# Therapeutic Evaluation of Zinc and Copper Supplementation in Acute Diarrhea in Children: Double Blind Randomized Trial

**Archana B. Patel, *Leena A. Dhande and *Manwar S. Rawat**

*From the Clinical Epidemiology Unit, Indira Gandhi Medical College, Nagpur 440 018 and Department of Pediatrics*, Government Medical College, Nagpur, India.*

*Correspondence to: Dr.Archana B.Patel, 125, Raj Nagar, Katol Road, Nagpur 440 013, Maharashtra, India.*
*E-mail: archana_patel@vsnl.com*

***Objective:*** *To test the hypothesis that daily supplementation of zinc and copper mixed with the oral rehydration solution(ORS) reduces the duration and the severity of acute diarrhea in children.* ***Methods:*** *In a randomized, double blind, placebo controlled trial children aged 6 months to 59 months in an urban hospital with acute diarrhea, were assigned to receive the intervention of once daily 40 mg of zinc sulfate and 5 mg of copper sulfate dissolved in a liter of standard ORS (n = 102) or placebo (50 mg of standard ORS powder) dissolved in a liter of ORS (n = 98).* ***Results:*** *The baseline characteristics in the two groups were similar. The mean survival time(days)(SE) with diarrhea was not significantly different in the treatment(4.34 ( 0.2) as compared to the placebo group(4.48(0.2), nor was there any difference in the median time to cure. Cure was less likely with longer duration of diarrhea prior to enrollment (P <0.001), if the time taken for rehydration was more (P = 0.001) and if intravenous fluids were used (P = 0.03) regardless of the micronutrient supplementation. The proportion of children with diarrhea >4 days was 46% in the placebo group with an adjusted odds ratio (OR)(95%CI) of 1.19(1.58, 0.9; P = 0.2) as compared to 39% in the supplemented group. The most important risk factor for diarrhea > 4 days was diarrheal duration prior to enrolment with OR = 6.25(3.7, 11.1). The supplemented group however had less severity of diarrhea with a lower proportion of children requiring unscheduled intravenous fluids (OR = 0.4; 95 % CI 0.05, 2.2), with weight loss (OR = 0.7; 95% CI; 0.4, 1.3), with complications (OR = 0.15; 0.01, 1.3) and had no deaths as compared to two in the placebo group.* ***Conclusions:*** *This study showed that the most important predictor for duration of diarrhea in children was the severity of the disease at enrollment, and, not the supplementation. There were clinical beneficial effects of supplementation on rate of any complications and mortality. A larger trial is warranted before supplementation of micronutrients mixed with ORS are recommended for management of acute diarrhea.*

**Key words:** *Acute Diarrhea, Copper, ORS, Zinc.*

**DIARRHEA** remains a major cause of morbidity and mortality in developing countries and is often the commonest cause of death in the first few years of life accounting for 10-20% of childhood deaths(1). Significant proportion of children who suffer from diarrhea are malnourished with depleted micronutrient stores. Diarrhea also leads to excess loss of micronutrients such as zinc and copper. Therefore children with marginal nutritional status are also at significant risk of developing zinc and copper depletion with an episode of diarrhea.

Zinc has a direct effect on intestinal villus, brush border disaccharidase activity and intestinal transport of water and electrolytes(2,3). Zinc also has a marked effect on T cell function and its supplementation improves immunity. Thus it may also reduce the severity of diarrhea(4). Copper is present in cytosolic erythrocyte superoxide dismutase (Cu, Zn-SOD) which is an important

scavenger of $O_2^-$, a free radical that causes damage to membranes and biological structures. A clear association between a history of diarrheal episodes and low plasma copper and zinc levels has been demonstrated, with copper deficit being more prevalent than zinc deficit(5).

The therapeutic effects of zinc supplementation in acute diarrhea have been demonstrated in a community-based randomized controlled trial in India and in a smaller hospital based study in Bangladesh(6,7). However, even pharmacological doses of zinc can interfere with the absorption of copper(8). Little is known about the role of copper in limiting the duration and severity of diarrhea. There are no studies on the effects of zinc with copper supplementation on acute diarrhea in children. One of the methods of supplementation of trace minerals is to incorporate it in the oral rehydration solution which is used to correct the negative electrolyte balance in a child of diarrhea in the hope that it will also correct the negative balance of trace minerals. Therefore we conducted a randomized, double blind, placebo controlled trial to test the hypothesis that daily supplementation of zinc and copper in the oral rehydration solution (ORS) shortens the duration and reduces the severity of acute diarrhea in children. Economic analysis of this intervention was the secondary outcome in this trial.

## Subjects and Methods

### *Study area and population enrollment*

The study was conducted at the Nagpur city's Government Medical College and Hospital, India, after approval by the human research ethical committee of the Government Medical College, Nagpur. In this trial children aged 6 months to 59 months who presented to the hospital with more than three unformed stools in 24 hours and diarrheal duration of <7 days were eligible after written informed consent. Patients with no signs of dehydration were also monitored in diarrheal treatment unit. A child could be enrolled only once and any child with severe malnutrition (kwashiorkar and marasmus), intractable vomiting, pre-renal or renal failure, respiratory distress, altered sensorium or any such co-morbid condition that precludes the use of oral rehydration solution(ORS) was excluded from the trial.

### *Baseline assessment*

The following base line data was collected: diarrheal duration, character of the stool (watery, mucoid or bloody), degree of dehydration (no, some or severe according to standard World Health Organization guidelines), age, gender, maternal education, number of children in the family, monthly parental income, diet of the child (mostly breast-fed, partially breast fed, formula or cows milk, mixed feeding or on family diet), immunization status (up to date, incomplete or not immunized according the Universal Immunization Program in India), history of fever or vomiting, prior use of ORS, prior use of medications and the nutritional status. The weight and height were measured on the UNICEF Detecto Scale by a trained person. In children under two years the length was measured on an infantometer in the supine position. Wasting was defined as having weight for height z-scores ≤2 (on gender-based percentile charts of Indian children from the All India Institute of Medical Sciences, New Delhi). All the information was recorded on a pre-designed and pre-tested form.

### *Randomization and Blinding*

The children were allocated to treatment groups following a preliminary clinical assessment to determine if they had any exclusion criteria. Children who had severe

dehydration (as per standard national guidelines for diarrheal management) or inability to drink were temporarily excluded for 4 hours during which they received standard treatment. At the end of this time period they were reassessed for possible inclusion. The treatment was randomized at an individual level using a fixed randomization scheme with equal allocation of patients to the intervention and placebo group. A computer based psuedo-random number generator was used with randomization schedules in permuted blocks of eight. The child, their guardians and the care givers were blinded to the subject's treatment status which was assigned by the research assistant. The outcomes were recorded in the case report forms by the caregivers. The study codes was available to the investigators only at the end of the data collection and analysis.

*Interventions*

Two identical coded waterproof sachets of the intervention or the placebo were dissolved in the ORS by the research assistant and then provided to the nurse for administration to the patients in the treatment and the placebo group, only once in a day. The treatment sachet contained 40 mg of Zinc sulphate and 5 mg of Copper sulphate. The placebo sachet contained 50 mg of standard ORS powder. Each day a fresh solution was prepared till the diarrheal episode lasted. There was no difference in color or the taste of ORS in the two groups. The children were encouraged to take their routine feeds. Patients were also provided other usual supportive care with antipyretics and antibiotics for bloody diarrhea. If a child was dehydrated after 6 hours of oral rehydration or if signs of severe dehydration appeared despite appropriate ORS administration then they were administered intravenous fluids and this was recorded as an "unscheduled intravenous fluid".

*Follow-up Data Collection*

The children were assessed at the same time every 24 hours till discharge. The time taken to rehydrate the child from time of admission, episodes of vomiting, amount of ORS consumed, use of intravenous fluids during rehydration and the use of unscheduled intravenous fluids during the maintenance of hydration was measured daily. We measured the number of children who disliked the taste of the oral rehydration fluid. Any complications such as pre-renal or renal failure, convulsions, electrolyte imbalance, bronchopneumonia and septicemia were recorded. The use of other medication such as antibiotics was also recorded. Weight was recorded on admission and at discharge. A child was discontinued from the study if the child experienced any of the above complications, died or if the parent withdrew consent.

*Outcomes Variables*

The primary outcome was the duration of diarrhea from the time of onset. A diarrheal day was defined as a 24-h period with passage of at least four unformed stools and this episode was considered terminated on the last day of diarrhea followed by a 24-h diarrheal free period. The number and proportion of patients with diarrhea >4 days and the mean length of hospital stay was also estimated. The proportion of children with diarrhea > 4 days was estimated based on the results of the Indian community based study of zinc supplementation which indicated that the reduction in the duration of diarrhea was evident on the fourth day(6). The severity of diarrhea was measured by the use of unscheduled intravenous fluids expressed as the number of subjects who received intravenous fluid at any time after randomization, weight loss at discharge, presence of

complications or mortality.

*Sample size and power estimation*

The necessary sample size was determined based on detecting a 20% decrease in the mean duration of diarrhea in those supplemented with zinc and copper as compared to the estimated mean diarrheal duration of 3.75 (1.6 days in those on standard ORS)(9). A total sample size of 220 patients with a 10% rate of possible attrition would give a 90% power to detect the above difference for two-tailed test. This sample size would have a power of 80% for two-tailed test if the estimated probability of diarrhea >4 days was 0.2 in those with treatment as compared to 0.4 in the placebo group.

*Data analysis*

Exploratory data analysis was carried out to assess the distribution of study variables. Baseline characteristics of treatment groups were compared using chi square test for categorical variables and ANOVA for continuous variables. This primary outcome was analyzed in two ways: the mean and median time to cure by plotting Kaplan-Meier curves, cox proportional hazard model, and, the proportion of children with diarrhea >4 (multiple logistic regression to calculate the adjusted odd). We also estimated the density function for the probability of cure over time.

## Results

A total of 220 children were enrolled in the study. Overall the non-participation rate was 9% and 200 children were randomized to treatment ($n$ = 102) and placebo ($n$ = 98) groups. Observations were made over a cumulative total of 443 child days in the treatment group and 432 days in the placebo group. In the placebo group, one patient left the hospital against medical advice after enrolment. The baseline characteristics of the children in the two groups were similar (*Table I*).

*Duration of diarrhea*

Most (90%) of the diarrheal episodes lasted between 6-7 days. The acceptability of the oral rehydration solution was similar in both study groups. All children consumed more than one packet of oral rehydration solution (1.2 liters $\pm$ 0.15). The duration of diarrhea and the length of stay in the hospital are depicted in the Kaplan-Meier curves. The cumulative probability of diarrhea is higher at any point of time before 6 days in the placebo group, but the mean survival time (SE) with diarrhea was not significantly different in the treatment (4.34 $\pm$ 0.2) as compared to the placebo group (4.48 $\pm$ 0.2), nor was there any difference in the median time to cure ($P$ = 0.3) (*Fig. 1*). Similarly, the Kaplan-Meier curve for length of stay showed that the cumulative probability of hospital stay was more in the placebo group especially after the 4th day, however the difference was insignificant. The mean length of stay(SE) in the supplemented group was 1.6 $\pm$ 0.2 as compared to 2 $\pm$ 0.2 in the placebo group (P = 0.2). *Figure 2* shows the estimated density function for cure, which indicates the probability of cure over time. Cure was more likely by the 4th day in the supplemented group.

The cox hazard model using stepwise variable selection identified weight of child, the prior duration of diarrhea, the time taken for rehydration and the use of intravenous fluids for rehydration as important explanatory variables for the duration of diarrhea (*Table II*). Even thought the relative risk for cure was greater than unity for the supplemented group, it was statistically insignificant. In fact, duration of diarrhea prior to randomization was the best predictor of total diarrheal duration.

**TABLE I**– *Base line Demogrpahic Characteristics and Features of the Diarrheal Episode with Respect to Study Group.**

| Characteristics | Treatment group ($n$ = 102) | Placebo ($n$ = 98) | P value |
|---|---|---|---|
| Age in months | 21.2 ± 14.2 | 21.4 ± 13.7 | 0.8 |
| Male sex (%) | 58.8 | 48.9 | 0.16 |
| Monthly income | Rs. 1497 ± 1719 | Rs. 1436 ± 1428 | 0.62 |
| No. of children in the family | 2.0 ± 0.9 | 2.1 ± 0.9 | 0.4 |
| Educational status of mother | | | |
| Illiterate(%) | 12.7 | 23.4 | 0.11 |
| Primary | 7.8 | 12.2 | |
| Middle | 14.7 | 14.2 | |
| High | 42.1 | 37.7 | |
| College | 22.5 | 12.2 | |
| Child's diet (%) | | | |
| Predominantly breast fed | 15.6 | 21.4 | 0.4 |
| Predominantly formula fed | 5 | 3 | |
| Both | 1 | 3 | |
| Other solid foods | 78.4 | 72.6 | |
| Immunization status (%) | | | |
| Unimmunized | 3.9 | 7.1 | 0.4 |
| Incomplete | 14.7 | 10.2 | |
| Complete | 81.3 | 82.6 | |
| Weight(Kg) | 8.2 ± 2.2 | 7.8 ± 2.1 | 0.2 |
| Height (cm) | 77.3 ± 10.9 | 75.8 ± 9.9 | 0.3 |
| Wasting (%) | 58.2 | 51 | 0.3 |
| Duration of diarrhea before enrollment(days) | 2.2 ± 1.4 | 2.2 ± 1.3 | 0.8 |
| No. of stools in the previous 24 hr | 9.9 ± 6.3 | 9.3 ± 7.1 | 0.5 |
| Vomiting in 24 hr before enrollment (%) | 61.7 | 55.1 | 0.3 |
| Fever during this episode (%) | 39.2 | 37.6 | 0.8 |
| Dehydration (%) | | | |
| No | 85.2 | 82.6 | 0.8 |
| Some | 11.7 | 14.2 | |
| Severe | 2.9 | 3 | |
| Type of stool | | | |
| Watery or mucoid | 89.3 | 87.8 | 0.2 |
| Bloody | 10.7 | 12.2 | |
| Hospitalized (%) | 57.8 | 66 | 0.2 |

*Plus-minus values are mean ± SD.

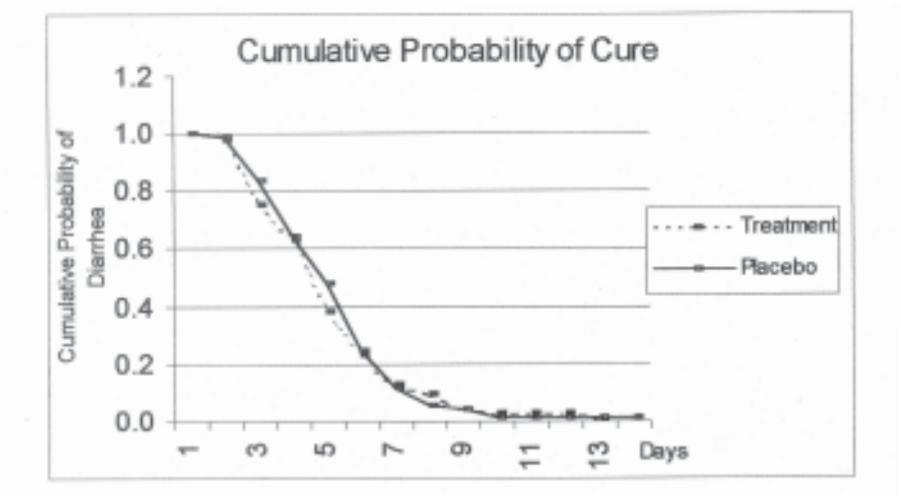


*Fig. 1. Kaplan-Meier curve for time to cure in the treatment and placebo groups.*

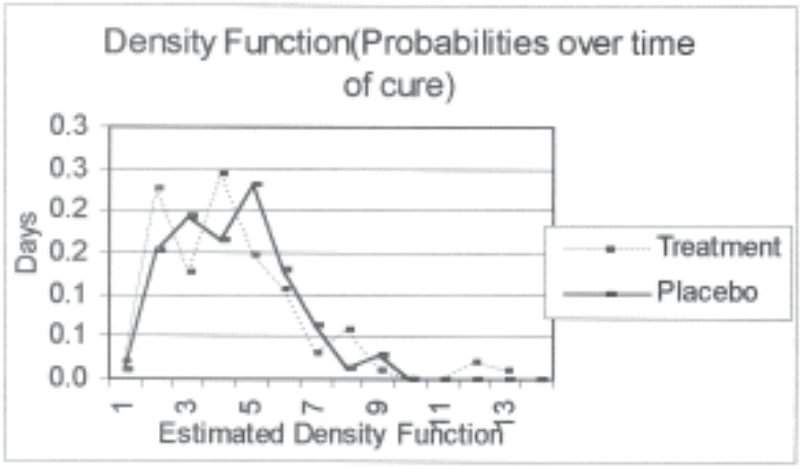


*Fig. 2. The probability of cure over time in treatment and placebo group.*

The proportion of children with diarrhea > 4 days was 46% in the placebo group with an adjusted odds ratio (95% CI) of 1.19 (1.58, 0.9; P = 0.2) as compared to 39% in the supplemented group. The univariate analysis showed that diarrhea > 4 days was associated with younger children of mean (± SD) age 18.7 ± 13.1 months (P = 0.03), lighter children of mean (± SD) weight 7.8 (± 2.2) Kg, children with a mean (± SD) duration of diarrhea of 3.3 (± 1.4) days (P = <0.001) before enrollment, with bloody diarrhea (P = 0.05), and, when intravenous fluids were used during rehydration(P = 0.006). Children with fever on admission were less likely to have diarrheal duration >4 days. On multivariate logistic regression, using the variables that were significant on univariate analysis, the most important variable that predicted diarrhea >4 days was diarrheal duration prior to

**TABLE II**–*Cox Hazard Model for the Relative Risk of Cure.*

| Variable | Coefficient | Error | Coeff. / S.E. | Relative risk (95% CI) | P-value |
|---|---|---|---|---|---|
| Treatment | 0.0803 | 0.1461 | 0.5491 | 1.083 (0.8, 1.2) | 0.35 |
| Weight | 0.0598 | 0.0333 | 1.7958 | 1.06 (1,11) | 0.29 |
| Diarrhea prior to enrolment | –0.5533 | 0.0621 | –8.9044 | 0.57 (0.45, 0.7) | 0.000 |
| Hours for rehydration | –0.0516 | 0.0166 | 3.1130 | 0.95 (0.91,0.98) | 0.001 |
| Use of IV fluids for rehydration | –0.7033 | 0.2332 | –3.043 | 0.49 (.04, 0.94) | 0.03 |

log likelihood = –828.4531, Global Chi square = 92-82, P.F. = 5, P value = 0.000.

enrolment with a OR = 6.25 (3.7, 11.1). The proportion of children with wasting were 51% in the placebo group as compared to 58% in the treatment group. However there was no difference in the mean duration of diarrhea and the proportion diarrhea >4 days in the wasted children with respect to their treatment status.

*Severity of diarrhea*

Fewer children in the supplemented group (34% as compared to 44%, OR 0.7 (95% CI; 0.4, 1.3; P = 0.2) lost weight at the end of the treatment. The unscheduled use of intravenous fluids was also less (2% as compared to 5%, OR 0.4; 95 % CI 0.05, 2.2; P = 0.2). There were less episodes of complications and mortality in the supplemented patients (OR 0.15; 95% CI 0.01, 1.3; P = 0.06). There was only one episode of convulsions in the treatment group whereas of the six patients in the placebo group one had pre-renal failure, one electrolyte imbalance, four had septicemia, two of which died.

## Discussion

Our intent was to evaluate the therapeutic effects of trace mineral by replenishing the immediate losses during the diarrheal episode, rather than restoring body stores. We considered it pragmatic to mix these trace minerals in the ORS so that the trace mineral deficits would be corrected in addition to correction of electrolyte imbalance. The advantage was two-fold. It reduced the burden of an additional supplement and hence compliance. Secondly, the trace minerals were delivered over a period of time along with the ORS rather than one single dose, and thereby exposing the mucosal epithelium to a more continuous dose of zinc and copper.

This study showed a statistically insignificant effect on reduction of duration of diarrhea but a 19% reduction in the risk of diarrhea >4 days. The most important predictor of diarrheal duration was the duration of diarrhea prior to enrolment and its severity. Therefore, regardless of supplementation, children with more severe diarrhea were less likely to experience a shorter episode. There were important differences in reduction of severity of diarrhea in the study group. There was a 60% less chance of receiving an unscheduled intravenous fluid after rehydration and a 17% reduction in proportion of children who lost weight at the end of diarrhea. Though the reductions in these parameters were statistically insignificant, they are clinically important. The rate of septicemia and other complications was

significantly more in the placebo group with two deaths. Zinc has marked effects on T cell function and its supplementation improves immunity and may help in reducing rate of sepsis and mortality(4).

The therapeutic effect of zinc in reducing duration of acute diarrhea has been documented in a pooled analysis of randomized controlled trials(9). Some of the studies supplemented zinc with multivitamins and should be interpreted as an effect of zinc supplementation in presence of multivitamins. It is possible that there are biological interactions between vitamins and zinc specifically of vitamin A and zinc(10,11).

Evidence from these trials on effectiveness of zinc in acute diarrhea is compelling. Failure to see a significant difference in duration of diarrhea in our study could be due to a number of reasons. The most important predictor of continued diarrhea in our study was its severity at randomization. A number of studies did not report the effect of zinc after adjusting for duration of diarrhea and its severity at randomization. Therefore it is unclear from these studies whether zinc would be effective despite the severity of diarrhea. Most of our study population are hospital referrals and therefore differ in disease severity as compared to home-based study population. Perhaps the trace mineral supplementation is more useful in children with a less severe illness. Secondly, we did not measure the plasma zinc and its response to the supplementation. Although plasma zinc may not reflect the zinc stores, it is known to increase with supplementation(7). If measured, it would have been an indication of the bioavailability of the trace minerals when mixed with ORS, which is currently undocumented. Another home-based study examined the effect of zinc syrup and ORS, zinc-mixed with ORS (same dose as in this study) and placebo on diarrhoeal duration. Zinc syrup group had decreased duration of diarrhea and though zinc-ORS group had lesser stool output there was no difference in duration of diarrhea as compared to placebo(12). It is also possible that a brief period of zinc supplementation during an episode of diarrhea along with ORS may not be adequate. This study thus draws attention to the fundamental question of what are the adequate doses and duration of supplementation especially if the trace elements are to be administered mixed with ORS. Thirdly, our study was powered to detect a 20% difference in mean duration but the observed difference was small but with important clinical implications. Therefore, a larger study would be more appropriate to study the therapeutic role of zinc and copper for different disease severity and types of diarrhea.

It could also be argued that copper may have reduced zinc absorbtion and obliterated the effect of zinc(13). We supplemented zinc and copper in the same ratio as in a customary diet and therefore this interaction would be unexpected(14). However, simultaneous measure of plasma minerals and fecal minerals would have helped in determining this interaction but it was not the objective of this study. There are no published studies of the effect of copper on diarrheal duration and severity. A clear association between a history of diarrheal episodes and low plasma copper and zinc levels has been demonstrated, with copper deficit being more prevalent than zinc deficit(5). Pharmacological doses of zinc can interfere with the absorption of copper and also chelate to enterocyte MT(8). Furthermore, copper balance remains negative one week after hospital admission for acute diarrhea(15). Copper deficiency causes neutropenia, hypochromic anemia by impairing the transport of iron by cerulo-

**Key Messages**

- The most important predictor for duration of diarrhea in children was severity of the disease.
- Zinc and copper supplementation resulted in beneficial effects on rate of diarrhea complications.

plasmin to the erythropoietic sites and depression of cytosolic erythrocyte superoxide dismutase (Cu, Zn-SOD) which is an an important scavenger of $O_2^-$, a free radical that causes damage to membranes and biological structures(16). These can influence the diarrheal morbidity. Even though zinc supplementation has been found to be beneficial, there are concerns on administering zinc alone to children with acute diarrhea.

## Conclusion

There appears to be ample evidence that a physiologic basis exists to explain the efficacy of trace minerals in reducing diarrheal severity and duration. A clinically relevant but statistically insignificant reduction in duration and proportion of children with diarrhea >4 days was observed in the supplemented group with significant reduction in complications and mortality. The most important predictor for duration of diarrhea in children was the severity of the disease, regardless of the supplementation and therefore larger trials are needed to study the dose, frequency and duration of administration of trace minerals particularly if they are to be administered mixed in ORS. This study was not powered for mortality or the number of complications so larger trials are also needed to detect a significant difference in diarrheal duration and its morbidity in different types and severity of acute diarrhea.

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*Contributors:* AP participated in the design of the study, its coordination, performed the statistical analysis and drafted the manuscript. LD supervised the data collection. MSR conceived of the study, participated in its design and coordination. All authors read and approved the final manuscript.

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