


Effect of Zinc Supplementation in Children Less Than 5 Years on Diarrhea Attacks: A Randomized Controlled Trial

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Abstract

Recurrent infections and weak immunity were found to be linked with zinc deficiency especially in children. The goal of this study is to measure the level of blood zinc in children aged 6 months to less than 5 years old, and to assess the effect of 4 months of daily zinc supplements on the incidence and the severity of diarrheal morbidity. A randomized controlled trial study was conducted in Pediatric Outpatient Clinic in Ain Shams University Hospital on 140 children less than 5 years old who are apparently healthy. Random assignment of the sampled children to group receiving zinc (70 children) or group receiving placebo (70 children) was done. A questionnaire containing questions about occurrence of diarrhea was used during follow up. Serum zinc at baseline was measured. The mean age was 25.26 ± 15.7 months. Mean of serum zinc was $51.08 \mu\text{g/dL}$, with 70% had low baseline serum zinc level. Cumulative incidence of diarrhea, number of diarrhea episodes per child and frequency of stools/day decreased significantly in zinc group ($P < .05$). Risk ratio was found to be 0.79, 95% CI: 0.64 to 0.97. we concluded that incidence of diarrhea and its severity reduced in children received zinc daily for 4 months.

This trial was registered at www.clinicaltrials.gov as NCT05002101.

Keywords

Diarrhea, Malnutrition, Prevention, Zinc

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Introduction

Many functions of the body systems and organs require the presence of adequate level of a micronutrient called zinc. Zinc is important for protein and lipid processing and metabolism, cell integrity and maturation. Moreover, besides its regulatory roles, zinc has an essential function in metabolism of different nutrients, for example it helps in catalysis, synthesis and breaking down of nutrients.¹ The role of zinc in protection and response of the body to infections is well documented especially in children. It is known that every cell in the immune system, either humoral or cell mediated, is influenced by zinc. Immune cells development and functioning require the presence of zinc. Moreover, zinc is an integral part of a thymic hormone, thymulin, which is required for maturation of T cell.^{2,3}

Zinc is required to be included in everyday diet because it was not stored in the human body.⁴

For children under 5 years, they should obtain about 3 to 5 mg of zinc from diet every day; that is to fulfill the dietary requirements of nearly all (97%-98%) healthy persons (Recommended Dietary Allowance [RDA]). The main food containing zinc includes meat, meat products, milk, dairy products, and eggs. Plant-based

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food as grains and cereals are rich in zinc also; but contain phytate as well which decrease the bioavailability of zinc in diet.^{5,6}

In less developed nations, low zinc consumption in the diet is a common problem (affecting around 2billions) where they rely mainly on grains and cereals in their diet, while consume animal products in modest quantities.⁷

Repeated infections and weak immunity were found to be associated with zinc deficiency especially in under 5 years children. Common infectious diseases which are linked with zinc deficiency were diarrhea, pneumonia, and malaria which all can affect the growth of the children negatively.^{7,8}

The World Health Organization reported in 2009 that zinc deficiency below 5 years resulted in 13% of lower respiratory tract infections (basically pneumonia), 10% of malaria bouts, and 8% of diarrhea.⁹

Around the world, diarrhea is considered as one of the major killers of children under 5 years; it is the second common cause of death in this age group, killing about 525 000 children annually.¹⁰

The passing of 3 or more loose or watery stools daily is considered diarrhea. From clinical point of view, acute diarrhea is considered if symptoms occur only for few days (less than 2 weeks); while in persistent diarrhea, symptoms last for long duration (more than 2 weeks). Passing 3 or more watery stools with blood is known as dysentery.¹¹

A meta-analysis published by Aggarwal et al¹² concluded that supplements of zinc markedly decreased diarrheal incidence, severity, and duration.

World Health Organization (WHO) recommended zinc supplements for children under 5 years who suffer from diarrhea as an adjuvant to their treatment regimens in developing countries.¹¹ On the other hand, there is limited evidence on the effects of zinc supplementation on prophylaxis against morbidities in this age group.

By searching results of the current reviews, it was found that the effect of zinc supplements on prevention of morbidities was not studied on Egyptian children under 5 years. Accordingly, the present study aimed to measure the level of serum zinc among healthy children between 6 months and 5 years attending Pediatrics Outpatient Clinic in Ain Shams University Hospital, and to assess the effect of daily zinc supplements for 4 months on the occurrence of diarrhea among these children.

Material and Methods

Study design and setting: In the Outpatient Clinic of Pediatrics at Ain Shams University Hospital, a single blinded, randomized controlled trial was performed.

Study duration: The current study was carried out in a 2 years duration starting from August 2018 to August 2020.

Study population and sampling method: A consecutive sample of 140 children (70 children in the study group and 70 children in the control group) aged from 6 months and less than 5 years was enrolled from the Pediatrics Outpatient Clinic in Ain Shams University Hospital. Apparently healthy children of both sexes were included. The exclusion criteria included refusal of consent, severe diseases and malnutrition, requiring urgent hospitalization on the scheduled enrollment day.

Sample size: By using G power program (version 3.1.9.4) for sample size calculation, setting power at 80%, alpha error at 5%, and assuming medium effect size difference (0.5) in diarrheal frequency and duration between children took zinc supplements and those did not take zinc supplements, and after considering 20% dropout rate; a sample size of at least 140 apparently healthy children divided randomly into 2 groups (intervention by zinc supplements and placebo group) (65 children in each group) would be sufficient to achieve the study objectives.

Randomization and allocation: A computer-generated random number table was used to randomly assign the children to one of the 2 groups, either zinc, or placebo groups. The computer-generated random number table with a block size of 5 was used to create an allocation list. According to the randomization plan, each zinc, or placebo bottle was marked with a distinct child identification code. The exact dose and the child's name were also written on the label of each bottle. The shape, taste and wrapping of bottles of both zinc and placebo syrups were similar.

Blinding: The study was single-blinded, where the children's care givers didn't know either their children were enrolled in the experimental group or the control group.

Data collection tools: Study was conducted in 3 levels (baseline, intervention, and follow up).

An interview questionnaire was conducted at the start of the study, before zinc administration, and then at end of each month for 4 months of zinc intervention. Laboratory investigations for serum zinc before start of intervention was also conducted.

At Baseline

A baseline questionnaire: Interview structured questionnaire in Arabic language was used at the first meeting with the care giver, the questionnaire included 2 main sections:

- 1- **Basic personal and sociodemographic characteristics:** before intervention start, like age, gender, birth weight and child feeding practices (breastfeeding). Exclusive breastfeeding was defined as receiving breast milk only by the infant, without any other liquids or solids—not even water—with the exception of oral rehydration solution, or drops/syrups of vitamins, minerals, or drugs. Low birth weight was defined by WHO as weight of infant at birth of less than 2500 g.¹³
- 2- **The occurrence of diarrhea:** The baseline questionnaire before start of the intervention (supplantation of zinc) was used to assess the occurrence of diarrhea in the past month. All questions were admitted to the caregiver in Arabic. Selected questions from the Demographic and Health Survey (DHS) questionnaire were used. Regarding diarrhea, questions included occurrence of diarrhea in the past month, number of episodes, duration of each episode and frequency of stools on the worst day of the episode. Diarrhea was defined as the passage of 3 or more liquid or watery stools in a 24-hour period. Recovery was defined after a 72-hour period of no diarrhea.¹⁴ Other questions included in the questionnaire were about hospital admission during the diarrheal episode and treatment received especially the intake of oral rehydration solution. If the stool contained blood, the episode was classified as dysentery.¹⁵

Laboratory investigation for Serum Zinc: Before intervention started, blood sample was collected during morning hours from every child to measure the serum zinc. After following hand hygiene procedures by the phlebotomist and child skin antiseptics, blood samples were collected by puncturing a forearm or dorsum of the hand vein without using a tourniquet. Venous blood samples (3 mL) were collected in heparinized tubes. Each tube was labeled by the child's name, number, the time, and date of getting the sample. After collection, the tubes were sealed and kept in icebox until being transported to the laboratory. The tubes centrifuged for plasma and cell element separation and then were stored in a refrigerator. The lab technician used atomic absorption spectrophotometer graphite to measure serum zinc.

Children with serum zinc level between 60 and 100 µg/dL are considered normal. While children below 60 µg/dL were considered having low serum zinc.¹⁶

- **Intervention:** Randomly, supplements of zinc or placebo were received every day by the sampled children. The dose of the elemental zinc was determined according to the child's weight, such that 3 mg/day was given to those whose weight was under 10 kg, and 7 mg/day those whose weight was 10 kg or more.^{17,18} This dosage was calculated such that it was higher than the recommended dietary allowance for zinc supplements. The reason for this dosage was that these children mainly consume phytate-rich cereal-based diet and may suffer from bacterial overgrowth, and infestations of parasites or protozoa, accordingly this dosage would help to cover these problems. In the meanwhile, the dosage did not surpass the upper limit of tolerability for zinc.¹

The zinc was prepared such that in each 5 mL solution there was a 7 mg zinc sulfate. Children weighing >10 kg were instructed to consume 5 mL of syrup daily, while children weighing ≤10 kg were instructed to take 2.5 mL. A 150 mL bottle was stored in the child's home and changed every month.

The placebo was similar to the zinc syrup in color, odor, consistency, and taste, but it was devoid from the vitamin or the mineral. The zinc and placebo syrups came in the identical bottles.

Follow Up

The follow up period was 4 months. Telephone calls were done on weekly bases to ensure compliance of the care givers. On monthly bases, each mother was asked about the occurrence of diarrhea in her child in the previous month during their visit to the Pediatrics Outpatient Clinic or via a telephone call. For standardization, questions asked at each month throughout the follow up duration were the same as in the baseline questionnaire.

Data Management and Analysis: After collection of data, it was reorganized, coded, tabulated, and entered into a computer then finally analyzed using SPSS program (Statistical Package for Social Sciences) for windows Version 24.

For Descriptive statistics, quantitative data was analyzed by mean and standard deviation, whereas qualitative data was analyzed by frequency and its corresponding proportion.

For inferential statistics, based on the type of data collected for each variable, the appropriate statistical analysis was done. The statistical significance difference of a parametric variable between 2 independent means of 2 groups was determined using the independent sample

t-test. The Chi-square test was conducted to examine the association between 2 qualitative variables, but when the expected count in more than 20% of the cells was less than 5; Fisher's Exact test was the test of choice. Statistical significance was defined as a 2-sided *P*-value of less than .05.

For incidence density calculation, person day was used. The person-day for the total studied population was $(140 \times 4 \times 30) = 16800$. The person-day for either zinc or placebo group was $(70 \times 4 \times 30) = 8400$.

- *Cumulative incidence*: probability of developing disease over a stated period of time.¹⁹
- *Incidence density*: the number of diarrhea episodes per person-time.¹⁹
- *Risk ratio*: the cumulative incidence in exposed group (zinc group) divided by the cumulative incidence in the unexposed group (placebo group).¹⁹
- *Rate ratio*: the ratio of the incidence density in the exposed group (zinc group) to the incidence density in the unexposed group (placebo group).²⁰
- *Risk difference*: the difference between the cumulative incidence in the unexposed group (placebo group) and the cumulative incidence in the exposed group (zinc group).²⁰
- *Rate difference*: the difference between the incidence density in the unexposed group (placebo group) and the incidence density in the exposed group (zinc group).²⁰
- *Preventive Fraction*: the risk difference is divided by the cumulative incidence in the unexposed group.²⁰

Ethical Consideration: Administrative and Ethical committee board approvals were obtained to carry out the study at Pediatrics Outpatient Clinic in Ain Shams University Hospital (the approval number: 180/2018). This study was registered at www.clinicaltrials.gov as NCT05002101. An informed consent was obtained from the care givers after a comprehensive explanation of the study. Confidentiality and privacy of the participants was assured, collected data was dealt in complete confidentiality and no one had access to the participants' collected data except the main researchers. Participants might choose not to continue the study anytime if they feel inconvenience without any sequels.

Results

In the current study, apparently healthy 178 children were approached for participation and assessed for eligibility, and 28 children from them were excluded. Among those excluded, there were 13 children didn't meet the

inclusion criteria and 15 declined to participate in the study. From the 150 children who were allocated at random to the 2 groups, 10 children (5 in zinc group and 5 in placebo group) lost to follow up within the first month. Data from 140 children were included into the final analysis as presented by flow chart Figure 1.

Out of 140 studied children, (58.6%) of the studied children were 2 or less years old, while (41.4%) of the children were more than 2 years. The mean of the age of the studied children was 25.26 ± 15.701 months. Males represented (53.6%) of the children. Most of children (90.7%) were living in urban areas. About (12.1%) of children's mothers were illiterate, while (37.1%) of them completed secondary school. Regarding breast-feeding history in the first 6 months of age, most of the children (84.3%) were breastfed. Among those who were breastfed, 65% were exclusive breastfed. Regarding birth weight, most of the studied children (79.3%) had normal birth weight with mean of 2.81 ± 0.53 kg (Table 1).

The difference between children in zinc group and placebo group was not statistically significant as regard age, gender, residence, mother's education, breast feeding, exclusive breast-feeding duration and weight at birth of the studied children ($P > .05$).

In the total population, measuring the zinc in the serum revealed a mean of 51.08, with no significant difference from statistical point of view between children in zinc group and placebo group (Table 2). A serum zinc level of 60 to 100 $\mu\text{g}/\text{dL}$ was regarded normal. While children below 60 $\mu\text{g}/\text{dL}$ were considered having low serum zinc. In the current study, 70% of the studied children had low baseline serum zinc level (below 60 $\mu\text{g}/\text{dL}$), whereas only 30% had normal baseline serum zinc level. There was statistically insignificant difference between zinc and placebo groups regarding baseline serum zinc level ($P > .05$). Higher percent of the children in both groups had low baseline serum zinc level (below 60), (67.1% in zinc group and 72.9% in placebo group).

Regarding the cumulative incidence of diarrhea, there was a statistically significant difference between children in zinc and placebo groups after the third and the fourth month of follow up period and throughout the whole follow up period (ie, throughout the 4 months of the study) ($P < .05$). In a 24 hours period, severe diarrhea was determined if there were 6 or more liquid or semi-liquid stools. It was found that there was a statistically significant difference between children in zinc group and placebo group regarding percent of children with severe diarrhea throughout all the follow up period ($P < .05$). Among zinc group, 8.6% got severe diarrhea, while among placebo group more children (25.7%) got severe diarrhea (Table 3).

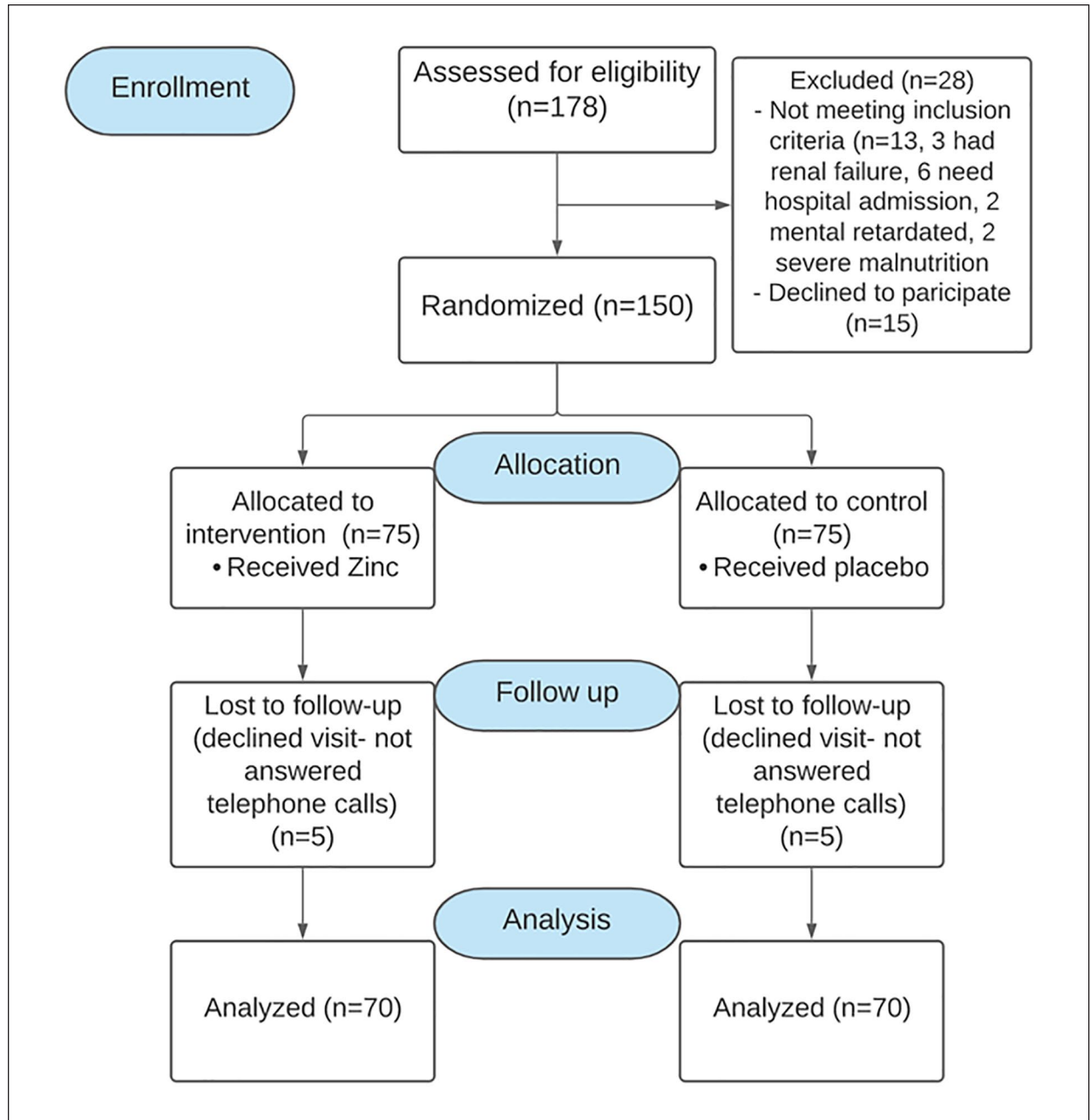


Figure 1. CONSORT flow chart for the study.

In all studied children, mean number of diarrheal episodes per child throughout the period of follow up was significantly lower in zinc group (1.1 episode) than in placebo group (1.69 episode). This difference was also significant in children with low serum zinc, but not in children with normal serum zinc.

Regarding mean of duration of diarrhea episode, there was a statistically insignificant difference between children in zinc group and placebo group

throughout the whole period of follow up (by summation of all durations of diarrheal episodes for each child in the 4 months of follow up) ($P > .05$). On the other hand, there was a statistically significant difference between children in zinc group and placebo group after the third and the fourth month of follow up period separately ($P < .05$).

Severity of diarrhea episodes was measured by the frequency of stools/day. Concerning mean number of

Table 1. Socio-Demographic Characteristics of the Zinc and Placebo Groups of the Studied Children.

Child characteristics	Zinc group (n=70)		Placebo group (n=70)		Total (n=140)		P value [#]
	No.	%	No.	%	No.	%	
Age in months							.303
6-24	38	54.3	44	62.9	82	58.6	
25-59	32	45.7	26	37.1	58	41.4	
Gender							.611
Male	36	51.4	39	55.7	75	53.6	
Female	34	48.6	31	44.3	65	46.4	
Mother's education							.192
Illiterate	7	10	10	14.3	17	12.1	
Read & write or primary school	11	15.7	10	14.3	21	15	
Preparatory school	14	20	11	15.7	25	17.9	
Secondary school	21	30	31	44.3	52	37.1	
University education	17	24.3	8	11.4	25	17.9	
Breast feeding							.353
Breast feed	61	87.1	57	81.4	118	84.3	
Never breast feed	9	12.9	13	18.6	22	15.7	
Birth weight							.532
Normal	57	81.4	54	77.1	111	79.2	
Low	13	18.6	16	22.9	29	20.8	

[#]Chi square test was used.

Table 2. Comparison Between Zinc and Placebo Groups Regarding Baseline Serum Zinc level ($\mu\text{g/dL}$).

	Zinc group (n=70)		Placebo group (n=70)		Total (n=140)		P value
	Mean \pm SD		Mean \pm SD		Mean \pm SD		
Baseline Serum Zinc level	52.58 \pm 24.84		49.56 \pm 21.85		51.08 \pm 23.37		.445 [#]
	No.	%	No.	%	No.	%	
Normal baseline serum zinc level	23	32.9	19	27.1	42	30	.461 [§]
Low baseline serum zinc level	47	67.1	51	72.9	98	70	

[#]Independent t test was used.

[§]Chi square test was used.

stools/day, there was a statistically significant difference between children in zinc group and placebo group over the whole 4 months of the follow up period (for each child, the highest number of stools/day in any of the episodes occurred in the 4 months was considered). The mean number of stools per day was 2.77 ± 2.346 stools/day in the zinc receiving group and 3.66 ± 2.45 stools/day in the placebo group. Also, there was a statistically significant difference between children in zinc group and placebo group per day after the third and fourth month of follow up period ($P < .05$) (Table 4).

Regarding oral rehydration solution, among children with diarrhea, there was a statistically insignificant

difference between children in zinc group and placebo group regarding receiving oral rehydration solution throughout all the follow up period ($P > .05$).

Calculating measures of risk, association, and impact of the zinc on occurrence of diarrhea in comparison to the placebo was done. Cumulative incidence and incidence density were lower in zinc group than in placebo group, where zinc supplement is protective against diarrhea (risk ratio was found to be 0.7895, which means that children of zinc group had **0.7895 times the risk** of developing diarrhea compared to children of placebo group). (Note that this risk ratio was less than 1 suggests a reduced risk of diarrhea in the zinc group) (Table 5).

Table 3. Comparison Between Zinc and Placebo Group Concerning Cumulative Incidence of Diarrhea and Percent of Children With Severe Diarrhea at Baseline and During Follow Up Period.

	Zinc group (n=70) (%)	Placebo group (n=70) (%)	Total (n=140) (%)	P value [#]
Cumulative incidence of diarrhea				
At baseline	45.7	40	42.9	.495
After first month	41.4	34.3	37.9	.384
After second month	31.4	30	30.7	.855
After third month	14.3	32.9	23.6	.01
After fourth month	10	40	25	<.001
Throughout follow up period	64.3	81.4	72.9	.023
Children with severe diarrhea				
At baseline	14.3	11.4	12.9	.614 [#]
After first month	7.1	12.9	10	.26 [#]
After second month	1.4	4.3	2.9	.62 [§]
After third month	0	2.9	1.4	.496 [§]
After fourth month	0	5.7	2.9	.12 [§]
Throughout follow up period	8.6	25.7	17.1	.007 [#]

[#]Chi square test.

[§]Fisher exact test were used.

Table 4. Comparison Between Zinc and Placebo Groups Concerning Mean Number of Diarrheal Episodes Per Child, Duration of the Diarrhea Episodes and Frequency of Stools/Day.

	Zinc group (n=70)	Placebo group (n=70)	P value [#]
	Mean ± SD	Mean ± SD	
Mean no. of diarrheal episodes/child throughout the period of follow up			
All children (n=140)	1.1 ± 1.19	1.69 ± 1.3	.006
Children with normal serum zinc (n=42)	1.3 ± 1.55	1.7 ± 1.05	.306
Children with low serum zinc (n=98)	1 ± 0.98	1.67 ± 1.39	.008
Mean duration of diarrhea episode (days)			
At baseline	1.91 ± 3.238	1.30 ± 1.852	.171
After first month	1.47 ± 2.062	1.19 ± 1.928	.399
After second month	1.23 ± 2.751	0.90 ± 1.515	.383
After third month	0.37 ± 1.092	1.2 ± 2.288	.007
After fourth month	0.2 ± 0.734	1.33 ± 1.775	<.001
Throughout follow up period	3.27 ± 4.98	4.61 ± 3.92	.079
Number of stools/day [§]			
At baseline	2.19 ± 2.799	1.90 ± 2.509	.526
After first month	1.84 ± 2.418	1.83 ± 2.864	.975
After second month	1.29 ± 1.979	1.17 ± 1.880	.727
After third month	0.5 ± 1.260	1.17 ± 1.761	.01
After fourth month	0.39 ± 1.183	1.71 ± 2.335	<.001
Throughout follow up period	2.77 ± 2.346	3.66 ± 2.45	.028

[#]Independent t test was used.

[§]The highest number of stools/days was considered.

Discussion

In the present study, serum zinc level was measured before the intervention and the calculated mean was $51.08 \pm 23.37 \mu\text{g/dL}$ in all studied children, with

no statistically significant difference between zinc and placebo groups. Accordingly, there would be a fair comparison between the intervention and control groups during the follow up. Low baseline serum zinc level was found in 70% of the studied population. This result was

Table 5. Effectiveness of Zinc Supplements on Diarrhea.

	Zinc group (n=70) No. of child/days (person/ time) (8400)	Placebo group (n=70) No. of child/days (person/ time) (8400)	Total (n=140) No. of child/days (person/time) (16800)	Risk ratio or rate ratio (95% CI)	Risk difference or rate difference (95% CI)	Preventive fraction (95% CI)
Cumulative incidence (95% CI)	64.3 (52.57 to 74.52)	81.4 (70.74 to 88.95)	72.9 (64.93 to 79.56)	Risk ratio: 0.7895 (0.6416 to 0.9714)	Risk difference: -17.14 (-31.6 to -2.687)	21.05 (2.861 to 35.84)
Incidence density per 1000 child/days (95% CI)	9.167 (7.234 to 11.46)	14.05 (11.63 to 16.82)	11.61 (10.04 to 13.36)	Rate ratio: 0.6525 (0.489 to 0.869)	Rate difference: -4.88 (-8.139 to -1.623)	

Abbreviation: CI, confidence interval.

similar to Barffour et al¹⁴ who found that 71% of the studied children had low serum zinc at the baseline.

There are some Egyptian studies measured serum zinc in different populations and revealed different concentrations. The mean of serum zinc concentration in these studies ranged from 57 up to 117.4 µg/dL.²¹⁻²³

These differences and wide variations between studies regarding serum zinc level may be due to its normal physiological fluctuations; serum zinc level fluctuate by about 20% throughout the day, mostly because of the ingestion of food. After meals, there was immediate increase in serum zinc concentration, after which this concentration decreases for 4 hours and then increase again. However, the fluctuation pattern is changed during fasting, whereby it decreased from morning for some hours and then increase again. Moreover, serum zinc concentration was affected by presence of several conditions like acute infections inflammation, hemolysis, malnutrition, dehydration, and fever.^{24,25}

In the present study, zinc supplementation was examined to evaluate its impact on diarrhea morbidity.

In the present study, the cumulative incidence of diarrhea in zinc group was significantly lower than that in placebo group after the third and the fourth month of follow up period and throughout the whole follow up period. Liberato et al¹⁷ also concluded that prophylactic zinc was effective in decreasing incidence of diarrhea.

Risk of developing diarrhea in the zinc group was lower than that in placebo group, thus, risk ratio was found to be 0.7895; 95% CI: 0.6416 to 0.9714. Also, the risk difference was found to be -17.14%, which means that children of zinc group had 17.14 fewer cases of diarrhea per 100 children compared to children of placebo group. These results agreed with Aggarwal et al¹² who found that supplementation of zinc decreased the incidence of diarrhea significantly by 14% with a relative risk of 0.86. In addition, Brown et al²⁶ found that zinc supplementation caused a significant 20% lower incidence of diarrhea (relative risk=0.80). The pooled analysis by Yakoob et al²⁷ revealed a significant reduction of

13% on the incidence of diarrhea in children receiving zinc in comparison to children in the control group, and the relative risk was found to be 0.87; 95% CI: 0.81 to 0.94.

Inconsistent with the previous studies, Luabeya et al reported that supplementation with zinc did not reduce diarrhea morbidity in rural South African children. The authors of this trial suggested that the cause of this inconsistency was that studies that found an effect on diarrheal morbidity had often included poor nourished children. Children enrolled in Luabeya et al²⁸ trial had better anthropometric indices than those reported on in most other studies. Also Barffour et al¹⁴ found no benefits of daily preventive zinc supplements on occurrence of diarrhea.

The current study revealed that the zinc supplements significantly decreased the overall number of diarrheal episodes in children of zinc group in comparison to the children of placebo group. This impact was more prominent in children with low baseline serum zinc level. This result agreed with Bhandari et al²⁹ who found a significant decline in the number of children who had recurrent diarrhea with significant decrease in the number of diarrheal episodes in children of zinc group in comparison to the children of placebo group. Moreover, Umata et al³⁰ found that, in response to zinc supplements, there were better improvements in the incidence of diarrhea in children whose serum zinc was low at the beginning of the study, in comparison with children with higher baseline serum zinc levels.

On the other hand, Bhandari et al²⁹ found the observation in the opposite direction, such that the trend of lower diarrhea incidence was prominent only in children who had serum zinc higher than 60 µg/dL. Moreover, a clinical trial was performed by Rahmati et al³¹ reported that, in terms of the mean number of diarrheal episodes, there was no substantial difference present between zinc and control groups.

Regarding duration of diarrhea episodes, there was no statistically significant difference between children

in zinc group and placebo group throughout the whole period of follow up (by summation of all durations of diarrheal episodes for each child in the 4 months of follow up) ($P > .05$). Consistent with the current study, a review conducted by Brown et al²⁶ found that, in community-based trials, there was no substantial effect of prophylactic zinc supplementation on the duration of diarrhea. Similarly, Rahmati et al³¹ found no apparent difference between the groups in terms of the duration of diarrheal episodes.

On the other hand, Garenne et al³² revealed shorter length of diarrheal episodes in boys who get supplements of zinc. Meta-analysis conducted by Aggarwal et al¹² found that supplementation of zinc shorten the duration of diarrhea episodes.

Concerning frequency of stools/day, the present study found that the zinc group had significantly lower frequency of stools/day than that in the placebo group. These results are inconsistent with the clinical trial of Rahmati et al³¹ who found that there was no difference of statistical importance between zinc receiving group and the other group regarding the number of stools/day.

Number of stools/day reflects the severity of the diarrhea episode, such that, in a 24 hours period, if the number of liquid or semi-liquid stools equals to or exceeding 6 times daily, it was considered as severe diarrhea. The present study found that percent children with severe diarrhea in zinc group was significantly lower than that in placebo group throughout all the follow up period. This result was inconsistent with the randomized, double-blind placebo-controlled trial conducted by Barffour et al¹⁴ who found that there was no effect for the preventive zinc supplements on the severity of the diarrhea episodes.

Generally, the diversity in effects of zinc on prevention of diarrhea could be due to a varied patient population, the underlying zinc status of the population, study designs (community based or hospital based), the dose and form of zinc administrated, the methods of monitoring diarrhea, the outcome measured and possible confounding effects of child feeding practices.

Conclusions and Recommendations

The study found that daily zinc supplementation for 4 months to children aged 6 months to 5 years is beneficial in reducing diarrhea and may be used as a suitable preventative supplement. Zinc reduced the number of diarrheal episodes and its severity but had no effect on the duration of each episode in the current study. Thus we recommend the following: (1) Nationally nutritional assessment surveys need to be conducted to assess the population zinc status, (2) Further studies to be conducted

to assess the effect of preventive zinc on different age groups, and (3) Prompt measures to be done to improve the zinc status of the population as: fortification of foods with zinc, cultivation of zinc rich plants, supplements of certain children with zinc and health education to the parents about foods containing zinc and proper weaning practices

Author Contributions

Conceptualization and design: A.E.-S., S.A.-H. and Y.G.-A.; acquisition, analysis, or interpretation of data: Y.S.-A., Y.G.-A. and M.M.-W.; assembled the data under Y.G.-A. supervision; writing—original draft preparation: Y.S.-A. and M.M.-W.; writing—review and editing: A.E.-S., Y.S.-A., Y.G.-A and S.A.-H.; supervision: A.E.-S. and S.A.-H. All authors read and approved the final version of the manuscript. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Declaration of Conflicting Interests

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References

1. World Health Organization. *Vitamin and Mineral Requirements in Human Nutrition*. World Health Organization; 2004.
2. Gammoh NZ, Rink L. Zinc and the immune system. In: Mahmoudi M and Rezaei N (eds) *Nutrition and Immunity*. Springer; 2019;127-158.
3. Prasad AS. Zinc: role in immunity, oxidative stress and chronic inflammation. *Curr Opin Clin Nutr Metab Care*. 2009;12(6):646-652.
4. Ferreira CR, Gahl WA. Disorders of metal metabolism. *Transl Sci Rare Dis*. 2017;2(3-4):101-139.
5. Trumbo P, Yates AA, Schlicker S, Poos M. Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. *J Am Diet Assoc*. 2001;101(3):294-301.
6. EFSA Panel on Dietetic Products, Nutrition and Allergies. Scientific opinion on dietary reference values for zinc. *EFSA J*. 2014;12(10):3844.

7. Brown KH, Rivera JA, Bhutta Z, et al. International Zinc Nutrition Consultative Group (IZiNCG) technical document #1. Assessment of the risk of zinc deficiency in populations and options for its control. *Food Nutr Bull.* 2004;25:S99-203.
8. Prasad AS. Discovery of human zinc deficiency: its impact on human health and disease. *Adv Nutr.* 2013;4(2):176-190.
9. World Health Organization. *Global Health Risks: Mortality and Burden of Disease Attributable to Selected Major Risks.* WHO; 2009.
10. World Health Organization. Diarrhoeal disease fact sheet. Published 2017. <http://www.who.int/mediacentre/factsheets/fs330/en/>. Accessed September, 2020.
11. Khan WU, Sellen DW. Zinc supplementation in the management of diarrhoea. Biological, behavioural and contextual rationale e-Library of Evidence for Nutrition Actions (eLENA). Published 2011. Accessed May 11, 2015. https://pajand-darou.com/wp-content/uploads/2021/06/WHO-_Zinc-supplementation-in-the-management-of-diarrhoea.pdf
12. Aggarwal R, Sentz J, Miller MA. Role of zinc administration in prevention of childhood diarrhea and respiratory illnesses: a meta-analysis. *Pediatrics.* 2007;119(6):1120-1130.
13. World Health Organization. *Nutrition Landscape Information System (NLIS) Country Profile Indicators: Interpretation Guide.* World Health Organization; 2019.
14. Barffour MA, Hinnouho GM, Wessells KR, et al. Effects of therapeutic zinc supplementation for diarrhea and two preventive zinc supplementation regimens on the incidence and duration of diarrhea and acute respiratory tract infections in rural Laotian children: A randomized controlled trial. *J Glob Health.* 2020;10(1):010424.
15. Ministry of Health and Population of Egypt. Egypt demographic and health survey 2014. Published 2015. <http://dhsprogram.com/pubs/pdf/FR302/FR302.pdf>. Accessed October, 2020.
16. Javed F, Asghar A, Sheikh S, Butt MA, Hashmat N, Malik BA. Comparison of serum zinc levels between healthy and malnourished children. *Ann Punjab Med Coll.* 2009;3(2):139-143.
17. Liberato SC, Singh G, Mulholland K. Zinc supplementation in young children: A review of the literature focusing on diarrhoea prevention and treatment. *Clin Nutr.* 2015;34(2):181-188.
18. Wuehler SE, Sempértegui F, Brown KH. Dose-response trial of prophylactic zinc supplements, with or without copper, in young Ecuadorian children at risk of zinc deficiency. *Am J Clin Nutr.* 2008;87(3):723-733.
19. Centers for Disease Control and Prevention. *Principles of Epidemiology in Public Health Practice, An Introduction to Applied Epidemiology and Biostatistics*, 3rd ed. Centers for Disease Control and Prevention; 2012.
20. LaMorte WW. Measures of Association. Boston University School of Public Health. Published 2018. https://sphweb.bumc.bu.edu/otlt/MPH-Modules/EP/EP713_Association/EP713_Association_print.html. Accessed October, 2020.
21. Eissa MA, Abdulghani KO, Nada MA, Elkhawas HM, Shouman AE, Ahmed NS. Serum zinc and copper levels in a sample of Egyptian epileptic children. *Egypt J Neurol Psychiatry Neurosurg.* 2020;56(1):1-9.
22. Mahmoud MM, El-Mazary AA, Maher RM, Saber MM. Zinc, ferritin, magnesium and copper in a group of Egyptian children with attention deficit hyperactivity disorder. *Ital J Pediatr.* 2011;37(1):60.
23. Azab SF, Saleh SH, Elsaheed WF, Elshafie MA, Sherief LM, Esh AM. Serum trace elements in obese Egyptian children: a case-control study. *Ital J Pediatr.* 2014;40(1):20-27.
24. Nasehi MM, Sakhaei R, Moosazadeh M, Aliramzany M. Comparison of serum zinc levels among children with simple febrile seizure and control group: a systematic review. *Iran J Child Neurol.* 2015;9(1):17-24.
25. Roohani N, Hurrell R, Kelishadi R, Schulin R. Zinc and its importance for human health: an integrative review. *J Res Med Sci.* 2013;18(2):144-157.
26. Brown KH, Peerson JM, Baker SK, Hess SY. Preventive zinc supplementation among infants, preschoolers, and older prepubertal children. *Food Nutr Bull.* 2009;30(1 Suppl):S12-S40.
27. Yakoob MY, Theodoratou E, Jabeen A, et al. Preventive zinc supplementation in developing countries: impact on mortality and morbidity due to diarrhea, pneumonia and malaria. *BMC Public Health.* 2011;11(3):S23.
28. Luabeya KK, Mpontshane N, Mackay M, et al. Zinc or multiple micronutrient supplementation to reduce diarrhea and respiratory disease in South African children: a randomized controlled trial. *PLoS One.* 2007;2(6):e541.
29. Bhandari N, Bahl R, Taneja S, et al. Substantial reduction in severe diarrheal morbidity by daily zinc supplementation in young north Indian children. *Pediatrics.* 2002;109(6):e86.
30. Umeta M, West CE, Haidar J, Deurenberg P, Hautvast JG. Zinc supplementation and stunted infants in Ethiopia: a randomized controlled trial. *Lancet.* 2000;355(9220):2021-2026.
31. Rahmati MB, Barchinezhad M, Houshmandi MM, Hamayeli Mehrabani H, Mahboubi HR, Hosseini S. Effect of zinc supplementation on preventing diarrhea in children. *Hormozgan Med J.* 2015;19(1):11-17.
32. Garenne M, Becher H, Ye Y, Kouyate B, Müller O. Sex-specific responses to zinc supplementation in Nouna, Burkina Faso. *J Pediatr Gastroenterol Nutr.* 2007;44(5):619-628.