>
<th>Hyperzincaemia n (%)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild dehydration</td>
<td>10 (27.8)</td>
<td>24 (66.7)</td>
<td>2 (5.6)</td>
<td>36 (100.0)</td>
</tr>
<tr>
<td>Moderate dehydration</td>
<td>2 (10.0)</td>
<td>17 (85.0)</td>
<td>1 (5.0)</td>
<td>20 (100.0)</td>
</tr>
<tr>
<td>Severe dehydration</td>
<td>6 (50.0)</td>
<td>6 (50.0)</td>
<td>0 (0.0)</td>
<td>12 (100.0)</td>
</tr>
<tr>
<td>Total</td>
<td>18 (26.5)</td>
<td>47 (69.1)</td>
<td>3 (4.4)</td>
<td>68 (100.0)</td>
</tr>
</tbody>
</table>

LR = 7.29; df = 4; p = 0.121

Forty-five (32.1%) of the 140 Subjects who had 3 - 4 episodes of watery stool per day had hypozincaemia, while 92 (65.7%) had normozincaemia and 3 (2.1%) hyperzincaemia. Nineteen (27.5%) of those who passed 5 - 6 episodes daily had hypozincaemia, while 49 (71.0%) had normozincaemia and 1 (1.4%) hyperzincaemia. The respective prevalences were 12 (29.3%), 26 (63.4%), and 3 (7.3%) among those who passed ≥ 7 episodes per day. The differences were not statistically significant (LR = 3.47; df = 4; p = 0.483).

**Relationship between the distribution of serum zinc levels and immunization status:**

Among the 216 Subjects that were appropriately immunized for age, 61 (28.2%) had hypozincaemia, 148 (68.5%) normozincaemia and 7 (3.2%) hyperzincaemia. Of the 27
incompletely immunized Subjects, 11 (40.7%) had hypozincaemia, while none had hyperzincaemia. Of the seven who had no immunization, 4 (57.1%) were hypozincaemic, while none had hyperzincaemia. The differences in distribution of zinc levels among the Subjects in relation to immunization status was not statistically significant ($\chi^2 = 5.57; \text{df } = 4; \ p = 0.234$).

Among the Controls, the prevalences of hypo-, normo- and hyperzincaemia were 11.3%, 76.2% and 12.6%, respectively among appropriately immunized children and 26.3%, 63.2%, 10.5%, respectively among the incompletely immunized ones. The differences in distribution between appropriately and incompletely immunized children were not statistically significant ($\chi^2 = 2.97; \text{df } = 2; \ p = 0.234$).

**Relationship between breastfeeding and serum zinc levels:**

The mean serum zinc level was $80.9 \pm 33.4$ µg/dl among the Subjects who were still breastfeeding, $77.2 \pm 38.1$ µg/dl among those who had stopped breastfeeding and $44.7 \pm 9.2$ µg/dl among those who never breastfed. The differences were not statistically significant (ANOVA $F = 1.74, \ p = 0.177$). Table IX shows the relationship between breastfeeding and the distribution of serum zinc levels. The differences in distribution were statistically significant among the Subjects (LR = 10.63; df = 4; $p = 0.031$). All the three Subjects that were never breastfed versus 73/247 (29.6%) breastfed Subjects had hypozincaemia [OR (95% CI) = 23.57 (1.79, 310.87); FET $p = 0.013$]. The difference in prevalence of hypozincaemia between those that were still breastfeeding (34/135, 25.2%) and those that were not breastfeeding (42/115, 36.5%) approached statistical significance [OR (95% CI) = 1.71 (0.99, 2.94); $\chi^2 = 3.77, \ p = 0.052$].

The mean serum zinc level was $114.63 \pm 45.79$ µg/dl among the Controls who were still breastfeeding and $98.05 \pm 46.53$ µg/dl among those who had stopped breastfeeding. The
The difference was statistically significant [difference (95% CI) = 16.6 (5.0, 28.1); t = 2.82, p = 0.005]. The difference in distribution of serum zinc levels in relation to breastfeeding status was not statistically significant among the Controls ($\chi^2 = 2.56$, df = 2, p = 0.279) as shown in Table IX.

The correlation coefficient ‘r’ of serum zinc levels with the duration of exclusive breastfeeding among the Subjects was 0.12 (p = 0.018), while the correlation coefficient with the total duration of breastfeeding was -0.14 (p = 0.036). Scatter-plots of the relationships are shown in Figures 1 and 2, respectively. The correlation coefficient ‘r’ of serum zinc levels with the duration of exclusive breastfeeding among the Controls was -0.04 (p = 0.453), while the correlation coefficient with the total duration of breastfeeding was -0.02 (p = 0.800). Scatter-plots of the relationships among the Controls are also shown in Figures 1 and 2, respectively.

Table IX: Relationship between breastfeeding and the distribution of serum zinc levels.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Hypozincaemia</th>
<th>Normozincaemia</th>
<th>Hyperzincaemia</th>
<th>Total</th>
<th>Statistical comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Still breastfeeding</td>
<td>*34 (25.2)</td>
<td>Ф98 (72.6)</td>
<td>@3 (2.2)</td>
<td>135 (100.0)</td>
<td>LR = 10.63, df = 4, p = 0.031</td>
</tr>
<tr>
<td>No longer breastfeeding</td>
<td>*39 (34.8)</td>
<td>Ф69 (61.6)</td>
<td>@4 (3.6)</td>
<td>112 (100.0)</td>
<td></td>
</tr>
<tr>
<td>Not breastfed at all</td>
<td>*3 (100.0)</td>
<td>Ф0 (0.0)</td>
<td>@0 (0.0)</td>
<td>3 (100.0)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>76 (30.4)</td>
<td>167 (66.8)</td>
<td>7 (2.8)</td>
<td>250 (100.0)</td>
<td></td>
</tr>
</tbody>
</table>

* LR = 9.95, df = 2, p = 0.007  
Ф LR = 10.06, df = 2, p = 0.007  
@ LR = 0.57, df = 2, p = 0.751
## Controls

<table>
<thead>
<tr>
<th>Breastfeeding status</th>
<th>Hypozincaemia n (%)</th>
<th>Normozincaemia n (%)</th>
<th>Hyperzincaemia n (%)</th>
<th>Total n (%)</th>
<th>Statistical comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Still breastfeeding</td>
<td>15 (10.8)</td>
<td>103 (74.1)</td>
<td>21 (15.1)</td>
<td>139 (100.0)</td>
<td>$\chi^2 = 2.56$ df = 2 $p = 0.279$</td>
</tr>
<tr>
<td>No longer breastfeeding</td>
<td>16 (14.4)</td>
<td>85 (76.6)</td>
<td>10 (9.0)</td>
<td>111 (100.0)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>31 (12.4)</td>
<td>188 (75.2)</td>
<td>31 (12.4)</td>
<td>250 (100.0)</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1: Scatter-plot illustrating the correlation between serum zinc levels and the duration of exclusive breastfeeding.

$r = 0.12, p = 0.018$
$r = -0.04, p = 0.453$
Figure 2: Scatter-plot illustrating the correlation between serum zinc levels and the total duration of breastfeeding.

$r = -0.14, p = 0.036$

$r = -0.02, p = 0.800$
**Relationship between nutritional status and serum zinc levels:**

The mean serum zinc level was 78.0 ± 37.2 µg/dl among the Subjects who were underweight, 78.3 ± 35.0 µg/dl among those with normal weight and 86.8 ± 21.2 µg/dl among the overweight ones. The only obese Subject had a serum zinc level of 177.0 µg/dl. The differences were statistically significant (ANOVA F = 2.70, p = 0.046). However, the difference in mean serum zinc levels between the underweight (n = 45) and non-underweight (n = 205) Subjects was not statistically significant (78.0 ± 37.2 µg/dl versus 79.0 ± 35.2 µg/dl; t = 0.17, p = 0.866). The difference between the overweight and obese Subjects (n = 6) and those who were neither overweight nor obese (n = 244) was also not statistically significant (101.8 ± 41.4 µg/dl versus 78.2 ± 35.3 µg/dl; t = 1.61, p = 0.109).

Among the Controls, the mean serum zinc level was 85.0 ± 29.3 µg/dl among the underweight children, 109.4 ± 47.8 µg/dl among those with normal weight and 107.6 ± 38.1 µg/dl among the overweight ones. The differences were not statistically significant (ANOVA F = 2.65, p = 0.073). However, the underweight Controls (n = 21) had a significantly lower mean value, compared to the 229 non-underweight ones (85.0 ± 29.3 µg/dl versus 109.3 ± 47.6 µg/dl; t = 2.31, p = 0.022). The difference between the overweight Controls (n = 5) and those who were not overweight (n = 245) was not statistically significant (107.6 ± 38.1 µg/dl versus 107.3 ± 47.0 µg/dl; t = 0.016, p = 0.987).

Table X shows the distribution of serum zinc levels in relation to nutritional status. The differences in the distribution were statistically significant among the Controls (LR = 12.65, df = 4, p = 0.013), but not among the Subjects (LR = 11.47, df = 6, p = 0.075). The prevalence of hypozincaemia was significantly higher among the underweight Controls [OR (95% CI) = 4.27 (1.57, 11.62); \( \chi^2 = 9.25, df = 1, p = 0.002 \)], but not among the underweight Subjects (\( \chi^2 = 0.22, df = 1, p = 0.637 \)).
Relationship between the distribution of serum zinc levels and the need for admission, presence of electrolyte derangements and outcome:

Sixteen (35.6%) of the 45 Subjects who required admission had hypozincaemia, while 2 (4.4%) had hyperzincaemia. Of the 205 that did not require admission, 60 (29.3%) had hypozincaemia and 5 (2.4%) hyperzincaemia. The differences in distribution of zinc levels were not statistically significant ($\chi^2 = 1.39$, df = 2, $p = 0.499$).

The prevalences of hypo- and hyperzincaemia were 33.3% and 6.7% among the 15 Subjects who had electrolyte derangements at admission. The respective prevalences among those without electrolyte derangements (n = 30) were 35.6% and 4.4%. The differences in distribution of zinc levels between them were not statistically significant ($\chi^2 = 0.267$, df = 2, $p = 0.875$).

The difference in the prevalence of hypozincaemia between the Subjects that died (100%) and those that survived (73/247, 29.6%) was statistically significant [OR (95% CI) = 16.62 (0.86, 322.57); FET $p = 0.027$].

RESULTS OF LOGISTIC REGRESSION ANALYSIS TO DETERMINE THE INDEPENDENCE OF FACTORS ASSOCIATED WITH HYPOZINCAEMIA

The results of logistic regression to determine the independence of factors associated with hypozincaemia are shown in Table XI. Among the Subjects, low socio-economic status was the only factor that had significant independent association with hypozincaemia [OR (95% CI) = 3.06 (1.25, 7.48); $p = 0.014$]. Among the Controls, nutritional status was the only factor that had

Table X: Relationship between nutritional status and the distribution of serum zinc levels.

<table>
<thead>
<tr>
<th>Nutritional Status</th>
<th>Hypozincaemia</th>
<th>Normozincaemia</th>
<th>Hyperzincaemia</th>
<th>Total</th>
<th>Statistical</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>status</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>comparison</td>
</tr>
<tr>
<td>-------------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>------------</td>
</tr>
</tbody>
</table>
| Underweight | 15 (33.3) | 29 (64.4) | 1 (2.2) | 45 (100.0) | LR = 11.47  
df = 6  
p = 0.075 |
| Normal weight | 61 (30.7) | 133 (66.8) | 5 (2.5) | 199 (100.0) |            |
| Overweight  | 0 (0.0) | 5 (100.0) | 0 (0.0) | 5 (100.0) |            |
| Obese       | 0 (0.0) | 0 (0.0) | 1 (100.0) | 1 (100.0) |            |
| Total       | 76 (30.4) | 167 (66.8) | 7 (2.8) | 250 (100.0) |            |

### Controls

<table>
<thead>
<tr>
<th>Nutritional status</th>
<th>Hypozincaemia n (%)</th>
<th>Normozincaemia n (%)</th>
<th>Hyperzincaemia n (%)</th>
<th>Total n (%)</th>
<th>Statistical comparison</th>
</tr>
</thead>
</table>
| Underweight         | *7 (33.3)           | 14 (66.7)            | 0 (0.0)              | 21 (100.0)  | LR = 12.65  
df = 4  
p = 0.013 |
| Normal weight       | *24 (10.7)          | 170 (75.9)           | 30 (13.4)            | 224 (100.0) |            |
| Overweight          | 0 (0.0)             | 4 (80.0)             | 1 (20.0)             | 5 (100.0)   |            |
| Total               | 31 (12.4)           | 188 (75.2)           | 31 (12.4)            | 250 (100.0) |            |

*LR = 8.13, df = 2, p = 0.017  
ΦLR = 0.89, df = 2, p = 0.641  
@LR = 5.99, df = 2, p = 0.50

Table XI: Logistic regression model for factors independently associated with hypozincaemia.

### Subjects
<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low social class</td>
<td>1.12</td>
<td>3.06</td>
<td>1.25, 7.48</td>
<td><strong>0.014</strong></td>
</tr>
<tr>
<td>Lack of breastfeeding</td>
<td>0.42</td>
<td>1.52</td>
<td>0.82, 2.83</td>
<td>0.184</td>
</tr>
<tr>
<td>Dysentery</td>
<td>0.61</td>
<td>1.83</td>
<td>0.76, 4.39</td>
<td>0.175</td>
</tr>
<tr>
<td>Nutritional status (Underweight)</td>
<td>0.34</td>
<td>1.41</td>
<td>0.66, 3.02</td>
<td>0.380</td>
</tr>
<tr>
<td>Age &gt; 24 months</td>
<td>0.23</td>
<td>1.26</td>
<td>0.51, 3.07</td>
<td>0.619</td>
</tr>
<tr>
<td>Sex</td>
<td>0.12</td>
<td>0.88</td>
<td>0.50, 1.57</td>
<td>0.675</td>
</tr>
<tr>
<td>Dehydration</td>
<td>0.44</td>
<td>0.65</td>
<td>0.32, 1.29</td>
<td>0.214</td>
</tr>
</tbody>
</table>

**Controls**

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutritional status (Underweight)</td>
<td>1.37</td>
<td>3.95</td>
<td>1.41, 11.05</td>
<td><strong>0.009</strong></td>
</tr>
<tr>
<td>Low social class</td>
<td>0.28</td>
<td>0.75</td>
<td>0.34, 1.67</td>
<td>0.486</td>
</tr>
<tr>
<td>Lack of breastfeeding</td>
<td>0.07</td>
<td>0.94</td>
<td>0.37, 2.36</td>
<td>0.887</td>
</tr>
<tr>
<td>Age &gt; 24 months</td>
<td>0.68</td>
<td>1.97</td>
<td>0.65, 5.94</td>
<td>0.228</td>
</tr>
<tr>
<td>Sex</td>
<td>0.19</td>
<td>0.83</td>
<td>0.38, 1.81</td>
<td>0.639</td>
</tr>
</tbody>
</table>

B – Coefficient of regression

significant independent association with hypozincaemia [OR (95% CI) = 3.95 (1.41, 11.05); p = 0.009].
RELATIONSHIP BETWEEN SERUM ZINC LEVELS AND THE
SOCIODEMOGRAPHIC CHARACTERISTICS OF THE SUBJECTS AND CONTROLS

The mean serum zinc level of Subjects aged ≤24 months was not significantly different from that of older Subjects (79.2 ± 35.0 µg/dl versus 76.4 ± 39.5 µg/dl; t = 0.42, p = 0.677). Among the Controls, those aged ≤24 months had a significantly higher mean serum zinc level [109.8 ± 45.5 µg/dl versus 93.0 ± 51.4 µg/dl; difference (95% CI) = 16.9 (0.7, 33.0); t = 2.06, p = 0.040].

Table XII shows the distribution of serum zinc levels in relation to age. The difference in distribution of zinc levels between children aged ≤24 months and older children was not statistically significant among either the Subjects ($\chi^2 = 1.17, p = 0.556$) or the Controls ($\chi^2 = 3.25, p = 0.197$). The correlation between age and serum zinc levels in the Subjects and Controls is shown in Figure 3. The correlation coefficient ‘r’ among the Subjects was -0.11 (p = 0.016) and that among the Controls -0.14 (p = 0.001).

The mean serum zinc levels were 78.7 ± 35.2 µg/dl versus 78.9 ± 36.2 µg/dl (t = -0.04, p = 0.970) among the male and female Subjects and 107.6 ± 48.6 µg/dl versus 106.9 ± 44.4 µg/dl (t = 0.11, p = 0.911) among the Controls. Among the male Subjects, 43 (29.3%) had hypozincaemia, 99 (67.3%) normozincaemia and 5 (3.4%) hyperzincaemia while among the females, 33 (32.0%) had hypozincaemia, 68 (66.0%) normozincaemia and 2 (1.9%) hyperzincaemia. The difference in distribution of zinc levels between males and females was not statistically significant ($\chi^2 = 0.65, p = 0.722$). Among the male Controls, 16 (11.2%) had hypozincaemia, 109 (76.2%) normozincaemia and 18 (12.6%) hyperzincaemia. The respective prevalences among the female Controls were 15 (14.0%), 79 (73.8%) and 13 (12.1%) respectively. The male-female difference in distribution was not statistically significant ($\chi^2 = 0.45, p = 0.798$).
There was no significant difference in mean serum zinc levels between those in the low and high social classes among the Subjects (82.7 ± 31.4 µg/dl versus 77.9 ± 36.5 µg/dl; t = -0.81, p = 0.419) and Controls (113.0 ± 52.8 µg/dl versus 104.4 ± 43.3 µg/dl; t = -1.37, p = 0.174). The distribution of zinc levels in relation to socio-economic status is shown in Table XIII. The difference between low and high socio-economic status was significant among the Subjects ($\chi^2 = 6.46$, df = 2, p = 0.040) but not among the Controls ($\chi^2 = 3.13$, df = 2, p = 0.209). The prevalence of hypozincaemia was significantly higher among the Subjects with low socio-economic status (p = 0.013).

RESPONSE OF SERUM ZINC LEVELS TO ADMINISTRATION OF ZINC SUPPLEMENTS

Eighty-eight (35.2%) of the Subjects presented for follow-up serum zinc assay. The mean ± SD serum zinc level among them was 78.0 ± 37.7 µg/dl pre-supplementation and 95.2 ± 37.6 µg/dl post-supplementation. The difference was statistically significant [difference (95% CI) = -17.22 (-18.32, -16.14); t = -31.41; p <0.001]. Thirty-four (38.6%) of these Subjects had hypozincaemia pre-supplementation compared with 14 (15.9%) post-supplementation. The difference was statistically significant ($\chi^2 = 11.46$, p = 0.001). The prevalence of hyperzincaemia was 3.4% (n = 3) pre-supplementation and 5.7% (n = 5) post-supplementation. The difference was not statistically significant ($\chi^2 = 0.52$, p = 0.469). The mean ± SD increase in serum zinc levels was

<table>
<thead>
<tr>
<th>Age-group (months)</th>
<th>Hypozincaemia n (%)</th>
<th>Normozincaemia n (%)</th>
<th>Hyperzincaemia n (%)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 - 24</td>
<td>63 (29.2)</td>
<td>147 (68.0)</td>
<td>6 (2.8)</td>
<td>216 (100.0)</td>
</tr>
<tr>
<td>25-59</td>
<td>13 (38.2)</td>
<td>20 (58.8)</td>
<td>1 (2.9)</td>
<td>34 (100.0)</td>
</tr>
<tr>
<td>Age-group (months)</td>
<td>Hypozincaemia n (%)</td>
<td>Normozincaemia n (%)</td>
<td>Hyperzincaemia n (%)</td>
<td>Total n (%)</td>
</tr>
<tr>
<td>-------------------</td>
<td>---------------------</td>
<td>----------------------</td>
<td>----------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>6 - 24</td>
<td>23 (10.8)</td>
<td>161 (75.9)</td>
<td>28 (13.3)</td>
<td>212 (100.0)</td>
</tr>
<tr>
<td>25-59</td>
<td>8 (21.1)</td>
<td>27 (71.1)</td>
<td>3 (7.9)</td>
<td>38 (100.0)</td>
</tr>
<tr>
<td>Total</td>
<td>31 (12.4)</td>
<td>188 (75.2)</td>
<td>31 (12.4)</td>
<td>250 (100.0)</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 1.17, \text{df} = 2, p = 0.556 \]

\[ LR = 3.25, \text{df} = 2, p = 0.197 \]
Figure 3: Scatter-plot illustrating the correlation between age and serum zinc levels.

Table XIII: Relationship between socio-economic status and the distribution of serum zinc levels.
<table>
<thead>
<tr>
<th>Subjects</th>
<th>Socio-economic status</th>
<th>Hypozincaemia n (%)</th>
<th>Normozincaemia n (%)</th>
<th>Hyperzincaemia n (%)</th>
<th>Total n (%)</th>
<th>Statistical comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>*69 (33.8)</td>
<td>Ф129 (63.2)</td>
<td>@6 (2.9)</td>
<td>204 (100.0)</td>
<td>$\chi^2 = 6.46$ df = 2 p = 0.040</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>*7 (15.2)</td>
<td>Ф38 (82.6)</td>
<td>@1 (2.2)</td>
<td>46 (100.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>76 (30.4)</td>
<td>167 (66.8)</td>
<td>7 (2.8)</td>
<td>250 (100.0)</td>
<td></td>
</tr>
</tbody>
</table>

* $\chi^2 = 6.14; df = 1; p = 0.013; OR = 2.85; 95\% CI = 1.22 – 6.64$

Ф $\chi^2 = 6.35; df = 1; p = 0.012$

@ FET ‘p’ = 1.000

<table>
<thead>
<tr>
<th>Controls</th>
<th>Socio-economic status</th>
<th>Hypozincaemia n (%)</th>
<th>Normozincaemia n (%)</th>
<th>Hyperzincaemia n (%)</th>
<th>Total n (%)</th>
<th>Statistical comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>19 (11.4)</td>
<td>131 (78.4)</td>
<td>17 (10.2)</td>
<td>167 (100.0)</td>
<td>$\chi^2 = 3.13$ df = 2 p = 0.209</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>12 (14.5)</td>
<td>57 (68.7)</td>
<td>14 (16.9)</td>
<td>83 (100.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>31 (12.4)</td>
<td>188 (75.2)</td>
<td>31 (12.4)</td>
<td>250 (100.0)</td>
<td></td>
</tr>
</tbody>
</table>
19.1 ± 3.9 µg/dl among those who had hypozincaemia pre-supplementation (n = 34) and 16.0 ± 5.5 µg/dl in those who did not have hypozincaemia. The difference was statistically significant (t = -2.84, p = 0.006).

The mean ± SD serum zinc among the 18 Controls who presented for follow-up serum zinc assay was 54.2 ± 11.8 µg/dl pre-supplementation and 71.7 ± 11.0 µg/dl post-supplementation. The difference was statistically significant [difference (95% CI) = -17.55 (-19.31, -15.80); t = -21.07, p <0.001]. All (100.0%) of these Controls had hypozincaemia pre-supplementation, while only 3 (16.7%) of them had hypozincaemia post-supplementation. The difference was statistically significant ($\chi^2 = 25.71$, p <0.001). The remaining 15 (83.3%) had normozincaemia post-supplementation and none had hyperzincaemia.

One child complained of vomiting about 15 minutes after administration of the first dose of zinc, but there was no other episode of vomiting thereafter. No other adverse effect was reported in children taking zinc supplements, including those who had hyperzincaemia before treatment.
DISCUSSION

The present study was carried out to determine the relationships between serum zinc levels and the presence, type and severity of acute diarrhoea among under-five children. The mean serum zinc level of the Subjects was significantly lower than that of the Controls and the prevalence of hypozincaemia was significantly higher in the Subjects. Dysentery was associated with a lower mean serum zinc level and a higher prevalence of hypozincaemia. There was no statistically significant difference in the mean serum zinc level or the prevalence of hypozincaemia between Subjects with signs of dehydration and those with no signs of dehydration. There was a significant rise in the mean serum zinc levels following zinc supplementation.

During the present study, diarrhoeal diseases accounted for only a small proportion of out-patient consultations (4.6%), as well as admissions (7.8%). Yilgwan et al.\textsuperscript{102} also reported a low prevalence of diarrhoea (2.7%) among children seen at the Paediatrics Unit of Jos University Teaching Hospital, Jos, Nigeria. The widespread use of oral rehydration salt (ORS) solution may be responsible for the low number of diarrhoeal cases. The appropriate use of ORS can obviate the need for hospital consultation, since dehydration from acute diarrhoea can be safely and effectively treated in over 90% of cases using ORS solution.\textsuperscript{1} As at 1980, diarrhoea was the leading cause of childhood mortality globally, accounting for 4.6 million under-five deaths annually.\textsuperscript{103} The annual number of deaths attributable to diarrhoea among under-five children dropped to about 1.5 million in year 2000.\textsuperscript{103} This reduction was largely attributable to increases in the use of ORS over those years.\textsuperscript{103} An estimated 0.6 million under-five deaths were attributed to diarrhoea in year 2014,\textsuperscript{7} indicating that further reductions in the burden of diarrhoea are still on-going. Ojuawo et al.\textsuperscript{104} also reported a significant reduction in the prevalence of severe
dehydration in children with diarrhoeal disease over a four-year period in Ilorin, Nigeria, due to significant improvement in the use of ORS at home before presentation in the hospital. There was also a large reduction in the number of childhood diarrhoea cases seen in the hospital over the period in that study,\textsuperscript{104} although the prevalence of diarrhoeal diseases was not specified.

Most (86.4\%) of the children with diarrhoea were two years of age and below. This is similar to the proportion reported by Ogunlesi et al\textsuperscript{105} from a previous study in the same centre. There was also a male preponderance among children with diarrhoea in the present study as well as in the earlier study.\textsuperscript{105} The preponderance of children below two years may be because children in this age group are in the oral phase of development when they insert virtually all available objects into the mouth.\textsuperscript{106} Various forms of weaning diets are also introduced to them at about this age.\textsuperscript{107} Furthermore, the immaturity of the immune system is an important predisposing factor to infections in this age-group.\textsuperscript{106} The male preponderance may be related to the more adventurous nature of male children who are therefore, likely to insert more objects into the mouth, putting them at greater risk of infection.

A higher proportion of the Subjects (81.6\%) belonged to the lower social classes compared with the Controls (66.8\%). The distribution of social classes among the children with diarrhoea is similar to that reported by Ogunlesi et al.\textsuperscript{105} The greater prevalence of diarrhoea among the children from low socio-economic status families may be related to the higher prevalence of poor hygiene, water shortages, poor weaning practices and undernutrition, which predispose to diarrhoea in the lower social classes.\textsuperscript{1} On the other hand, the higher prevalence of high socio-economic status families among the Controls may be due to the fact that apparently healthy children presenting for routine tests, immunization or minor complaints are more likely to be from the higher social classes, since such tests and complaints may rather be ignored in
children from the lower social classes to minimize demands on the already inadequate income of such families.\textsuperscript{95}

The 10.8\% prevalence of dysentery in this study is in keeping with the 10\% average reported by the World Health Organization (WHO)\textsuperscript{21} from the developing world. It is, however, lower than the 13.7\% reported by Ogunlesi \textit{et al}\textsuperscript{108} from the same centre 11 years ago, though the difference is not statistically significant ($41 / 300$ versus $27 / 250$; $\chi^2 = 1.03$, $p = 0.309$). The decline in prevalence of dysentery over the years may be due to increase in the misuse of antibiotics for children with diarrhoea before presentation in the hospital.\textsuperscript{109} The absence of ova, cysts or trophozoites of parasites in the stool samples of the Subjects with dysentery in the present study is not in keeping with the previous report in Ilesa children.\textsuperscript{108} The organisms isolated on stool culture were also different between these studies. The frequent misuse of antibiotics may also partly explain these differences, but variations in laboratory technique and expertise of laboratory personnel are additional factors that may be contributory.\textsuperscript{109}

The children who did not have any routine immunization or had incomplete immunization were mostly from the lower socio-economic classes. This suggests that some parents, usually the poor ones, are still reluctant to take their children for immunization regularly, despite the fact that routine immunization is free. This may be due to the fear of adverse reactions, long waiting time at health facilities, lack of money for transportation, false contra-indications or socio-cultural beliefs that discourage immunization.\textsuperscript{110, 111}

The prevalence of undernutrition was relatively high among the Subjects. This is in keeping with the known predisposition of malnourished children to diarrhoea.\textsuperscript{9} The proportion of underweight children in the present study (18.0\% in the Subjects and 8.4\% in the Controls) is lower than the 23.1\% reported about a decade ago from Ifewara, another town in Osun State.\textsuperscript{112} The prevalence of stunting was also lower (14.4\% vs 26.7\%), whereas the prevalence of wasting
was higher (17.6% vs 9.0%) than in Ifewara children. The disparity is probably due to the fact that severely undernourished children were included in the Ifewara study,\textsuperscript{112} and children with z-scores of -2 were classified together with those who had z-scores < -2 as being undernourished in that study.\textsuperscript{112} The present study excluded severely undernourished children, and considered children with z-scores of -2 as normal according to WHO standards.\textsuperscript{6} In addition, 75% of the under-five children in the Ifewara study\textsuperscript{112} were older than two years, whereas 86.4% of the children in the present study were two years and below, with about half of them still breastfeeding, thereby putting them at a lower risk of malnutrition. The longer period of malnutrition required for stunting to develop may also explain the higher prevalence of stunting among the Ifewara children, since they were mostly older than two years. Furthermore, Ifewara is a more rural area than Ilesa, and whereas the Ifewara study was community-based, the present study was hospital-based. The latter may also partly explain the lower prevalence of malnutrition in the present study because malnourished children who have no symptoms of illness may not present in the hospital, but such children may be identified during nutritional screening in the community.

Only about a quarter of the Subjects had signs of dehydration at presentation. This is much less than the 59.0% reported by Ogunlesi \textit{et al}\textsuperscript{105} about a decade ago. In fact, the prevalence of dehydration reported by Ogunlesi \textit{et al}\textsuperscript{105} was in reference to moderate and severe dehydration only. The proportions of diarrhoea patients without signs of dehydration and those with mild dehydration were not stated in that study. Furthermore, details of the methods of assessment of dehydration used in the study were not available for comparison. Similarly, Ojuawo \textit{et al}\textsuperscript{104} reported a significant reduction in the prevalence of severe dehydration from 11\% to 5\% (p <0.001) among children with diarrhoea in Ilorin over four years. The low prevalence of dehydration among children with diarrhoea in the present study may be related to
the increasing awareness and use of oral rehydration salt (ORS) solution over the years. ORS is readily available as an over-the-counter medication, and many care-givers resort to using it as a first line treatment for children with diarrhoea even before presentation in the hospital.\textsuperscript{113}

There was a high prevalence (33\%) of electrolyte derangements and azotaemia among the Subjects who were admitted, including the three Subjects that died. These derangements as well as the accompanying dehydration are preventable with the use of ORS solution. When prepared and given correctly, ORS solution provides sufficient water and electrolytes to correct the deficits associated with acute diarrhoea.\textsuperscript{1} Since many diarrhoeal deaths are caused by dehydration,\textsuperscript{1} early institution of oral rehydration therapy should be a priority among the efforts being made to reduce diarrhoeal morbidity and mortality. Mothers should be taught how to prevent dehydration at home, including the correct preparation and administration of ORS solution. They should also know about the signs which indicate that the child should be taken to a health worker. Such signs include repeated vomiting, increasing thirst, increasing frequency of watery stools, presence of fever, passage of bloody stools, eating or drinking poorly, and lack of improvement in three days.\textsuperscript{1}

The mean serum zinc level of the Subjects was significantly lower than that of the Controls in the present study. The prevalence of hypozincaemia was also higher in the Subjects. Baqui \textit{et al}\textsuperscript{114} similarly reported a significantly higher prevalence of hypozincaemia among children with acute diarrhoea, compared with healthy Controls. Both Okolo \textit{et al}\textsuperscript{34} and Rani \textit{et al}\textsuperscript{35} reported lower mean serum zinc levels in children with diarrhoea, but did not report the prevalence of hypozincaemia among them. The prevalence of hypozincaemia in the Subjects in the present study suggests that zinc deficiency is quite common among children with diarrhoea in the study community. In addition, the fact that more than one-tenth of apparently healthy children in the study had hypozincaemia, which could put them at risk of diarrhoea and other
problems associated with zinc deficiency, should be of concern. It would therefore be imperative
to put in place programmes that will help to make zinc-rich foods more available and affordable
to children in Ilesa and in the country at large, so as to reduce the burden of zinc deficiency.

Although the prevalence of hypozincaemia in the Controls appears to be much lower than
that reported from other parts of Nigeria, the difference may be more apparent than real
because of differences in the definition of hypozincaemia used. Using a cut-off value of 80 µg/dl,
a national prevalence of 20% was reported from the Nigerian Nutrition survey in 2004, whereas using a cut-off value of 70 µg/dl, a prevalence of 21% was reported among 5-13 year old children in Lagos in 2011. However, using the same cut-off value of 65 µg/dl as in the present study, a prevalence of 41.5% (35% in urban areas and 48% in rural areas) was reported from under-five children in Imo State in 2011. The prevalence reported in Imo was even higher than that observed in children with diarrhoea in the present study (30.4%). The disparity could be related to differences in methods of analysis, as well as the health, socio-economic and nutritional status of the study participants, which were not specified in the Imo study. Differences in the zinc content of the soil as well as staple foods across regions may also be contributory. The disparities in prevalence across these studies underscore the importance of the use of similar definitions or cut-off values in studies of the prevalence of trace element deficiencies. The IZiNCG recommends the adoption of a cut-off value of 65 µg/dl in the definition of hypozincaemia in children.

The occurrence of hyperzincaemia in seven (2.8%) of the Subjects is noteworthy. Although haemolysis during serum separation could cause spuriously high serum zinc levels, necessary precautions were taken to avoid haemolysis while handling blood samples in the present study. Haemolysis may therefore not be an appropriate explanation for the prevalence in this study. Hyperzincaemia has not been reported previously in children with diarrhoea to the
best knowledge of this Researcher, but hyperzincaemia could be associated with diarrhoea as one of the manifestations of zinc toxicity in humans.\textsuperscript{11, 23} Hyperzincaemia may be related to high dietary intake of zinc in the affected children.\textsuperscript{11, 23} However, the dietary intake was not assessed in the present study.

Dysentery was associated with significantly lower serum zinc levels than acute watery diarrhoea. This observation is in keeping with the relationship reported by Strand \textit{et al}\textsuperscript{115} in Nepalese children. The prevalence of hypozincaemia was also higher in children with dysentery. Possible explanations for this finding include the loss of significant amounts of zinc through the red blood cells that are passed in the stools in dysentery, as well as increased loss of zinc through the damaged intestinal mucosa.\textsuperscript{115} Strand \textit{et al}\textsuperscript{115} did not report the difference in prevalence of hypozincaemia in relation to dysentery, and this Researcher is not aware of other studies that did so.

In the present study, there were no statistically significant relationships between mean serum zinc level, or the prevalences of hypo- and hyperzincaemia and the presence and degree of dehydration. In contrast, higher zinc concentrations were reported in dehydrated children compared with non-dehydrated ones by Strand \textit{et al}\textsuperscript{115} and Olmez \textit{et al},\textsuperscript{116} while Arora \textit{et al}\textsuperscript{47} reported decreased levels of serum zinc after ORS therapy. This pattern is probably due to haemoconcentration in the dehydrated children.

The association between hypozincaemia and low socio-economic status is consistent with the findings in the report from Imo state.\textsuperscript{86} Tanzer \textit{et al}\textsuperscript{117} and Chomeili \textit{et al}\textsuperscript{118} also reported a similar relationship between serum zinc and socio-economic status. This may be related to the higher intake of zinc-rich but expensive food items, such as red meat, by children in the higher social classes.\textsuperscript{24} The lower social classes are often unable to afford such food items, relying more
on the cheaper food items such as cereals and legumes which have low zinc bio-availability due to a high phytate content.\textsuperscript{24}

It should be expected that hypozincaemia would be more common amongst undernourished children since malnutrition is a known risk factor for zinc deficiency.\textsuperscript{10, 41-44, 46} Also, the severity of malnutrition correlates with the severity of zinc deficiency.\textsuperscript{41, 43, 44} Surprisingly however, there was no significant difference between the zinc status of malnourished and well-nourished children with diarrhoea in the present study. A possible reason for this finding is that diarrhoea itself, through zinc losses in the stools,\textsuperscript{34} could have been the precipitating factor for hypozincaemia in the non-malnourished Subjects. In contrast, the relationship between undernutrition and serum zinc levels among the Controls is in keeping with that in previous reports.\textsuperscript{10, 41-44}

Patients with hypozincaemia are at risk of increased severity of diarrhoea, including a more frequent passage of stools and longer duration of diarrhoeal illnesses.\textsuperscript{33, 47} However, the present study did not confirm this relationship as there was no significant relationship between the prevalence of hypozincaemia and the frequency of passage of stools. Similarly, there was no significant relationship between the prevalence of hypozincaemia and the duration of diarrhoea.

Breastfeeding is helpful in preventing diarrhoeal diseases and in reducing the morbidity and mortality associated with diarrhoea in young children.\textsuperscript{58, 59} Breastfeeding was associated with a lower prevalence of hypozincaemia in the present study and all the three Subjects who were never breastfed had hypozincaemia. Similarly, lack of breastfeeding was associated with hypozincaemia in the report from Imo State.\textsuperscript{86} The low zinc levels observed among the non-breastfed children is probably due to the fact that most weaning diets given to children in developing countries are based on low-zinc food items, due to the high cost of zinc-rich food items.\textsuperscript{24} The absence of breastmilk as an additional zinc source in non-breastfed children is a
likely contributor to their low serum zinc levels, moreso that the effects of ingested zinc have been found to last up to three months in the body, and the biological half-life of zinc in the body may be up to five months. In view of this, greater effort should be made to encourage and promote breastfeeding.1

The positive correlation observed between the duration of exclusive breastfeeding and serum zinc levels, though in support of the previously reported relationship between breastfeeding and zinc status, was weak. The negative correlation observed between serum zinc levels and the total duration of breastfeeding, which is contradictory to the earlier finding, was also weak. These correlations are not important because of the very small values of the correlation coefficients, 0.12 and 0.14, respectively. Their occurrence was probably due to the large sample size, since it has been reported that very small correlation coefficients can sometimes appear to be significant when the sample size is large.119

About two-thirds of the Subjects did not return for follow-up post-supplementation zinc assay. Poor compliance with follow-up appointments is frequently encountered in clinical practice in Nigeria and may be due to lack of funds, lack of time, or failure to appreciate the need for follow-up.120 Increased serum zinc levels have been reported in studies that measured the outcome of zinc supplementation in children with acute diarrhoea.68, 77, 123 The results of the present study are, therefore, in keeping with the earlier observations.

Vomiting was the only side-effect of zinc therapy reported during the present study and this was observed in only one child. No other adverse effects were reported, even in children who had high serum zinc levels pre-supplementation. Roy et al, Fajolu et al and Trivedi et al did not observe any adverse effect of zinc supplementation, but Strand et al and Boran et al reported that vomiting occurred in a few children shortly after the administration of zinc.
supplements. These results could mean that zinc supplements are safe in children with diarrhoea and can be administered routinely as recommended by the WHO.\textsuperscript{1,20}

Low socio-economic status had significant independent association with hypozincaemia among the Subjects in the present study. For children, socio-economic status is derived from the parents’ highest educational attainments and their occupations.\textsuperscript{95} Thus, strengthening of the nation’s educational system and creation of more employment opportunities could go a long way in reducing the burden of zinc deficiency in Nigeria. Furthermore, in line with the identification of malnutrition as an independent predictor of hypozincaemia among the Controls, breastfeeding and improved weaning practices should be emphasized for the care of young children.

\section*{CONCLUSIONS}

From the present study, it is concluded that:

1. Diarrhoea in under-five children is associated with lower serum zinc levels and a high prevalence of hypozincaemia.

2. Dysentery is a risk factor for hypozincaemia among under-five children with diarrhoea.

3. There is no significant relationship between the prevalence of hypozincaemia and the presence or degree of dehydration in under-fives with acute diarrhoea.
4. Among children with acute diarrhoea, the prevalence of hypozincaemia is lower in breastfed children and in those from high socio-economic status families.

5. Zinc supplementation during childhood diarrhoea results in increased serum zinc levels.

6. Dehydration, electrolyte derangement(s) and hypozincaemia are risk factors for mortality in children with diarrhoea.

RECOMMENDATIONS

1. Breastfeeding for up to two years should be encouraged for all children, so as to reduce the risks of zinc deficiency and diarrhoea.

2. Zinc supplements should be given to children with diarrhoea, particularly those with dysentery, those from low socio-economic status families and those who were not breastfed.
3. Considering the fact that one in eight Controls had hypozincaemia, mothers and caregivers should be educated to give zinc-rich food items to their children to reduce the risk of zinc deficiency.

LIMITATIONS OF THE STUDY

1. Dietary zinc intake, which can significantly influence serum zinc levels, was not assessed in this study. High dietary intake might have explained the occurrence of hyperzincaemia in some of the Subjects and Controls.

2. The time of the day during which blood samples for zinc assay were collected from the Subjects varied, since the samples were collected at presentation. Thus, the effect of diurnal fluctuations in serum zinc levels cannot be ruled out.
3. The time of the last meal before blood sampling, which may affect serum zinc levels, was not taken into consideration in this study.

4. Blood culture was done only for selected patients, but not routinely for all the Subjects. Thus the presence of bacteraemia could have been missed in some of the Subjects, who could then have been excluded from the study.

5. Not all the Subjects returned for follow-up post-supplementation zinc assay, thus limiting the number included in the analysis of the response to zinc supplementation.

**LINES OF FUTURE RESEARCH**

1. A study on the effects of seasonal variation in food supply on serum zinc levels could be worthwhile.

2. Comparison of zinc levels in the serum and breastmilk of lactating mothers with the serum zinc levels of their breastfeeding children could shed more light on the place of breastfeeding in zinc homeostasis in infants.
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APPENDIX I

ETHICAL CLEARANCE CERTIFICATE
APPENDIX II

SUBJECT INFORMATION SHEET AND CONSENT FORM

Researcher:

- Name: Dr. Abolurin O.
- Telephone number: 08035176432
- E-mail: funlyt@yahoo.com

Institution: Wesley Guild Hospital unit, Obafemi Awolowo University Teaching Hospital, Ilesa.

Department: Paediatrics

Title of study: Prevalence of Zinc deficiency among under-five children with diarrhoea at Wesley Guild Hospital unit, Obafemi Awolowo University Teaching Hospitals Complex, Ilesa, Nigeria.

Some general things to know about the study:

This study is set out to determine the serum zinc levels of children less than five years of age presenting at the Wesley Guild Hospital with diarrhoea. We also want to compare the serum zinc levels in these children with those of other children without diarrhoea. Children between 6 to 59
months of age having diarrhoea will be invited to join the study. Apparently healthy children within the same age group will also be required to participate as controls.

**Purpose of the study:**

The purpose of the study is to find out how common low blood zinc level is among children having diarrhoea. This will help to know how important it is to give oral zinc tablets to children having diarrhoea in our environment, since oral zinc tablets have been generally found to improve the condition of children with diarrhoea.

**Procedures:**

The doctor will ask you some questions about your child/ward and yourself, and will examine your child. 2.5 ml of blood will be collected from your child for a test (serum zinc level) in the laboratory.

**Benefits:**

If your child/ward is found to have low blood zinc level, you will be contacted to ensure that the child is given oral zinc tablets, which will be prescribed for him accordingly.

**Cost of participation:**

Participation in this study will be at no cost to you or your child. All materials that will be required will be provided free, and the blood test will also be done for your child free.

**Risks:**
Your child/ward will feel some pain during blood collection, but he/she will not be harmed. No injury is anticipated.

**Compensation:**

No payment will be given to you or your child/ward for participating in the study.

**Confidentiality:**

All information collected for the study will stay private and will not be accessible or disclosed to any person that is not involved in the study. Biodata will be coded using numbers, and all information will be stored in a passworded computer (only the investigator will have access to the password).

**Respondents’ Rights:**

You are free to allow your child/ward to take part in this study or not to. You will still have normal access to health care for your child/ward whether you choose to join the study or not. You also have a right to withdraw from the study at any time if you wish to.

**Conflict of Interest:**

Opinions concerning the study will be respected and treated considerably well without any sentiment.

**For the Records:**

A copy of this document will be given to you.
Further clarifications:

If you need further clarifications concerning the study, please contact the investigator whose contact details are indicated above. Thank you.

CONSENT FORM

PREVALENCE OF ZINC DEFICIENCY AMONG UNDER-FIVE CHILDREN WITH DIARRHOEA AT WESLEY GUILD HOSPITAL UNIT, OBAFEMI AWOLOWO UNIVERSITY TEACHING HOSPITALS COMPLEX, ILESA, NIGERIA.

I have read and understood the information provided in the subject information sheet (or the information has been read to me and I understand it).

I have had the opportunity to ask questions about it and any questions I have asked have been answered to my satisfaction.

I consent voluntarily to permit my child/ward to participate in this study/ agree that blood sample be taken for the study and understand that I have the right to withdraw my child/ ward from the study at any time.

Name and signature (or thumb impression) of mother/ father/ legal guardian-------------------------
-----------------------------------------------------------------------------------------------

Name of the child----------------------------------------Study no-----------------------

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This form has been read by (or I have read the above to) ------------------------------- in a language that he/she understands. I believe that he/she understood what I explained and has freely agreed that his/her child/ward should take part in the study.

Signature of the personnel obtaining consent-----------------------------------------------

Name of the personnel-----------------------------------------------Date-----------------

APPENDIX III

DATA PROFORMA

PREVALENCE OF ZINC DEFICIENCY AMONG UNDER-FIVE CHILDREN WITH DIARRHOEA AT WESLEY GUILD HOSPITAL UNIT, OBAFEMI AWOLOWO UNIVERSITY TEACHING HOSPITALS COMPLEX, ILESA, NIGERIA.

DATE____________ STUDY NUMBER_____________

SECTION A- BIODATA

1. Name: _______________________
2. Age:________________________
3. Sex:________________________
4. Hospital number:____________
5. Address:____________________
6. Ethnicity: ____________________
7. Parental occupation and Educational background:
   Father__________________________________________
                                                  ____________________
   Mother__________________________________________
                                                  ____________________
8. Socio-economic status (derived from above): ______
9. Contact phone number:________________________

SECTION B- HISTORY

10. Duration of Diarrhoea: __________days
11. Number of episodes per day (average): __________
12. Nature of stool (contains blood)?  Yes___________ No_________
13. Vomiting  Yes___________ No_________
14. Fever  Yes___________ No_________
15. Urinary output: Normal_______ Reduced_______ Absent_______
16. Immunization  None______________________
                       Appropriate for age________
                       Not appropriate for age_______
17. Breastfeeding or Breastfed?  Yes___________ No_________
   If Yes, still breastfeeding?  Yes___________ No_________
   Duration of exclusive breastfeeding: _______ months
   Total duration of breastfeeding: ____________ months

SECTION C – EXAMINATION

18. Weight (in Kg)____________________
19. Height/ Length (in cm)______________
20. Mid-arm circumference (in cm)_______
21. Pedal oedema:  Yes___________ No_________
22. z – score →Weight-for-age (WFA): ____________
23. z – score →Height/ Length-for-age (HFA): ____________
24. z – score →Weight-for-Height (WFH): ____________
25. Nutritional status:
               Well nourished___________________
               Underweight (WFA z-score < -2 but ≥ -3): ______
               Wasted (WFH z-score < -2 but ≥ -3): __________
               Stunted (HFA z-score < -2 but ≥ -3): __________

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Overweight (WFH z-score > +2 but ≤ +3): ____
Obese (WFH z-score > +3): ______

26. Temperature _______________°C

27. Pulse rate and volume ________ beats/min. Normal_____ Weak_____ Thready______

28. Blood pressure ________________ mmHg

29. Signs of dehydration
   General condition: Alert/Irritable/Lethargic/Unconscious
   Dry mouth: Yes________ No____________
   Drinking water: Eagerly_____ Normally_______ Unable________
   Skin pinch returns: immediately ______
      slowly (mild delay in elasticity) __________
      very slowly (>2 secs) __________
   Capillary refill: Normal (<1.5secs) ______ Delayed (1.5 - 3 secs) ______
      very delayed (>3 secs) ______
   Sunken eyes: Yes________ No____________
   Anterior fontanelle: Normal_____ Sunken_____ Very sunken _____ Closed____
   Producing tears when crying: Yes________ No_____

30. Degree of dehydration:
   No evidence of dehydration________
   Mild dehydration____________________
   Moderate dehydration________________
   Severe dehydration__________________

SECTION D

31. Investigation result (serum zinc level): ________________ µg/dl

32. Final diagnosis and outcome:
   Admission: Yes________ No __________
If yes, duration of admission: ___________ days.

Final diagnosis: __________________________________

Outcome: Discharged_________ DAMA_________ Died________

APPENDIX IV

OYEDEJI CLASSIFICATION OF SOCIAL CLASS

<table>
<thead>
<tr>
<th>Points</th>
<th>Occupation</th>
<th>Level of Education</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Senior public servants, Professionals, Managers, Large scale traders, Businessmen and contractors</td>
<td>University graduates and equivalents</td>
</tr>
<tr>
<td>2.</td>
<td>Intermediate grade public servants and senior school teachers</td>
<td>School certificate GCE O/L with Teaching or other professional training</td>
</tr>
<tr>
<td>3.</td>
<td>Junior school teachers, Drivers, and Artisans</td>
<td>School certificate or Grade II teachers’ certificate holders or equivalents</td>
</tr>
<tr>
<td>4.</td>
<td>Petty traders, Labourers, messengers, and similar grades</td>
<td>Modern three and primary six certificates</td>
</tr>
<tr>
<td>5.</td>
<td>Unemployed, Full-time house-wives, students</td>
<td>Can just read and write or illiterate</td>
</tr>
</tbody>
</table>

The mean of four scores (two for the Father and two for the Mother) to the nearest whole number is assigned as the social class for the child.
APPENDIX V

WHO GROWTH CHARTS
APPENDIX VI

CLINICAL EVALUATION OF DEHYDRATION\textsuperscript{5} \textsuperscript{6} \textsuperscript{7} \textsuperscript{1}

- **Mild dehydration (<5\% in an infant; <3\% in an older child or adult):** normal or increased pulse; decreased urine output; thirsty; normal physical findings.

- **Moderate dehydration (5-10\% in an infant; 3-6\% in an older child or adult):**
  - tachycardia; little or no urinary output; irritable/lethargic; sunken eyes and fontanelle; decreased tears; dry mucous membranes; mild delay in elasticity (skin turgor); delayed capillary refill.

- **Severe dehydration (>10\% in an infant; >6\% in an older child or adult):** rapid and weak or absent peripheral pulses; decreased blood pressure; no urinary output; very sunken eyes and fontanelle; no tears; delayed elasticity (poor skin turgor); very delayed capillary refill; depressed consciousness.

**APPENDIX VII**

**ATOMIC ABSORPTION SPECTROMETRY**

Atomic absorption spectrometry (AAS) is an analytical technique that measures the concentrations of elements in a solution. The technique makes use of the wavelengths of light specifically absorbed by an element, since atoms of different elements absorb characteristic wavelengths of light. The AAS machine has several lamps called hollow cathode lamps, each of which is specific for a particular element. The cathode lamp containing the element to be analyzed is used.

The process starts by aspiration of the liquid sample by the nebulizer of the machine. The sample is thereafter aerosolized and introduced into flame. The atomizer of the machine creates atoms of
the element of interest, after which there’s absorption of radiation from a light source by the free atoms. The intensity of the absorbed light is proportional to the concentration of the element in the flame.

A calibration curve is constructed by running several samples of known concentrations (standards) under the same conditions as the unknown. The amount the standard absorbs is compared with the calibration curve and this enables the calculation of the concentration of the element in the unknown sample.