Efficacy and safety of zinc sulfate to reduce the duration of acute diarrheic disease between 6 and 59 months of age

Project number 1474

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Abstract

Acute diarrheal diseases (ADD) continue to be a public health problem. Even though the oral rehydration salts (ORS) have reduced annual death rates related to this, from 4.5 to 1.8 million, in the world, it still is the second cause of death in children under the age of 5 years, and in Mexico the fifth, generating in the country 20.8% of all the appointments and 10% of all pediatric hospitalizations.

Worldwide in 2004 zinc deficiency was associated to 400,000 deaths in children under the age of 5, whilst in Mexico, in the ENSANUT-2006, 11.4% of children under the age of 5 had a low zinc ingest and 29.2% of children under the age of 2 years had zinc deficiency.

In Mexico the administration of ORS and immunization against rotavirus has positively impacted ADD mortality in children, nonetheless it continues to be a public health issue, being between the main causes of morbidity and mortality, with an important load for the health systems. Consequently there exists a necessity for the implementation of alternate strategies for this condition, nutrition being on of the pillars to strengthen.

Zinc administration during the ADD episode is a simple intervention for clinical picture reduction and relapse. The recommendation of including zinc in the treatment of ADD is not part of the Mexican normativity. The evaluation of the efficacy of zinc administration in Mexican children is insufficient and a program or national strategy for zinc treatment does not exist.

Objective. Evaluate the efficacy of oral zinc sulfate administration (20 mg/day for 10 days), during the ADD clinic picture in children 6 to 59 months of age treated in nine Sentinel Centers of Mexico City.

Methodology. Randomized clinical trial, multicenter, double risk, controlled. Including 516 children 6 to 59 months of age, in two groups, 258 in the zinc intervened group, and 258 in the controlled group.

For the analysis descriptive statistic will be used. The comparison of evaluation time of ADD and number of evacuations will be done by the use of the Student t test for independent groups in case the distribution is normal, or with the Manny-Whitney U test if the distribution is not normal. For the evaluation of risk of relapse relative risk will be estimated, reduction of relative risk and reduction of absolute risk. The efficacy of the intervention over the duration of the disease and the number of evacuations will be model with the lineal general model to adjust by the basal characteristics.

The present proposal will allow to respond to the necessity of counting with controlled studies in Mexican children to know the impact of zinc in the reduction of ADD severity, duration and relapse.

Backgrounds

Epidemiology and impact of acute diarrheic disease

Acute diarrheal diseases (ADD) are still an important public health problem; worldwide it is the second cause of death in children under the age of 5, preceded only by low respiratory infections. In Mexico it occupies fifth place amongst the causes of mortality in children under the age of five years and generates 20.8% of consultations in health services and 10% of pediatric hospitalizations in the country.[1]

The disease´s rates are very similar amongst the different states of the Mexican Republic. According to ENSANUT 2012, the proportion of ADD in rural areas was 12.3%; and in urban areas 10.5% respectively. When the proportion of children under the age of five years of age was analyzed by socio economic level, that presented diarrhea in the two weeks previous to the interview, it was reported a 10.5%, 11.9%, 11.4%, 10.9% and 10.1% in the socio economic levels 1, 2, 3, 4, 5, respectively, once again there are no differences between the different socio economic levels.[2]

Oral rehydration electrolytes and oral rehydration therapy, adopted by the WHO and UNICEF at the end of the 70s, have been key in mortality rate descend amongst children under the age of 5 with acute diarrhea because it went from 4.5 million to 1.8 million annual deaths.[3] Nonetheless, despite this notable descend, acute diarrhea still is one of the main infant mortality causes in developing countries. [4]

It is estimated that 21% of world population is at risk of zinc deficiency according to the food supply balance reports.[5]

The World Health Organization has recognized zinc deficiency as a risk factor for children´s health, since it is associated with diarrhea morbidity in a 10%, low respiratory infections in a 6%, and with malaria in 18%, plus 0.8 million deaths in children every year.[6]

Zinc use in ADD

Clinic evidence shows that, worldwide, frequent zinc consumption helps prevent ADD.[7] The new recommendations for the treatment of diarrhea, published by the Global Program of the WHO in Genève, are aimed to reduce morality in children under 5 years of age. This new recommendations have in mind two recent transcendental advances: the demonstration of the higher efficacy of the new formulation of the ORS with low concentrations of glucose and salt; and the good results of the administration od zinc supplements.[7 8 9]

The National Nutrition Survey of 1999 reported a prevalence of low levels of zinc of 34% in Mexican children under the age of 2 years. [10] Furthermore, in 2006 the National Nutrition Survey (ENSANUT) [11] found that the 11.4% of children under the age of 5 years had a zinc intake lower than the Estimated Required Average and 29.2% of the children under the age of 2 years presented zinc deficiency.[12] Zinc deficiency was associated with approximately 400,000 deaths in children under the age of 5 years old in 2004 worldwide.[13]

Zinc physiology

Moderated zinc deficiency in children of preschool age is common in developing countries; [12] [14] it may delay growth, inhibit immunological response, suppress appetite, and reduce the capacity to detect flavors.[15] [16]

Zinc has a crucial function in the metalloenzymes, the polyribosomes, the membrane and cellular function, which performs an important function in cellular growth and in the function of the immune system. Zinc use for the treatment of acute diarrhea affects immune functioning, the intestinal structure or its function, and the process of epithelial recuperation during diarrhea. Moreover, it has been highlighted that the losses of intestinal zinc during diarrhea aggravate the lack of preexisting zinc. [17]

Safety

In the extensive safety studies conducted on laboratory animals, it has been demonstrated that zinc is not carcinogenic, mutagenic, or teratogenic.[18]

The human body has efficient homeostatic mechanisms that regulate the absorption and retention of zinc, which diminishes the probability of toxic accumulation in the body.[19]

The toxicity of zinc in adults may occur following a moderately high zinc ingest (> 150mg / day, approximately 10 times the daily recommendation) during a long period of time or by ingest of more than 1 g of zinc, or by overdose via supplementation or intravenous feeding.[20]

High zinc dosage during long periods of time may conduct to a minor concentration of plasmatic lipoproteins and diminish the absorption of copper. The descend of copper status may also inhibit transportation of iron and give place to anemia.[21]

In the most extreme cases of ingest of more than 1 gram of zinc taken daily during many months, most of the patients recovered from all symptoms and signs, including fatigue, gastrointestinal discomfort and anemia as soon as the ingest of zinc was reduced, and serum zinc went back into the normal range.

The recommended dosage of 10mg in children under the age of 6 months and 20mg in children from 6 months to 59 months of age for 10- 14 days have demonstrated effectiveness and safe for treatment during diarrhea. Up to this day there has not been reports of serious adverse reactions of any kind of zinc supplements for the treatment of diarrhea. The trials have included more than 8.500 children that have participated in efficacy trails. The zinc dosages varied from 5- 45 mg a day, and have been well tolerated in diverse environments. The trails have not found differences in the adverse reactions based on the different zinc salts used in the supplement trails, which are acetate sulfate and zinc gluconate.

For the moment, the only secondary effect of the supplement administration has been vomit. Out of the seven trails that have informed about vomiting incidence, only two reported more vomiting in children with zinc supplements vs. placebo.[22] [23]

One trail found a significant tendency to diminish copper status when comparing children supplemented with zinc and children supplemented without zinc.[27]Nonetheless, this children were malnourished and with persistent diarrhea at the beginning of the study. In general, there

is no evidence that short-term zinc supplements as a treatment for diarrhea affect negatively copper concentrations.

Other studies where zinc efficacy has been evaluated for the treatment of pneumonia, malaria, measles, and common cold, have not reported serious adverse events related to zinc supplement.

In studies with long-term supplementation in children seeking to improve growth, and prevent pneumonia, diarrhea and malaria, it was used 5 to 20 mg Zn/day for a year; there were no reports of adverse events in those studies.[28]

Like with any treatment, medication or vitamin, zinc supplements should be kept at a safe location to prevent accidental ingestion of more of the recommended dosage. In the rare event that a child consumes multiple zinc supplements, it is probable that he or she will rapidly vomit. There is no evidence that suggest that more adverse events will occur, but like with any accidental medication ingestion, the child should be taken to a medical attention provider. The risk of more adverse events that are produced from the recommended supplementation schemes is extremely low, but the zinc supplement should be maintained at a safe location to prevent unnecessary accidents.

Zinc supplement is a safe and effective treatment fro diarrhea. Zinc also has demonstrated being safe in long-term supplementation studies. The most serious adverse events from the supplementation trails have been vomiting in some cases and a mild decrease in copper status in some children. None of them have demonstrated to cause long-term damage. Even though in adults there has been cases reported of excessive zinc consumption, even the adverse events in those cases have been limited to short-term morbidity and few have resulted in long-term sequels.

Efficacy of zinc usage

In the last two decades there has been multiple studies done focused on zinc use in diarrhea; they have concluded the following:

- Children treated with zinc have a significantly faster recovery than children that do not received it (the duration of diarrhea is reduced approximately a 20-25%). On the other hand, the risk of severe episodes of more than seven days of duration is reduced in a 20%. The decrease of the total amount of evacuations goes from 18% to 59% in children treated with zinc when compared with the placebo group.[29 30]
- The administration of zinc supplements to children in developing countries, being in a continuous way or in short duration treatments, is associated to substantial reductions of diarrhea rates.[8]
- Children with persistent diarrhea that took zinc supplements had a probability of continuing with diarrhea lower than 24% (Confidence Interval of 95% [CI]: 9% to 37%), and the failure rate of the treatment or death was 42% lower (CI of 95%: 10% to 63%) than the witness group.[8]
- The studies conducted in children with acute shigellosis have revealed that the administration of supplements of zinc favors significantly seroconversion, meaning the

appearance of antibodies against shigella, and increases the proportion of circulating B lymphocytes and plasmatic cells, as well as the specific response due to the A immunoglobulin production (IgA).[31] Consequently it is clear that zinc supplements must be administered as a complement to treatment with antibiotic for bloody diarrhea.

It has been concluded that the administration of zinc supplements has an important and beneficial effect in clinic evolution of acute diarrhea, because it reduces severity as well as its duration. The administration of zinc supplements for the treatment of diarrhea, together with educational programs may reduce inadequate use of antibiotics that are leading to the appearance of pathogenic agents resistant to antimicrobials.[32]

It has been concluded that the administration of zinc supplements, in a daily dosage from 10 to 20 mg during 10 to 14 days, is effective in terms of reducing significantly the severity of diarrhea and the duration of the episode. It has not been determined yet the optimal dosage, nonetheless it does not appear to be an increase in efficacy when passing from the usual daily dosage of 20 to a higher of 30 to 40. It has been demonstrated that a daily dosage of 20 mg of elemental zinc is innocuous and efficient, even for lactating infants, and consequently it is recommended.

It has been accomplished the same efficacy with the different zinc salts used (sulfate, acetate and zinc gluconate).[33] However, zinc sulfate is low cost, efficient and safe, and consequently could be optimal for a national program.[34]

Zinc effect in acute diarrheic disease

Zinc deficiency in immune response is likely to increase susceptibility of children with infectious diarrhea. There have been two populations phenotypically different of T lymphocytes identified that are zinc sensible: the response Th1, important in the protection against intracellular infections, and the Th2 response is important in the protection against non invasive infections such as helminthes. An adequate ingest of zinc and energy leads to a Th1 dominant response, and to a Th2 response regulated to the low.

In-vitro studies and studies in patients with zinc deficiency have demonstrated that zinc performs an essential part mediated by cells and humoral immunity. The findings during zinc deficiency are a decrease in lymphocytes numbers (lymphopenia), lymphocytic development deterioration, reduced proliferation, apoptosis increase and thymic atrophy. Zinc is an essential cofactor for timulin that induces various T cells markers and promotes the function of T cells, including allogenic cytotoxicity, suppressor functions and the production of Il-2. Also modulates the liberation of cytokines by the nuclear cells of peripheral blood and induces the proliferation of T CD8 cells, that function ass cytotoxic cells capable of recognizing and killing pathogens.

Zinc also plays a key part in the maintenance of cells from the intestinal mucous. Zinc blocks basolateral potassium channels (K^+) and therefore inhibits the secretion of liquid dependent of the chloride induced by cAMP, an important control point for liquid loss in the large intestine. The mechanisms by which zinc may act as an enteroprotector have not been determined yet. Zinc also inhibits ion secretion induced by enterotoxin induced the cholera toxin, but not the thermo stable enterotoxin induced by E.cola. The cAMP acts as the intracellular effector from

thermo labile enterotoxin inducing fluid secretion. So zinc efficacy will be limited to the diarrhea induced by thermo labile enterotoxin or to diarrhea mediated by cAMP, but not by cGMP or by intracellular calcium. It has also been reported that ZnT-1, zinc transporter, modulates cation permeability through the channel of L type calcium (LTCC), therefore the regulation of cationic homeostasis. Therefore, ZnT-1 may perform a part in cell ion homeostasis conferring protection against pathophysiological events related to cellular permeability of calcium or of zinc. A micromolar concentration of extracellular zinc might trigger a massive liberation of calcium from the intracellular reserves in colonocytes. A sustained increase of intracellular calcium level can also increase the flow of K and a hyperpolarization of the cellular membrane potential, giving place to an electrical gradient advantageous for chloride secretion.
Protozoan intestinal infections by *Giardia* and *Cryptosporidium* are common in humans all over the world. Especially important are infections in children, during pregnancy, and amongst people with HIV/AIDS. The associated morbidity and mortality are high, with more than 58 million cases of infant protozoan diarrhea every year. A recent study performed in children habitants of Mexico City informed that vitamin A and zinc reduced the incidence of *G.lamblia*, while zinc supplements only decrease diarrhea associated with *Entamoeba histolytica.*

Even though bacterial pathogens may cause diarrheic illnesses, a group of less than 10 (including *Shigella spp,* ETEC, *Vibrio cholerae* and possibly *Camplylobacter jejuni*) represent a significant percentage of these illnesses in developing countries.
Crane et al. informed that zinc decreased the adherence of enteropathogenic bacterias of *E.coli* (EPEC) the rabbit intestinal epithelium.[35] Also demonstrated that zinc inhibited a key enzyme, the ecto-5´-neuclotidase, implicated in the conversion of 5´-AMP in adenosine at the intestine lumen. Adenosine triggers fluid secretion of the intestinal cells of the host and also has growth promoting effects in the EPEC bacteria. The inhibition of zinc reduced the secretory response that triggers watery diarrhea activated by EPEC.
In animal testing, the net secretion of water and sodium induced by the cholera toxin was four times higher in zinc deficiency in comparison with animals suitable for zinc.
Zinc supplementation reduced significantly the duration of acute shigellosis, promotes a better weight increase during recovery and reduces diarrheic morbidity during the following 6 months.

Zinc as a public health strategy

To obtain maximum impact over diarrheic diseases, zinc and the ORS must be placed within the reach of all the community. In the market there are many vitamin preparations and other nutritional supplements that contain zinc. Nevertheless, they rarely contain the recommended dosage. Consequently, is required a product that contains only zinc, even though copper may be added. The product should ne elaborated in a manner that masks the metallic flavor that zinc can leave for children to accept it. Zinc salts in tablet form or syrup are the most convenient for infant administration.
Currently in Mexico in the Basic table and the Medication Catalogue 2014 diverse presentations exist (medical food, formulas, multivitamins, human milk substitutes and/or diets) that contain

diverse concentrations of Zinc Sulfate, fundamental Zinc, Zinc Chloride and/or Zinc Phosphate in combination with other active substances. There is only one presentation of multivitamins (polyvitamins) and Oral Solution Minerals for children of 6 to 24 months of age where every 100 ml contains among others vitamins and minerals; monohydrated zinc sulphate (2.744 g) equivalent to 1.0 g of fundamental zinc and its use is indicated in patients with specific deficiencies and/or pathologies related to deficiency, however in the treatment of Acute Diarrheic Disease it is necessary an oral solution as supplement that is zinc based (such as heptahydrate sulfate).[34] All the supplements distributed to "Prospera" beneficiaries ("Nutrisano", "Vitaniño" and "Nutrivida") already include a recommended amount of zinc for this population. The recommendation to include zinc in ADD treatment is not yet a part of the Mexican normativity in the matter. However, there exist background in the health sector that have carried out this practice, it has been recommended in the Attention to Nutrition Integral Strategy (EsIAN) from the CNPSS through the Promoters Manual and in the Manual for Acute Diarrheic Diseases, Prevention, Control, and Treatment of the Health Ministry. Therefore it is necessary to look for the way to include zinc in combination with "Vida Suero Oral" to perform a more efficient treatment for diarrhea amongst children under the age of five.

The WHO recommends giving zinc to any malnourished child. UNICEF and the WHO recommends zinc supplementation, 10mg, in children under 6 months of age and 20mg in lactating children older than 6 months old and preschoolers for 10 to 14 days, as a universal treatment for children with diarrhea.

In Mexico 78% of children population under the age of 5 years live in urban areas. Additionally, 50% of all the cases of ADD in 2015 in children under the age of 5 years were reported in the following states: Distrito Federal, Mexico, Guanajuato, Guerrero, Oaxaca, Jalisco, Puebla. A key step to evaluate the effect of zinc supplementation is to conduct a clinical study to measure the effect on ADD in children in Mexico. Therefore, the ideal scenery for this evaluation would be in an infant population that represents the majority of Mexican population, fundamentally constituted by urban or conurbated area inhabitants, where a high population density of the targeted group exists and counts with higher case reports.

Problem Statement

Acute diarrheic disease in our country occupies the fifth place amongst mortality causes in children under the age of 5 and generates 20.8% of consultations in health services and 10% of pediatric hospitalization.

Oral rehydration therapy, adopted by the WHO and UNICEF have been key in the decrease of the mortality rate amongst children under the age of 5 year with acute diarrhea, despite this decrease, acute diarrhea still is one of the main causes of infant mortality in developing countries. The administration of zinc supplements has an important and beneficial effect in the clinical evolution of acute diarrhea, because it reduces so much its severity as its duration.

The strategy has been efficient in malnourished children, but the universalization of this strategy is still controversial in places where there is a low prevalence of malnutrition.

In our country the ADDs are still a public health problem, being amongst the main causes of morbidity and mortality, with an important load to the health systems, so there is the need of implementation of alternate strategies for this condition, nutrition being one of the pillars to strengthen.

Justification

In Mexico there have been strategies placed, such as the administration of ORS and vaccination against rotavirus, which have impacted positively in ADD mortality amongst children. Even though the magnitude of the problem has decreased, acute diarrheic disease continues to be one of the first causes of infant morbidity and mortality, generating an important load to the health systems, being a latent public health problem; therefore there exist the necessity of implementation of alternate strategies to combat the incidence of this condition, being nutrition one of the pillars to strengthen.

Zinc administration during an ADD episode is a relatively simple intervention that may allow the reduction of severity, duration, and relapse. The anti infectious potential of zinc may be of high importance in the actual financial crisis scene of the health systems, and the new and re-emerging infectious diseases.

The Integral Strategy of Nutrition Attention (EsIAN) of the CNPSS through the Manual for Promoters and in the Manual for the Acute Diarrheic Diseases, Prevention, Control and Treatment from the Health Ministry consider zinc supplementations for ADD treatment. Nevertheless, to this day, studies that evaluate the efficacy of zinc administration in Mexican children are scarce and do not count with any national program or strategy of zinc treatment.

We consider that the present proposal will allow us to respond to the need of counting with controlled studies in Mexican children to know the impact of zinc use in the reduction of ADD severity, duration and relapse.

Research questions

Zinc supplement administration (20 mg/day) in acute diarrheic disease decreases the severity and duration of the clinic picture and relapse in children from 6 to 59 months of age, in relation with children from the same age group treated with placebo?

Objectives

General objective

Evaluate the efficacy of oral zinc sulfate administration (20 mg/day for 10 days), during the ADD clinic picture in children from 6 to 59 months of age attended in nine Sentinel Centers of Mexico City.

Primary Objectives

a) Determine zinc serum concentrations in children from 6 to 59 months of age.

b) Compare the effect of 20 mg of zinc oral administration in ADD in the duration of diarrhea, in relation with the group that received placebo.
c) Compare the effect of 20 mg of zinc oral administration in acute diarrheic disease in the number of evacuations during the acute diarrheic disease picture, in relation with the placebo group.

Secondary Objectives

a) Compare the effect of 20 mg zinc oral administration in acute diarrheic disease in the reduction of diarrheic disease recurrence in the subsequent months to the treated clinical picture (from 3 to 6 months), in relation with the placebo group.
b) Correlate the increase in the zinc serum concentration to the oral treatment for 10 days with 20mg of zinc with the duration of ADD picture, the number of total evacuations and relapse rates.

Hypothesis

- The oral administration of 20 mg of zinc will decrease at least 12 hours in average the duration time of ADD duration in children from 6 to 59 months of age in comparison with the placebo group.
- The oral administration of 20 mg of zinc will decrease at least 30% of diarrheic evacuations in children from 6 to 59 months of age in comparison with the placebo group.

Methods

General study design

Randomized clinical study, multicenter, double risk, controlled.

a) Participants: There will be a study done in 516 children, both sexes between 6 to 59 months of age, that are attended in the sentinel centers assigned for the study (258 for each length of the treatment).
b) Intervention duration: 10 days.
c) Inclusion period: 6 months (2017).
d) Average tracking duration to evaluate relapse: 6 months average.
e) Location: 9 Health Units from the Federal and State Ministries.

Table 1. Sentinel Centers

Medical unit
Instituto Nacional de Pediatria
Hospital Pediátrico de Tacubaya
Hospital Pediatrico de Iztapalapa
Centro de Salud Topilejo
Hospital Pediatrico de Coyoacan
Hospital Materno Infantil de Xochimilco

Table 1. Sentinel Centers

Medical unit
Hospital Pediatrico La Villa
Centro de Salud Cultura Maya
Centro de Salud San Andres Totoltepec

Study population

Children of both sexes, that have between 6 to 59 months of age and that attend a consultation for ADD.

Eligible population

Children of both sexes, that are between 6 to 59 months of age and that attend a consultation for ADD in the Sentinel Health Units (see Table 1) between June and December 2017.

Selection Criteria

Inclusion Criteria

Children, both sexes, from 6 to 59 years of age that attend a consultation in the sentinel centers, with a Acute Diarrheic Disease diagnose, that both parents or legal guardian agree to participate in the study and sign the informed consent letter. That parents have not planned moving to other location in a time period of at least a year.

Exclusion Criteria

Children with deficient intestinal absorption Syndrome, Acrodermatitis Enterohepatic, Zinc Sulfate Hypersensitivity, Leucine metabolic Disorders, secondary lactose intolerance, Galactosemia lactase primary deficiency, allergy to cow milk protein, and/or children that are supplemented with zinc for the last 6 months.

Elimination Criteria

Children that present hypersensitivity to zinc supplement, diagnosed subsequently to the intake with deficient intestinal absorption Syndrome, Acrodermatitis Enterotropic, Zin Sulfate hypersensitivity, leucine metabolism disorders, secondary lactose intolerance, Galactosemia lactase primary deficiency, cow milk protein allergy, informed consent withdrawal.

Sampling

Sequential sampling, non probabilistic, until completing the number of required participants.

Sample size

To respond to the objective of reduction of at least 12 hours of acute diarrheic disease evolution with the administration of zinc, a sample of 36 in each group is required (+20%=44), through the calculation of the Student-t sample for independent samples considering $\mu1=2.2$, $\mu2=1.7$, $\sigma=0.85$, $\alpha=0.05$, $\beta=0.2$, 1 tail, according to the report of Jiang CX, et. al.[37]

To respond to the objective of 15% or more reduction in the number of diarrheic evacuations in the group with the administration of zinc, a sample of 25 by group is required (+20%=30), through the calculation of Student-t sample for independent samples considering $\mu 1=9.06$, $\mu 2=10.46$, $\sigma=0.05$, $\alpha=0.05$, $\beta=0.2$, 1 tail, according to the reports of Agarwal, et.al.[38]

Finally to respond to the secondary objective of the reduction of acute diarrheic disease relapse in children that received zinc in their first clinic picture, considering $\alpha=0.05$, $\beta=0.20$, Ratio Exposed/ Non Exposed=1, Percentage of positive non exposed (basal recurrence) = 20%, risk ratio= 0.5 (efficacy), there is a sample required of N=215 by group (+20%=258).

Do to the above, to give a response to the stated objectives, a sample of 258 children in each group is required, considering 20% of loss, meaning a total of 516 children to be included.

Variable definition

VARIABLE (Index/indicator)	Type	OPERATIONAL DEFINITION	MEASUREMNT SCALE	QUALIFICATION
Identification sheet				
ID	Context	Unique alphanumeric indicator constructed with a consecutive number of 4 digits and the initials from de first last name, second last name and first name.	Nominal polytomous	0001AAA a9 999ZZZ
Date of fill	Context	Date in which the case study initiates and fills the Case Report Format.	Discrete	day/MONTH/year
Attention Hospital	Context	Medical attention unit where the case is identified and put in to the study	Nominal	1 to k hospitals
File	Context	Number or alphanumeric expression corresponding to the hospital file for the identification and gathering of medical information from the case.	Nominal	Alphanumeric, n categories individual
Name minor	Context	Complete name of the minor with last name 1, last name 2 and name(s)	Nominal	Open alphanumeric chain; n Names
Sex	Independent	Biological condition of the sex that the minor belongs to	Nominal dichotomous	1. Men 2. Women
Date of birth	Context	Calendar date registered with month, day and year in which the minor was born	Quantitative discrete	dd/MMM/yyyy
Age	Independent	Age of the minor in months and days turned since the date of birth up to the beginning of the case study	Numeric	X months, Y days
Address	Context	Physical address where the minor and the parents or legal guardians can be located, specifying street name, exterior number, interior number, neighborhood or borough or district, zip code, municipality or delegation, federal entity and particular references.	Nominal	Alphanumeric open with n categories

VARIABLE (Index/indicator)	Type	OPERATIONAL DEFINITION	MEASUREMNT SCALE	QUALIFICATION
Address references	Context	Places known that will help as reference for the easy localization of the patients address.	Nominal	Open
Telephones	Context	Telephone numbers in which the minor or parents or legal guardians could be found, corresponding to home telephone, work, cellphones or of family members or acquaintances.	Nominal	Numeric open with n amounts of telephones
Non pathological Personal Background				
Rooms in the house	Context	Number of rooms that the house has where the minor lives, taking into consideration the living room, kitchen and bathrooms.	Quantitative discrete	0-98
People living in the house	Context	Number of people with the minor that live on the house.	Quantitative discrete	1-98
Mother´s level of education	Context	Last academic grade the mother was in, and if it was completed or not.	Ordinal	a. None b. Elementary (complete/incomplete) c. Middle school (complete/incomplete) d. High school (complete/incomplete) e. Technical (complete/incomplete) f. Collage (complete/incomplete) g. Undergraduate (complete/incomplete) h. Unknown.
Fathers level education	Context	Last academic grade the father was in, and if it was completed or not.	Ordinal	a. None b. Elementary (complete/incomplete) c. Middle school (complete/incomplete) d. High school (complete/incomplete) e. Technical (complete/incomplete) f. College (complete/incomplete) g. Undergraduate (complete/incomplete) h. Unknown

VARIABLE (Index/indicator)	Type	OPERATIONAL DEFINITION	MEASUREMNT SCALE	QUALIFICATION
Television	Context	Existence of a television in the house minor lives in.	Nominal polytomous	1. Yes 2. No 99.Does not know
Refrigerator	Context	Existence of a refrigerator in the house minor lives in.	Nominal polytomous	1. Yes 2. No 99.Does not know
Automobile	Context	Existence of a automobile in the house minor lives in.	Nominal polytomous	1. Yes 2. No 99.Does not know
Computer	Context	Existence of a computer in the house minor lives in.	Nominal polytomous	1. Yes 2. No 99.Does not know
Water in the house	Context	Type of water disposition in the house the minor lives in	Nominal polytomous	1. Inside the house 2. Outside the house but in the building, community or land. 3. From a public tap 4. Well 5. Pipe or deposit 6. Not available 7. Other_____ 99.Does not know
Sewage	Context	Type of sewer of available in the house minor lives in.	Nominal polytomous	1. Connected to the sewer in the street 2. Connected to a septic pit 3. Connected to the ground, river or lake 4. No sewage 99.Does not know
Breast fed ever	Independent	At one point in their lives the minor received breast milk feeding.	Nominal polytomous	1.Yes 2.No 99.Does not know
Age maternal lactation	Independent	Age in months the minor was when he finished being breastfed.	Numeric continuous	Months 0-24
Still being breastfed	Independent	At the moment of the interview, the minor was still breastfed.	Nominal polytomous	1.Yes 2.No 99.Does not know
Actual feeding	Independent	Predominant diet with which the minor has been fed at the moment of the inclusion. .	Nominal polytomous	1. Only maternal breast 2. Dairy formula exclusive 3. Mix with predominant maternal breast 4. Mix with predominant dairy formula 5. Mix with predominant solids 99.Does not know

VARIABLE (Index/indicator)	Type	OPERATIONAL DEFINITION	MEASUREMNT SCALE	QUALIFICATION
Attends daycare currently	Control	If the child attends daycare regularly	Nominal dichotomous	1.Yes 2.No
Time attending daycare	Control	The time since the child attended the daycare for the first time up to the last day he attended daycare.	Numeric	X months, Y days
Immunization backgrounds				
National Health Record	Context	If there was a copy of minor´s National Health Record attached to the Case Report Form	Nominal	1=Yes 2=No
Dosage 1 rotavirus vaccine	Independent	If the first dosage of the rotavirus vaccine was applied.	Nominal	1=Yes 2=No 99=Does not know
Vaccine type dosage 1	Independent	Biological type of the rotavirus received at the moment of the first dosage of their immunization scheme	Nominal	1=RotaTeq (RV5) 2=Rotarix (RV1) 99=Does not know
Date of dosage 1	Independent	Day, month, year in which the patient received their first rotavirus vaccine dosage.	Numeric	n dates with day/MONTH/year
Dosage 1 source	Context	Source where it was obtained the date of application and the type of vaccine of rotavirus of dosage 1.	Nominal	1=National Health Record 2=Temporal immunization proof 3=Verbal 4=Nominal census (immunization unit)
Immunization unit dosage 1	Context	Health services establishments where the first dosage of the rotavirus vaccine was administered.	Nominal	Up to n immunization units
Dosage 2 rotavirus vaccine	Independent	If dosage 2 of the rotavirus vaccine was applied	Nominal	1=Yes 2=No 99=Does not know
Type of vaccine dosage 2	Independent	Biological type of the rotavirus received at the moment of the second dosage of their immunization scheme	Nominal	1=RotaTeq (RV5) 2=Rotarix (RV1) 99=Does not know
Daye dosage 2	Independent	Day, month, year in which the patient received their second rotavirus vaccine dosage.	Numeric	n dates con day/MONTH/year
Dosage 2 source	Context	Source where it was obtained the date of application and the type of vaccine of rotavirus of dosage 2	Nominal	1=National Health Record 2=Temporal immunization proof 3=Verbal 4=Nominal census (immunization unit)
Immunization unit dosage 2	Context	Health services establishments where the second dosage of the rotavirus vaccine was administered.	Nominal	Up to n immunization units

VARIABLE (Index/indicator)	Type	OPERATIONAL DEFINITION	MEASUREMNT SCALE	QUALIFICATION
Dosage 3 rotavirus vaccine	Independent	If dosage 3 of the rotavirus vaccine was applied	Nominal	1=Yes 2=No 99=Does not know
Type of vaccine dosage 3	Independent	Biological type of the rotavirus received at the moment of the third dosage of their immunization scheme	Nominal	1=RotaTeq (RV5) 99=does not know
Date dosage 3	Independent	Day, month, year in which the patient received their third rotavirus vaccine dosage	Numeric	n dates with day/MONTH/year
Source dosage 3	Context	Source where it was obtained the date of application and the type of vaccine of rotavirus of dosage 3	Nominal	1=National Health Record 2=Temporal Immunization proof 3=Verbal 4=Nominal census (Immunization unit)
Immunization unit dosage 3	Context	Health services establishments where the third dosage of the rotavirus vaccine was administered.	Nominal	Up to n immunization units
Vaccine observations	Context	If there exist observations about the immunization state that up to the doctors judgment must be registered in the case study.	Nominal	Alphanumeric Open
Personal Pathological backgrounds				
Health problem	Control	If the minor´s caregiver refers to a health problem.	Nominal dichotomous	1. Yes 2. No 99.Does not know
Specified health problem	Control	Detailed description in case there is a health problem	Nominal	Open
Hospitalizations background due to diarrhea	Control	Hospitalization appearance due to gastroenteritis in their lives without taking into accounts the actual, and the age of that event.	Nominal	1.Yes 2.No 99.Does not know
Age at the moment of the previous hospitalization due to diarrhea	Control	In case of having hospitalizations background due to diarrhea, indicate the age at the moment that happened.	Numeric	X months
Hospitalization background due to pneumonia.	Control	Hospitalization appearance due to pneumonia through their lives without counting the actual, and the age in which the event happened.	Nominal	1.Yes 2.No 99.Does not know

VARIABLE (Index/indicator)	Type	OPERATIONAL DEFINITION	MEASUREMNT SCALE	QUALIFICATION
Age at the moment of the previous hospitalization due to pneumonia	Control	In case of having any backgrounds of hospitalization due to pneumonia, indicate the age at the moment of the event.	Numeric	X months
Diarrheic disease pictures	Control	Number of acute diarrheic disease pictures that they have presented in the last year.	Quantitative discrete	1 It is the first episode 2 two to three pictures 3 four to five 4 more than five 99 Does not know
Acute respiratory infections pictures	Control	Number of acute respiratory infections pictures that they have presented in the last year.	Quantitative discrete	1 It is the first episode 2 two to three pictures 3 four to five 4 more than five 99 Does not know
Actual Condition				
Beginning of the diarrheic picture	Control	Date in which the clinical picture with diarrhea initiated.	Quantitative Discrete	dd / mmm/yyy
Time of the beginning of the diarrheic picture	Control	Time of the day in which the clinical picture with diarrhea started.	Quantitative Discrete	Hh:mm
Days with diarrhea	Control	Quantity of days with diarrhea, from the beginning of the episode to the medical attention.	Quantitative discrete	Number of days 1 to X
Number evacuations/ day	Control	Maximum amount of evacuations decreased in consistency by day	Quantitative discrete	1 to X
Consistency of evacuations	Control	Characteristics quality required in terms of firmness and stool complement.	Nominal polytomous	1 Pasty 2 Semi liquid 3 Liquid
Characteristics of the evacuations	Control	Presence of mucus or blood in the stool.	Nominal polytomous	1 No 2 Only mucus 3 Only Blood 4 Mucus and blood
Vomit	Control	Presence of vomit in the gastroenteritis episode.	Nominal dichotomous	1.Yes 2.No
Number of days with vomit	Control	Quantity of days with vomit, from the beginning of the episode up to the medical attention.	Quantitative discrete	1 to X
Number of vomits	Control	Maximum amount of vomit events a day	Quantitative discrete	1 to X
Temperature	Control	Maximum body temperature registered from the minor during the diarrheic episode.	Continuous	35.0ºC to 42.0ºC

VARIABLE (Index/indicator)	Type	OPERATIONAL DEFINITION	MEASUREMNT SCALE	QUALIFICATION
Place the temperature was taken.	Control	Anatomical place in which the thermometer was placed for the body temperature measurement.	Nominal polytomous	1 Rectal 2 Oral 3 Armpit 4 Otaca 5 Frontal
Infections or concomitant diseases	Control	Presence of concurrent diseases with the actual condition.	Nominal dichotomous	1.Yes 2.No
Observations actual condition	Context	Additional optional descriptive notes to the actual episode of intestinal intussusception.	Nominal	Open alphanumeric chain
Physical exploration at the moment of the initial assessment				
Weight	Control	Corporal weight of the minor in kilograms determined at the moment of the interview.	Continuous	Kilograms
Height	Control	Height of the minor in centimeters determined at the moment of the interview.	Continuous	Centimeters
General condition	Control	Clinical manifestation that the subject presents in relation with their alert state.	Qualitative nominal	1.Alert, active, smiling o crying, but easily comforted. 2.Thirsty, restless or lethargic but irritable when touched. 3.Sleepy, hypotonic, cold or sweaty or comatose. 99. Does not know.
Eyes	Control	Presence or absence of sunken eyes secondary to the dehydration caused by diarrhea.	Nominal polytomous	1.No changes in the usual appearance of the eyes (normal ocular tone) 2.Lightly sunken 3.Very sunken 99 Does not know
Oral mucosa	Control	Level of dehydration of the oral and tongue mucosa, excluding lips.	Nominal polytomous	1.Humid 2.Stiky, with viscose saliva 3.Dry 99. Does not know
Tears	Control	Presence of tears during crying of the minors an indicator of dehydrated state.	Nominal polynomial	1.Present 2.Scarce 3.Abstent 99.Does not know
Treatment				
Area of first contact	Control	Area of the hospital where the minor received the first medical attention for this episode od diarrhea.	Nominal polytomous	1.Doctors office 2.Urgent care

VARIABLE (Index/indicator)	Type	OPERATIONAL DEFINITION	MEASUREMNT SCALE	QUALIFICATION
Hydration plan	Context	Type of hydration plan according to the WHO that was administered to the minor on account of the actual gastroenteritis episode.	Nominal	1.Plan A 2. Plan B 3. Plan C 4.Other_____
Hydration Treatment	Control	The action of receiving a medical treatment consisting on the reposition of the liquids lost during the episodes of diarrhea via enteral before going to medical attention.	Nominal polynomial	1.Ambulatory with oral hydration 2.Urgent care with oral hydration 3.Urgent care with IV hydration 4. Hospitalized
Antibiotic treatment	Control	Presence or absence of the antibiotic prescription in their current condition.	Nominal dichotomous	1 Yes 2 No
Antibiotic name	Control	Type of antibiotic that was prescribed for their current condition.	Nominal	Alphanumeric
Antibiotic dosage	Control	Antibiotic dosage prescribed	Nominal	Alphanumeric
Days of treatment	Control	Number of days the antibiotic was prescribed.	Quantitative discrete	1 to X
Antiemetic treatment	Control	Presence or absence of antiemetic prescription in their current condition.	Nominal dichotomous	1 Yes 2 No
Name of antiemetic	Control	Type of antiemetic that was prescribed for their current condition.	Nominal	Alphanumeric
Antiemetic dosage	Control	Dosage of antiemetic that was prescribed.	Nominal	Alphanumeric
Days of antiemetic	Control	Number of days that the antiemetic was prescribed.	Quantitative discrete	1 to X
Antidiarrheal treatment	Control	Presence or absence of the antidiarrheal prescription in their current condition.	Nominal dichotomous	1 Yes 2 No
Antidiarrheal name	Control	Type of antidiarrheal that was prescribed for their current condition.	Nominal	Alphanumeric
Antidiarrheal dosage	Control	Dosage of the antidiarrheal that was prescribed.	Nominal	Alphanumeric
Days with antidiarrheal	Control	Number of days that the antidiarrheal was prescribed.	Quantitative discrete	1 to X
Other treatment	Control	Presence or absence of the prescription of other medications for their actual condition.	Nominal dichotomous	1 Yes 2 No
Name of lactobacilli	Control	Type of lactobacilli that was prescribed for their current condition.	Nominal	Alphanumeric

VARIABLE (Index/indicator)	Type	OPERATIONAL DEFINITION	MEASUREMNT SCALE	QUALIFICATION
Dosage of lactobacilli	Control	Dosage of lactobacilli that was prescribed	Nominal	Alphanumeric
Days with lactobacilli	Control	Number of days that the lactobacilli was prescribed.	Quantitative discrete	1 to X
Name of the other medication	Control	Type of other medication that was prescribed for their current condition.	Nominal	Alphanumeric
Dosage of the other medication	Control	Dosage of the other medication that was prescribed.	Nominal	Alphanumeric
Days with the other medication	Control	Number of days that the other medication was prescribed.	Quantitative discrete	1 to X
Sample of stool for the laboratory				
Collection of the stool sample	Control	Collection of the stool sample from the current episode was achieved.	Nominal	1.Yes 2. No 99.Does not know
Date sample of stool	Control	Specification of a day in the calendar, in which the fecal matter was taken.	Quantitative continuous	Day-month-year
Hour of recollection of the stool sample	Control	Hour of the day in which the recollection of stool samples initiated.	Quantitative continuous	Hour: min _AM/PM
Collection of blood sample	Control	The collection of the blood sample was achieved. .	Nominal	3.Yes 4. No 99.Does not know
Date blood sample	Control	Specification of a day in the calendar, in which the blood sample was taken.	Quantitative Continuous	Day-month-year
Hour of recollection of blood sample	Control	Hour of the day in which the recollection of blood sample started.	Quantitative continuous	Hour: min _AM/PM
Observation	Context	If there exists observations about the sample collection that in the physician´s judgment must be registered in the case study.	Nominal	Alphanumeric open
Responsible physician	Context	Name of the researcher physician responsible of filling the form of the hospital´s Case Report.	Nominal	Alphanumeric open
Tracking				
Date filled	Context	Date in which the case study initiated and the tracking Form is filled.	Discrete	Day/MONTH/year

VARIABLE (Index/indicator)	Type	OPERATIONAL DEFINITION	MEASUREMNT SCALE	QUALIFICATION
Type of tracking	Context	Manner in which the parents were contacted to perform the tracking.	Nominal	Telephone Domiciliary Inpatient Ambulatory in Sentinel Center, Other.
Informant	Context	Name of the person that provided the information of the minor.	Nominal	Alphanumeric
Relation with the minor	Context	Relation of the informant with the minor.	Nominal	Alphanumeric
ADD clinic picture evolution	Control	ADD clinic picture evolution.	Nominal	1 Has gotten worse 2 No improvement, persist the same symptomatology 3 Has improved 4 The picture has remitted totally
Medical assessment	Control	Specify the minor needed new medical assessment.	Nominal	1 yes, in urgent care 2 yes, in doctor´s office 3 no
Nausea	Control	Presence or absence of nausea.	Nominal dichotomous	1 yes 2 no
Fever	Control	Presence or absence of fever.	Nominal dichotomous	1 yes 2 no
General condition	Control	Clinical manifestation that the subject presents in relation with their alert state.	Qualitative nominal	1.Alert, active, smiling or crying, but is easily comforted 2.Thirsty, restless or lethargic but irritable when touched 3. Drowsy, hypotonic, cold or sweaty or comatose 99. Does not know
Oral tolerance	Control	Acceptance of the ingest of food and liquids.	Nominal	No Yes, only liquids Yes, liquids and solids
Tablet acceptance	Control	Ease for the minor to receive diluted tablet.	Nominal	Ingested with no problem Had difficulty, specify
Administration of the tablet from the study	Control	Administration of the tablet from the study (zinc or placebo).	Nominal	Yes No
Medication	Control	Medications that have been administered in the last 24 hours.	Nominal	Alphanumeric

VARIABLE (Index/indicator)	Type	OPERATIONAL DEFINITION	MEASUREMNT SCALE	QUALIFICATION
Home remedies	Control	The presence or absence of home remedies administration during the current condition.	Nominal	Yes No
Number evacuations/ day	Dependent	Maximum amount of evacuations decreased in consistency per day.	Quantitative discrete	1 to X
Consistency of evacuations	Dependent	Characteristics quality required in terms of firmness and stool complement.	Nominal polytomous	1 Pasty 2 Semiliquid 3 Liquid
Quantity of evacuations	Dependent	Quantity of evacuations, in relation to the surface that covers in the diaper.	Nominal polytomous	1 Usual 2 Scarce 3 Abundant
Vomit	Control	Presence of vomit in the gastroenteritis episode.	Nominal dichotomous	1.Yes 2.No
Number of days with vomit	Control	Quantity of days with vomit, from the start of the episode up to the medical attention.	Quantitative discrete	1 to X
Abdominal pain	Control	Presence of abdominal pain	Nominal	Yes, no
Laboratory results				
Enteropathogen	Control	Isolation od enteropathogens in stools.	Nominal	Alphanumeric open
Levels of basal Zinc	Independent	Concentrations of zinc in blood/serum at the beginning of study.	Quantitative continuous	0 to X μg/dL
Time tracking the sample	Control or independent	Time in days since the beginning of symptoms/start of the treatment/clinical cure/end of the supplementation? In which a sample of blood is taken for zinc level determination.	Discrete	Hours
Levels of zinc tracking	Dependent	Blood concentration/ zinc serum in μg/dl in a time X determined.	Quantitative Continuous	0 to X μg/dL
Basal albumin	Control	Serum concentrations of albumin in blood at the beginning of the study.	Quantitative continuous	0 to X gr/dL
Hemoglobin	Control	Hemoglobin concentration in blood at the beginning of the study.	Quantitative continua	0 to X g/dL

Analysis Plan

The analysis of the results will be by protocol and by intention to treat. Descriptive statistic will be used, with measurements of central tendency and dispersion of the numerical data according to the distribution of them. Continuous variables with normal distribution: mean and standard deviation. Continuous variables with non-normal distribution: median and inter quartile interval. The categorical data will be described with simple frequency, proportions and ratios.

The comparison of evolution time of ADD and the number of evacuations will be done through the Student t-test for independent groups in case the distribution was normal, or with Mann-Whitney U test if the distribution was non-normal.

In case there are non-homogenous factors between groups and that they may be potential confounders of the duration of the diarrhea and number of evacuations, there will be included in an analysis model of two-tailed variance and/or in a multiple lineal regression model in case there is more than one potential confounder. The potential confounders taking data from are:

a) Attention Hospital
b) Socioeconomic level
c) Lactation
d) Childcare
e) Vaccine
f) Previous hospitalizations
g) Temperature-fever
h) Dysentery
i) Malnourished
j) Antibiotic
k) Albumin
l) Hemoglobin

In case the distribution allows it, a stratified analysis for this variables would be perform.

For the evaluation of relapse risk the Attributable Risk Fraction, the Reduction of the Fraction of Attributable Risk and Absolute Risk Reduction would be estimated with the next formulas:

$$\text{Reduction of the fraction of attributable risk:} = \frac{\text{ADD relapse in control group} - \text{ADD relapse in zinc group}}{\text{ADD relapse in control group}}$$

$$\text{Absolute risk reduction:} = \text{ADD incidence in control group} - \text{ADD incidence in zinc group}$$

The efficacy of the intervention over the duration of the disease and the number of evacuations will be modeled with a general lineal model to adjust by the basal characteristics. A $p<0.05$ would be considered significant.

The efficacy estimators in case the treatment groups are not comparable for any non-homogenous factor in the randomization and/or associated with relapse, will be adjusted by logistic regression model considering the following potential confounders:

a) Age
b) Socioeconomic level
c) Lactation
d) Childcare
e) Vaccine
f) Previous hospitalization
g) Diarrhea severity

The correlation between the increment in the zinc serum concentration posterior to the oral treatment for 10 days with 20 mg of zinc with the duration of the ADD picture, the number of total evacuations and the relapse rate, will be done as follows:

Variable	Test
Zn concentrations and Duration	Simple Correlation (parametric or non-parametric)
Zn concentrations and number of evacuations	Simple Correlation (Parametric or non-parametric)
Zn concentrations and relapse	Student t or Mann-Whitney U

Specific process of the study

Study stages and data recollection.

Recruitment

The recruitment and tracking of children will be done in the Sentinel Centers assigned to the study. To all the children from 6 to 54 months of age that attend for acute diarrheic disease and comply with the selection criteria will be invited to participate in the project. In this first intervention there will be an explanation given, of the project and how it will be done (reason why the study pretends to be done, objective, inclusion criteria, exclusion, phases of the study and anthropometric measurements, dietetics and biochemical as well as the implementation of the socioeconomic questionnaire, distribution (randomization) and supplement consumption). It will try to clarify every doubt that the mothers present. Once the guardians or parents of the child accept to participate the intervention will be assigned randomly and the case study form will be filled.

Assignment of intervention

The assignment of treatment will be done under the randomization scheme in balanced blocks. The children will be assigned randomly to one od 2 treatment groups: one group will receive an oral dosage daily of 20 mg for 10 days and the other group will receive placebo in a similar way.

Baseline Visit

In each one of the health centers the children will be anthropometrically measured by weight, size, height, and arm and head circumference. A trained physician in the matter will perform these measurements. Also will obtain a sample of 5 mL of venous blood, with the purpose of determining afterwards the concentrations of zinc serum, hemoglobin and albumin. A Pediatric Nurse with a wide experience in sample taking from children will obtain the blood sample, and the corresponding registration will be done. There will also be a questionnaire applied about the characteristics of the house as well as the age, education, marital status, and number of domestic appliances like radio, television, blender, etc. In the same appointment, the children will be assigned randomly to one of the groups for treatment. A sample of stools will be taken from each child within 48 hours after being included. Trained and standardized staff will do all the interviews and measurements.

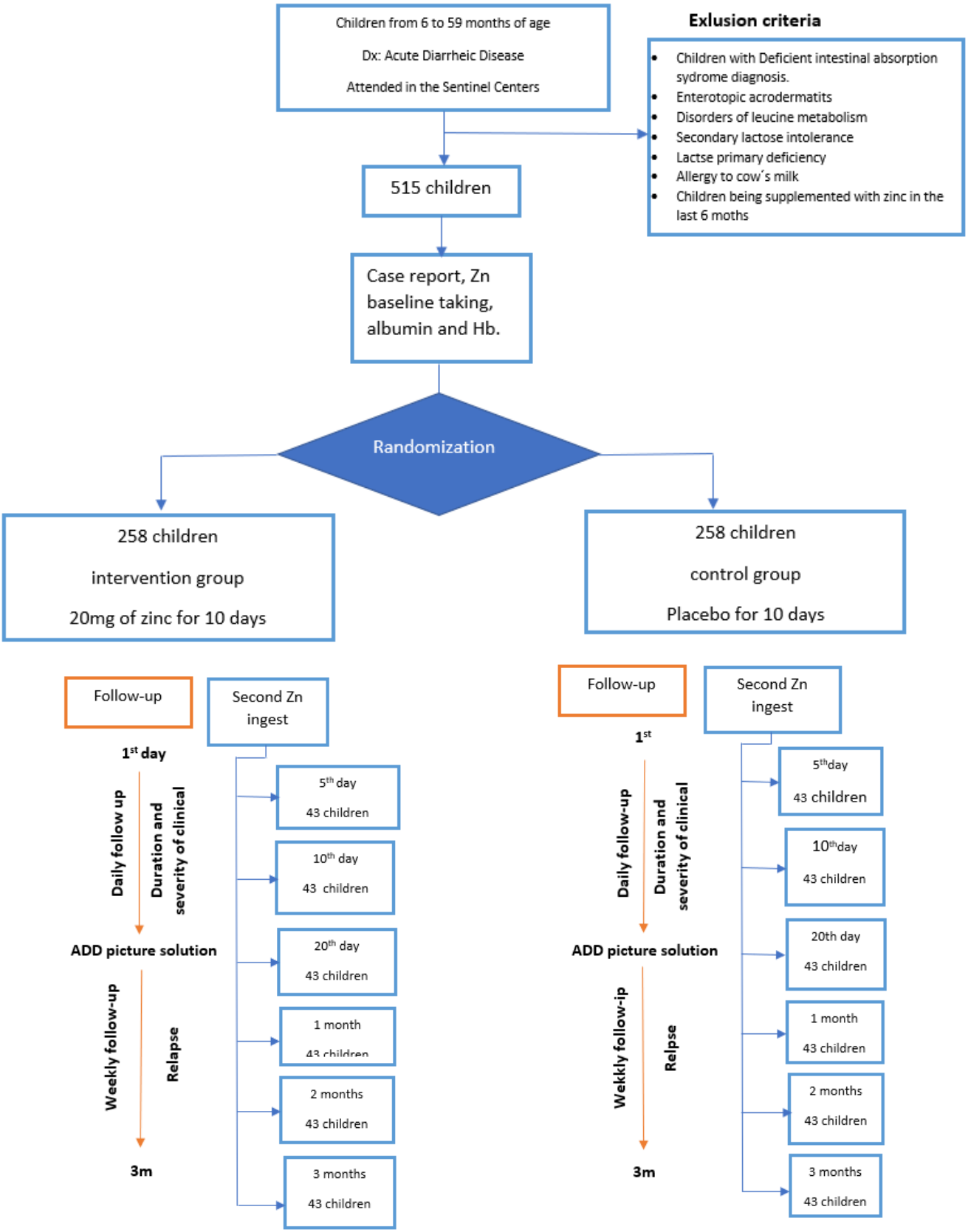
Follow-up

A daily telephone follow-up will be done for the ambulatory patients until the resolution of the episode, this is to know the number of evacuations, consistency, and administered treatment and strengthen the intervention. For the follow-up of relapses telephone calls will be done weekly until completing a maximum of twelve months after the inclusion episode. There will be two itinerant groups for domiciliary follow-ups for the failed telephone calls. Furthermore, the caretaker will be provided with a symptoms journal, which they will fill out daily during the time of the study.

A sample of 3 mL of blood will be taken from each child, to determine the control zinc levels. The total of participants will be randomized in 6 groups, in which a sample of blood will be taken from them, in different moments in the following scheme:

Group	1	2	3	4	5	6
Time when the control sample was taken, After starting the treatment.	5 days	10 days	20 days	1 month	2 months	3 months

Flowchart


Children from 6 to 59 months of age
Dx: Acute Diarrheic Disease
Attended in the Sentinel Centers
Exlusion criteria
Children with Deficient intestinal absorption sydrome diagnosis.
Enterotopic acrodermatits
Disorders of leucine metabolism
Secondary lactose intolerance
Lactse primary deficiency
Allergy to cow´s milk
Children being supplemented with zinc in the last 6 moths
515 children
Case report, Zn baseline taking, albumin and Hb.
Randomization
258 children
intervention group
20mg of zinc for 10 days
258 children
control group
Placebo for 10 days
Follow-up
Second Zn ingest
1st day
Daily follow up
Duration and severity of clinical
ADD picture solution
Weekly follow-up
Relapse
3m
5th day
43 children
10th day
43 children
20th day
43 children
1 month
43 children
2 months
43 children
3 months
43 children
Follow-up
Second Zn ingest
1st
Daily follow-up
Duration and severity of clinical
ADD picture solution
Wekkly follow-ip
Relpse
3m
5thday
43 children
10thday
43 children
20th day
43 children
1 month
43 children
2 months
43 children
3 months
43 children

Ethical considerations

Consent

To the parents or guardians, or to who exercise the parental responsibility of each potential participant, information related to the study will be provided in verbal and written form. The physician that will serve as Local Researcher in each Sentinel Center will conduct an initial invitation to the parents and/or guardians of the minor, for the offspring to participate in the study and will read the "Informed Consent Letter" to expose the purpose of the investigation and will do a description of the same, pointing out that the study will consist of a 15 to 20 minutes interview, in taking a blood sample from the minor, and the random allocation to one of the study groups. Children will receive the treatment that in the physician´s judgment will be more adequate for their disease, age, and clinical state. The intervention group will receive supplementation and placebo control, during 10 days. The signature of the parents or guardians of the minor should be obtained in a voluntary and free manner in the Informed Consent Declaration after assuring that the research objective has been comprehended and doubts have been cleared, and before the child is included in the study, if they wish that the minor participates. If any parent or guardian does not know how to sign, they will print their fingerprints, and another person designated by them will sign on their name. The Informed Consent Letter will be handed in duplicate, a copy being for the medical record of the minor in the study, and the other one for the parents or guardians. Do to the fact that the participants are lactating and to the fact that they are not directly related to the process of information collection, an assent request is not appropriate. The people responsible for the minor can reject the minor´s participation in the study. If the collaborating researcher is a physician treating a candidate to be included, the informed consent must be taken and handed by a third party, this way the investigator can still be their treating physician and perform the fill out of the "Case Report Form (CRF)". The foregoing in accordance with the legislation and the established recommendations regarding ethics.

Confidentiality

With the final purpose of guaranteeing confidentiality and participant´s privacy, the investigation documents, as well as the informed consent that contain the names of the participants and their parents or caretakers will be kept in drawers locked with key, to which only authorized personnel will have access to. More so, the electronic data will be stored in protected databases with a code or password, to which only authorized personnel will have access. The information of the minor recollected in this study will only be used to the same end.

Risk

The study is classified with major and minimum risk. The fact of receiving placebo is acceptable because the zinc supplement is not part of the usual diarrhea handling in Mexico, and in case that they get zinc does not represent a risk either because of the dosage that is going to be administered, which has been recommended and has proven to be safe in other countries. Adverse effects have not been described for this amount of zinc. The amount of blood that is

going to be taken from the minor is small, and will be only in two occasions in a year, the discomforts that they could present are pain at the moment of puncture or a small bruise that will disappear in a few days. The stool sample will be directly recollected from the diaper, so their child will not undergo an invasive diagnostic procedure.
The treatment or any attention provided will be standard or usual, so they will not suffer any damage directly related to the investigation.

Benefits

There is no immediate benefit for the patient, and there is no payment or financial compensation for the minor´s participation in the study. The parents or guardians of the participating minor may have access to the information collected from their child for the study, if needed. After completing the study, it will not be necessary to inform the results to the participants, but any relevant findings related to the health of any particular participant, must be informed to the parents or guardians of the same.

Conflict of interests

There is no conflict of interest involving researchers. The design, logistics, case and laboratory studies, analysis, report writing and publication of results will be in charge of the researchers.

Biosecurity

All personnel involve in the study must adopt the preventive measures for their protection during the collection, storing, transport and handling of the blood samples; taking into consideration the requirements set forth in the general provisions applicable in the matter, in particular the Mexican Official Norm for environmental Protection - environmental Health – biological-infectious dangerous waste - Handling Classification and Specifications, NOM-087-ECOL-SSA1-2002. The personnel involved in the study must know that the blood samples collected are potentially infectious.
Inappropriate manipulation of the samples may become a biological risk source for the people that are in contact with them or for the environment; therefore, it will be important to use the necessary personal protection elements to avoid exposure with biological risk, such as:

- Ocular protection: glasses or visor mask for the laboratory processing.
- Gloves (do not substitutes the regular and appropriate hands hygiene), for the collection and handling in the laboratory
- Coat, in the collection and handling in the laboratory.

Sample collection must be performed with the necessary personal protection, in a safe place, avoiding surface pollution. As part of the safety measures, all the samples must be tagged with the following basic data:

1. Patients ID.
2. Sample type that in every case will be fecal matter.
3. Hour and time of collection.
4. Initials of the person collecting the sample.

The time from the sampling to the shipment of the local laboratory for its preparation should be as short as possible.

Feasibility

Sentinel centers considered for the study have been selected based on the installed capacity to perform the study. In addition to the fact that the population of children under the age of 5 years is not so geographically dispersed and easy access to these sites, will ensure adequate monitoring of the study.

The sample analysis will be carried out by the laboratory that counts with the standardized test and with strict quality control for the determination of blood analytes.

The research team counts with a wide experience in coordination and tracking of research studies.

The study counts with a specific funding from UNICEF to accomplish the stated objectives.

Expected results

The efficacy evaluation of zinc administration over the duration of the episode and number of evacuations in children from 6 to 59 months of age with ADD, will allow to know the usefulness of such intervention in the treatment of one of the main causes of morbidity and mortality in infant population in our country. A scientific publication will be generated in the indexed international magazine, and presentation in conferences and congresses.

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