Symposium : Micronutrients and Health Indian J Pediatr 1995; 62 : 181-193

Zinc Supplementation in the Treatment of Childhood Diarrhoea

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Diarrhoea is major cause of childhood death and malnutrition in most of the developing as well as developed countries. The public health importance of control of diarrhoeal diseases is well recognised by World Health Organization, International health promoting agencies and Governments of various countries.[1] Yet, it is critical to identify the important determinants of diarrhoeal diseases and initiate appropriate measures to reduce the incidence and severity of diarrhoeal illness. Children are most vulnerable to diarrhoeal illness due to its direct and indirect effects. Diarrhoea causes death due to dehydration and complications, but for vast majority of diarrhoea affected children, recovery is the usual outcome. The after effects of diarrhoea episodes have received relatively less attention but malnutrition is one which has been taken with importance.[2] The mechanism of causation of malnutrition has been incompletely understood, as many issues on nutrition have remained unclear. There are substantial wealth of information to believe that in children, nutritional status is a determining factor for severity of diarrhoeal episodes,[3,4] whereas most of studies could not document that incidence of diarrhoea has any clear relationship with nutritional status.

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While anthopometric index of nutritional status has been taken as a proxy indicator of micronutrient status of individual, until recently this view has not been challenged. There are evidences now to assume that even in normal to mild malnutrition, trace element deficiency could exist, which has been related to inadequate dietary intake, inappropriate dietary habit and various metabolic disorders.[5] Along with the macronutrients i.e., fat, protein, carbohydrate, micronutrients are essential for growth and normal physiological function of children. Among the micronutrients, trace elements are important because of their essential functions despite the small amount required. Trace element deficiencies in children of most developing countries are related to their inabundance in the common food items.

Among the essential micronutrients for growth and morbidity, zinc has been known for long for its integral presence in more than 100 metaloenzymes which are regularly needed for protein synthesis, bone mineralization, physical growth and biological functions including immunocompetence.[6]

Role of zinc in maintenance of physiological functions

Severe zinc deficiency has been labelled as 'Acrodermatitis enteropathica' (AE) presented with severe diarrhoea, severe infec-

tion, erethematous skin lesions, alopecia, oral and anal scoriation and Moynehan reported that this severe disease condition could be returned to normal by zinc supplementation.[7] Zinc deficiency in experimental animals causes diarrhoea.[8] AE is an inherited autosomal recessive trait where severe diarrhoea, alopecia, skin lesion and frequent infection constitute a life threatening syndrome. Complete recovery and reversal of the mucosal abnormality, after large doses of oral zinc supplementation, has been reported.[7,9] Information on the relationship between zinc deficiency and diarrhoea in children is limited although, evidence of zinc deficiency in severely and marginally malnourished children has been reported in recent years. [10,11] The role of zinc in diarrhoea may be mediated through several mechanisms which include, membrane stabilization, mucosal integrity, electrolyte transport, water transport, immunecompetence, protein and essential enzyme synthesis. Several of these phenomena have been studied in animal models in the laboratory and some have been observed in supplementation studies in children with malnutrition and diarrhoea.

Factors that affect body zinc status include; nutritional status : severity and duration of diarrhoea, presence of systemic diseases, extent of small bowel enteropathy, absorption of zinc before or during diarrhoea, extent of pre-existing zinc deficiency, adequacy of dietary intake of zinc, bio-availability and utilization in the body. The maintenance of integrity of the mucosal cell membranes, repair of mucosal injury by increased protein synthesis, multiplication of epithelial cells, and improvement in sodium and water transport are likely to reduce fluid loss during diarrhoea. Repair of mucosal paracellular tight junctions would allow better absorption of water. The improvement in immunological function especially on secretory IgA and T-lymphocytes would be expected to limit the growth and multiplication of diarrhoeal pathogens within the intestinal lumen. Thus the physiological, ultrastructural and immunological role of zinc mentioned above offers a set of mechanisms which may explain why there may be a reduction in diarrhoeal severity and duration. The therapeutic effect of zinc is likely to be more in the subjects who are zinc deficient.

Role of zinc in water and electrolyte transport

Ultrastructural abnormalities in experimental zinc deficient animals have been described in intestinal paneth cells including crystalloid secretory granules, giant granules and dilated golgi vesicles, cytoplasmic vacuolation, few endoplasmic reticulum, mitochondria and ribosomes.[12] Functional abnormalities of sodium and water transport have been described in experimental studies. An *in-vivo* singlepass perfusion study showed that net water and sodium transport from the small intestine of zinc-deficient rats was significantly decreased compared to corresponding mean values of pair-fed controls and *ad libitum* fed rats.[13] These results are in agreement with other studies showing decreased sodium transport in leukocytes and renal tubules of zinc deficient animals and suggest that zinc may have a role in modulating membrane permeability.[14]

Changes in rat small intestine in zinc de-

ficiency have been described earlier. At the end of 2 weeks of zinc deficient diets (0.8g zinc/kg diet), mucosal mass, cell number and cell size of epithelium were significantly reduced in zinc deficient animals. Ultrastructural abnormalities in the eneterocytes of zinc deficient animals included degenerative changes in mitochondria; destroyed desmosome system; loss of tight junctions between mucosal epithelial cells; appearance of abnormal lateral space; reduced number of ribosomes and reduced rough endoplasmic reticulum.[8] These changes in small intestine were completely absent in *ad libitum* fed control animals with the same diet including adequate zinc (55 mg zinc/kg diet). The aforementioned changes were associated with drastically reduced absorption of water and sodium by the small intestine which was tested with single pass perfusion of glucose electrolyte solution containing ^{14}C-Polyethylenegycol (PEG 4000) as marker. Reversal of absorption defects was associated with concomitant repair of mucosal lesions by zinc repletion. 48 hours of repletion of dietary zinc, reversed the net transport of sodium and water to normal levels.[15] The same authors showed that *V. cholerae* enterotoxin induced secretion of water, sodium and potassium per unit length of mucosa was significantly higher in zinc-deficient animals compared to that of *ad libitum* zinc fed controls and the *V. cholera* enterotoxin induced secretion reduced significantly after dietary zinc supplementation for 48 hours.[16] The correlation between unit mucosal mass and water transport did not fully explained that absorption was only a function of mucosal mass, i.e. the cellular integrity and enzyme cotransport system could have been well related to the net transport of water and electrolytes.

Many of the previous work in animal models would support the view that zinc deficiency at relatively severe level would affect diarrhoeal susceptibility or severity due to specific effect in mucosal integrity and function. In zinc deficient animals, paneth cells contained plemorphic secretory granules of crystalloid structure, giant intracellular granules and dilated golgi vesicles.[17,18] However one study reported normal structures from such animals.[12] In the enterocytes, microvilli were reported to be normal by some workers[12] but others found them fragmented, scanty in number and destructed.[18] The reports were similar for enterocytes structure including supranuclear cytoplasmic vacuolations, increased number of lysosomes, scare granular endoplasmic reticulum, frequent membrane bound autophagic vacuoles, clear regions devoid of structure, vacuolated mitochondria, fewer ribosomes in cytoplasm and very few organelles inside the cells. However, the causal relationship between these abnormalities and cell functions was not further investigated.

Intestinal pathogens can colonize and infect animals for longer period. Such a phenomenon was observed by infecting rats with intestinal parasite *Strongyloides ratti*. Fenwick *et al.* have shown that zinc deficient rats could not expel an infecting dose of *Strongyloides ratti* from the inestine.[19] The zinc deficient animals had reduced thymus size and were considered to be deficient in T-cell activity. The impairment of thymus dependent immune responses in zinc deficient mice had been readily reversed by zinc supplementation.[20]

Changes in the intestinal mucosa in zinc deficiency in man

In human zinc deficiency syndrome of acrodermatitis enteropathica, severe mucosal lesions have been reported, such as flat mucosa infiltrated with plasma cells and lymphocytes, stunted villi, reduced number and necrotic crypts, cytoplasmic vacuolations, increased lysosomes, and reduced rough endoplasmic reticulum.[21,9,22] These patients were associated with low plasma zinc, low alkaline phosphatase, reduced serum cholesterol, marginal hair zinc level and reduced urinary excretion of zinc. All of the above abnormalities reversed to normal after zinc therapy. Ultrastructural abnormalities in paneth cells have also been described in acrodermatitis enteropathica.[23] These included pleomorphic secretory granules and abnormal fibrillar inclusion bodies.

Requirement of zinc in humans

Zinc balance studies in man have shown that children aged 8-12 years retain 4.9 g of elemental zinc per day on a dietary intake of 12-14 mg elemental zinc. It is indicated that growth and rehabilitation require higher amount of zinc than maintenance only. In young persons of 17-27 years, retention of zinc was reported to be 5-8 mg/day on an intake of 12-14 mg of dietary zinc.[24] Recommended dietary allowances by the Food and Nutrition Board of USA[25] are 3 mg for 0-5 months, 5 mg for 0.5-1.0 years, 10 mg for 1-10 years, 15 mg for children above 11 yrs and adults, 20 mg for pregnant women and 25 mg for lactating women. Protein content and source of protein in the diet are important for absorption and retention of zinc. Studies with labelled zinc have shown that the duodenum is the active site of zinc absorption.[26] It is important to consider the factor that zinc homeostasis is dependent on energy metabolism. In energy deficient state, zinc would be sequestrated through gut and excreted through stool. Such phenomenon would allow relatively minimal effect of the potential for maximum benefits of zinc supplementation in a situation where zinc supplementation is given to energy deficient malnourished children without provision of adequate dietary energy. Various dietary and physical factors which changes zinc requirements include dietary phytate, phosphate, fibre, calcium, copper, iron, heavy metals, alcohol and physical stress such as severe and moderate degree of malnutrition, blood loss, prolonged diarrhoea, malabsorption, prolonged parenteral nutrition, fasting, infection, nephrosis, chelating drugs, surgery, cirrhosis of liver, pregnancy, lactation, and genetic defect of absorption of zinc.[27]

Sources of zinc in diet

Zinc content of diet depends on the composition of diet. The absorption in small intestine is significantly dependent on presence of phosphate, phytate and concentration of other trace elements in the absorptive environment. Oxidative tissue such as muscles or red meat (3.4 mg/100 g) is a rich source. Sea and fresh water fish (2-4 mg/100 g), egg yolk (3.8 mg/100 g) are also rich sources. Pulses are good source of zinc (2.9-3.6 mg/100 g). Cereals including rice (1.4 mg/100 g), wheat (0.9-26 mg/100 g) due to high phytate are considered to have poor bioavailability. Cow's milk (0.6 mg/100 g) is a poor source but bioavailability of zinc from breastmilk (0.2

mg/100 g) is high.[28] Food habits and dietary pattern in young children should be considered to prepare the diet list to include zinc containing foods.

Zinc deficiency state in diarrhoea

At the cellular level of electrolyte transport, adequate zinc has been shown to be directly associated with increase in intracellular potassium and decrease in intracellular sodium as a reversal process in recovery from malnutrition. During nutritional rehabilitation, Patric *et al.* observed that zinc supplementation led to a small rise in intracellular potassium and a reciprocal fall in intracellular sodium.[14] The most striking changes in this study with zinc supplementation were the increases in leucocytes in measures of sodium pump activity, namely total and glycoside sensitive rate constants and efflux rates for sodium. Patients with chronic diarrhoea have been reported to have hypozincemia. Eleven of 30 patients with chronic inflammatory bowel disease (CIBD) showed to have serum zinc values less than 0.7 µg/ml, whereas none of the others had hypozincemia.[29] Among 14 patients with CIBD and growth abnormalities, seven were hypozincemic and four were hypozincuric. Children with severe persistent diarrhoea lost about 300 µg zinc/kg/day in stool and high dose of oral zinc (200-300 mg elemental zinc) was required to resolve the skin rash and to restore serum alkaline phosphatase to normal levels.[30] Castillo-Duran *et al.* have demonstrated that about 159 µg zinc/kg/day was lost in stool and zinc retention was negative during acute diarrhoea.[31] The negative balance of zinc occurred not only due to high zinc loss in diarrhoeal stool but also by the reduced dietary intake during acute phase of diarrhoea, a situation commonly experienced in childhood diarrhoea. Zinc balance became positive for this children at recovery mainly due to a significant reduction of zinc loss through stool, but the balance remained lower than that of the non-diarrhoea control group children.

Effect of zinc supplementation in diarrhoea

It needs critical examination of the existing evidences to arrive at a consensus on zinc supplementation to patients of diarrhoea. There are several aspects for evaluation of this issue. Firstly who are likely to benefit? Secondly what are the indicators for measurement of benefits? As far as public health concern, one would ask whether zinc supplementation would benefit the vulnerable groups such as children with malnutrition who encounter more risk of frequent and severe diarrhoeal illness. To document benefits from a single micronutrient supplementation, zinc would offer to be an unique one in term of its requirement for enzyme and protein synthesis. In more fundamental way, zinc deficiency would limit most of the biologically essential protein synthesis and growth. Diarrhoea as a consequence of abnormal intestinal function and deranged physiology due to enteropathogens, zinc has particular possibility to improve intestinal functions. It needs to be considered whether zinc supplementation reduces diarrhoeal episode or incidence. Even more, in patients with diarrhoea, whether zinc supplementation may have any effect on the duration of diarrhoea and severity of diarrhoea.

As the nutritional physiology has been reviewed above it is likely that zinc

supplementation might benefit those who are zinc deficient. However even if that is possible, it is difficult to have simple and feasible means in population groups, to diagnose a low zinc status. For individual cases, serum zinc level and hair zinc level are accessible tissue for rapid estimation of zinc. It would be important to mention here that the interpretation of plasma zinc level is not straightforward as only 3% of body zinc is present in plasma and 75% of plasma zinc is bound with albumin and alpha-2 macroglobulin. Zinc status may be further reflected by zinc containing metaloenzymes and metaloproteins such as alkaline phosphatase, carbonic ahydrase, pancreatic carboxypeptidase, glutamic dehydrogenase aldolase, malic dehydrogenase and metallothionein.[32]

To recommend zinc supplementation in acute or persistent diarrhoea in children, several factors are to be considered such as age, body weight, daily dose, type of zinc salt, dietary content of phytate, phosphate and other micronutrients. The malnourished children are often zinc deficient and during diarrhoea they are likely to face a greater deficit of zinc, hence the dose of supplementation needs to be adequate. It is needed to consider the amount of elemental zinc from the available zinc salts, its interaction and bioavailability with other elements and compounds. The foremost importance in diarrhoea patients are the factor of mucosal injury or integrity, absorption and endogenous secretion in diarrhoeal state. In invasive type of diarrhoea, zinc sequestration from plasma would be enhanced due to loss of protein bound zinc with protein loosing enteropathy. In some studies doses in the region of 2 to 3 times of recommended dietary allowances (RDA) were applied. Duration of supplementation may be well beyond the disease period. There are only a few reports on the effect of zinc supplementation in diarrhoea.

From India, Sachdev *et al.* reported that in zinc depleted subgroups of acute diarrhoea patients (judged by lower zinc concentration in rectal mucosa), mean diarrhoeal duration (65.5 vs 97 hr) and frequency (6.7 vs 10.0/day) were significantly less ($p < 0.05$ and $p < 0.01$, respectively) with zinc supplementation (20 mg elemental per child per day) compared to placebo group.[33] Serum alkaline phosphatase, serum zinc level and rectal zinc content were significantly elevated in the supplemented group and reduced in the placebo group at the end of supplementation period.

In persistent diarrhoea patients (20 infants in each group), the same authors found that diarrhoeal duration and average frequency in the supplemented group were lower than in the placebo group but the differences were not significant ($p = 0.078$ and $p = 0.076$, respectively).[34]

In Bangladesh, a double blind randomised trial of zinc or placebo in a multivitamin syrup was conducted among 4-24 months old children suffering from acute diarrhoea for less that 3 days. 5 mg of elemental zinc per kg body weight per day was administered for two weeks. When the results were compared, benefits of zinc supplementation were observed among the children who were hypozincemic and shorter.[35] The mean duration for recovery from acute diarrhoea was shorter (4.7 vs 6.0 days, $p < 0.04$) among the hypozincemic children (serum zinc < 14 umol/l). Similar trend was seen in children who were wasted (4.9 vs 5.8 days $p = 0.3$) and stunted (5.0 vs 6.0 days

TABLE 1. Impact of Zinc Supplementation on Duration of Diarrhoea in Days

Mean ± Sd

(95%) confidence interval

	Placebo	Zinc	Statistical significance*
All children	n = 37 5.80 ± 2.7 (4.93-5.84)	n = 37 50 ± 2.6 (4.16-5.84)	p = 0.33
Serum zinc < 14 µmol/1	n = 25 6.0 ± 2.9 (4.96-7.0)	n = 30 4.7 ± 2.0 (3.92-5.48)	p < 0.04
Wasted < 80% wt/ht	n = 18 5.8 ± 2.8 (4.53-7.07)	n = 11 4.9 ± 2.0 (3.72-6.07)	p = 0.35
Stunted < 95% ht/age	n = 33 6.0 ± 2.8 (5.0-6.94)	n = 37 5.0 ± 2.6 (4.16-5.84)	p = 0.13

p = 0.1) but differences were not statistically significant (Table 1). In these patients, significant reduction in median stool weight during recovery was noticed among the hypozincemic children (279 vs 329 g, p < 0.04) and shorter children (238 vs 325 g, p < 0.05) who were zinc supplemented (Fig. 1) compared to their to the placebo counterparts.

In another study of 90 patients with persistent diarrhoea, aged 3-24 months, were given a placebo or zinc (5 mg/kg/day) for two weeks. The duration of clinical recovery was significantly reduced by zinc supplementation among children who were underweight (≤ 70% wt/age, p < 0.01).[36] (Figure 2).

Mucosal integrity

Small intestinal mucosal injury due to enteropathogen infections or dietary allergy or protein sensitivity has been regarded as one of the underlying causes for continued diarrhoea. Micronutrients including zinc would then be considered to be limiting factor for usual healing. Zinc has been shown to enhance mucosal repair in patients with acrodermatitis enteropathica but its role on mucosal integrity in persistent diarrhoea patients is not known. Permeability tests using large and small molecular sugars have been validated as a useful test of mucosal integrity.[37] Abnormal results have been shown in children with persistent diarrhoea. Using a dose of 5 g lactulose and 1 g mannitol, there was a significant increase in lactulose excretion and decrease in mannitol excretion compared with healthy controls. Lactulose and lactulose/mannitol (L/M) ratio were higher in children with

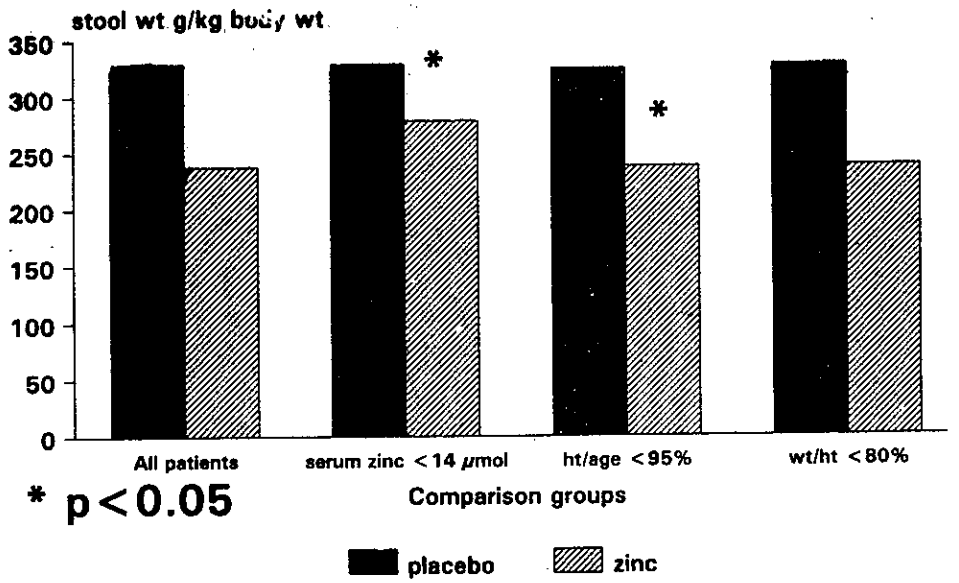

Fig. 1. Comparision of stool output in acute diarrhoea patients (median)

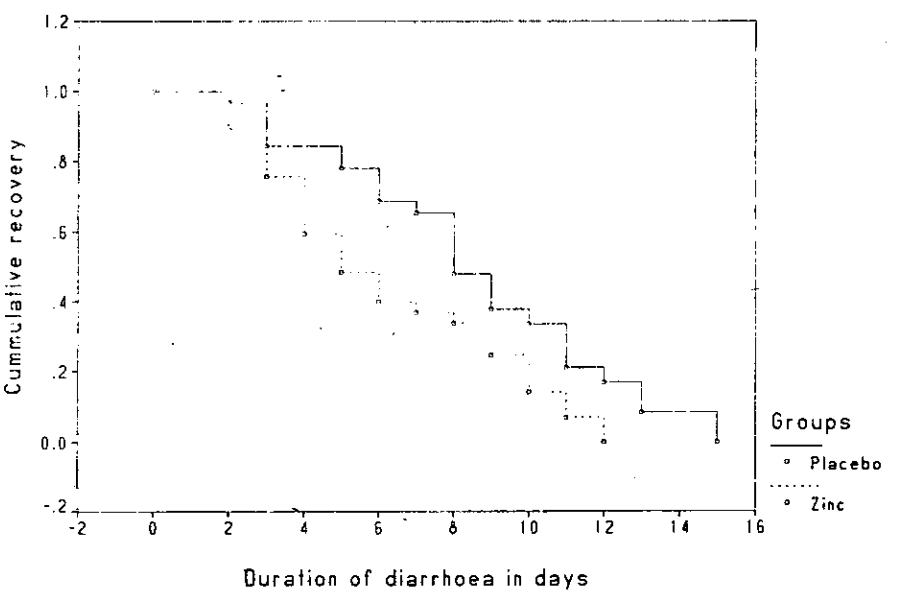

Fig. 2. Recovery of malnourished children (W/A < 70%) with persistent diarrhoea supplemented with zinc compared to placebo

diarrhoea caused by mucosa damaging pathogens compared with noninvasive pathogens. Zinc supplementation significantly reduced lactulose excretion in persistent diarrhoea and this effect was more marked in the malnourished patients. The results suggested that zinc has significant effect on intestinal integrity and is likely to contribute to better recovery.[38]

Absorption of zinc

The small intestine has central role in maintaining zinc homeostasis. Very substantial amount of endogenous zinc that are secreted into small intestine need to be reabsorbed to avoid negative zinc balance. During diarrhoea there is reduced absorption from food and endogenous secretion as well as reduced dietary intake thus raising the probability to have a zinc deprived state. If this negative zinc balance state is not corrected, growth and physiological function are likely to be affected. Duodenum and jejunum are primary sites for zinc absorption where pancreatice secretion provides a zinc binding ligand 'picolinic acid'. In addition, during acute diarrhoea with mucosal invasive pathogens such as rotavirus or enteroadhesive *E. coli*, zinc absorption may be reduced to a great extent. It is therefore important to consider that, not only diarrhoeal state but also the types of diarrhoea are important for zinc deprivation in the children. Invasive diarrhoea are known to affect the large bowel as well as part of the distal ileum. Many invasive diarrhoeas cause protein loosing enteropathy while the endogenous secretion in this occasion contain many plasma proteins and micronutrients. Zinc loss in invasive diarrhoea might appear to be more severe. It has been reported that dysenteric type of illness is frequently associated with growth retardation. Along with protein loss, significant among of zinc loss could be responsible for the stunting of this children following invasive diarrhoea.

Effect of zinc supplementation during diarrhoea on serum zinc level and weight gain

Patients with acute diarrhoea showed significant rise in serum zinc levels compared to controls when zinc supplementation was given.[35] Body weight of acute diarrhoea patients increased significantly with zinc supplementation. The effect of zinc was reflected in serum level and body weights. Patients with persistent diarrhoea receiving placebo had a significant reduction in body weight and serum zinc level after 2 weeks (13.6 μmol/1 vs 11.8 μmol/1, $p < 0.03$) whereas zinc supplementation maintained their body weight and serum zinc level (13.4 μmol/1 vs 13.6 μmol/1).[36] Evidence of increased new tissue synthesis with zinc supplementation has been confirmed from several studies, one of which showed a negative correlation between he rate of weight gain and the plasma zinc concentration.[39] Golden *et al* showed that fourteen of 16 children had an immediate and definite increase in their rate of weight gain with zinc supplementation. This was associated with a decrease in the energy cost of tissue deposition, growth of the thymus, and activation of the sodium pump. Severely malnourished children in Bangladesh received daily zinc supplement of 50 mg for 2 weeks; during the second week, weight gain was 73% more in the zinc supplemented group (8.83 ± 1.56 vs 5.09 ± .62 g/kg/day) compared to the unsupplemented group.[40]

Effect on zinc supplementation on mortality

There is scarcity of large field data on measuring the impact of zinc supplementation on mortality. However in a small scale clinical study, some protective effect was noticed. During zinc supplementation in children suffering from persistent diarrhoea, there was about 6.5 fold reduction in relative risk of mortality ($p = 0.06$) compared to unsupplemented patients.[41] About 40% of these young children were having various types of systemic infection. The immediate causes of death were systemic infections. These observations need to be thoroughly examined by further studies with large sample size.

Effect of zinc supplementation during diarrhoea on height of children

Retardation in linear growth has been documented as a syndrome of mild zinc deficiency in Iran,[42] Denver, [43] China,[44] Yougoslavia.[45] The height gain was dose dependent. These community studies showed a gain when 40 mg elemental zinc was given daily in Iranian children.[42] Zinc is utilised best in presence of energy and protein. In absence of dietary ingredients for growth, zinc can be diverted to synthesize essential proteins, immunoglobulins, and skeletal tissue synthesis.[46,47] The dose of zinc is a critical component in effects of linear growth. A number of studies in developing countries have confirmed that zinc has significant effect on growth. In Bangladesh, a double blind randomized trial of zinc supplementation to children with acute watery diarrhoea for 14 day period showed that the supplemented children continued to grow significantly taller for subsequent 2 months.[41] The dose of zinc used in that study was 5 mg/kg body weight of zinc per day.

In persistent diarrhoea patients zinc supplementation for 2 weeks also led to higher length gain in children who were stunted (< 91% length for age) (35 vs 24 mm, $p < 0.0$) or lighter (70% wt/age) (30 vs 24.4 mm, $p < 0.03$) over a subsequent period of 10 weeks.[41] A large number of studies have documented that zinc supplementation in growth retarded children can produce increased linear growth if dose and duration are adequate. Ronaghy and his colleagues showed significant increase in height in 13-year old Iranian school boys.[42] Talukder and co-workers have shown that acute respiratory tract infection and diarrhoeal attack rates in the supplemented and control groups were 8.69 ± 4.03, 1.46 ± 1.43 and 8.66 ± 4.44, 2.14 ± 1.88 per child per year respectively. This study has also shown that there is significantly better growth in weight ($p < 0.02$) and length ($p < 0.05$) and less diarrhoeal morbidity ($p < 0.05$) in the zinc supplemented group of infants.[48] The growth rate in growth retarded children who took 50 mg of oral zinc supplements daily for a year, together with their growth hormone injection increased from 5.1 to 7.3 cm/yr ($p < 0.01$).[47]

Effects of zinc supplementation on diarrhoea and other illnesses

Supplementation of zinc in deficient subjects can lead to improved immunity which may be expressed through reduced morbidity. Children who received zinc supplements during persistent diarrhoea had fewer attacks of diarrhoea compared to controls during the subsequent 3

months (0.8 vs 0.5 episodes, $p < 0.05$).[41] Castillo-Duran and his co-workers showed that, during nutritional rehabilitation, zinc supplementation significantly reduced the incidence of pyoderma, (3 vs 10, $p < 0.025$).[49] Plasme zinc levels negatively correlated with number of febrile days ($r = 0.66$, $p < 0.05$). The proportion of anergic infants decreased and serum IgA increased significantly with Zn supplementation. In this study, effect of zinc supplementation in patient with persistent diarrhoea on intestinal function has been reflected by earlier clinical recovery, improved intestinal permeability, reduced incidence of infection and increased weight and height gain.

The possible benefits of zinc supplementation in diarrhoea patients can be summarized as maintenance of normal serum zinc levels, mucosal integrity, reduction of diarrhoeal duration of malnourished children and prevention of weight gain during diarrhoea.

For further understanding of zinc supplementation effects the following recommendations can be made: (1) Studies on zinc and other micronutrient balance during diarrhoea using appropriate technology such as stable isotopes of zinc; (2) Determination of the most effective dose, duration and method of supplementation of zinc in diarrhoea; (3) Development of accurate and feasible methods of determining zinc status in patients with diarrhoea and malnutrition; (4) Trace element interaction during supplementation in diarrhoea; (5) Study on specific effect on immune status during and after diarrhoea; (6) Zinc supplementation at community level with feasible dose and duration; (7) Mechanism of effect on growth under different dietary conditions.

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