Role of Zinc in Shaping the Gut Microbiome; Proposed Mechanisms and Evidence from the Literature

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Abstract

Zinc is an important constituent of diet that regulates gut epithelial wall and modify gut microbiome in humans as well as animals. Zinc deficiency may affect 39% children in Pakistan, according to the recent National Nutritional Survey 2011. Although zinc has been used in the prevention and treatment of diarrhea, the relationship of plasma zinc status with potentially pathogenic bacteria has not been studied. In this review, we have discussed evidence suggesting the impact of zinc on gut microbiota and its interaction with gut epithelium. Furthermore, animal and human studies suggesting the role of zinc in modifying gut microbiota have been presented.

Introduction

Micronutrients are substances required by the body in minute quantities. These low concentration substances play a major role in our metabolism and normal tissue functions. Amongst other micronutrients, zinc is one of the most important micronutrient, required for several body functions such as it restricts the loss of barrier function under malnutrition condition [1], alcoholic liver disease [2], chronic inflammatory bowel disease or crohn's diseases [3], and is an influential causing agent of growth [4,5]. Daily dietary allowance for zinc is 11 mg/day for men and 8 mg/day for women [6]. But when these values are depleted than their normal range, deficiency symptoms arise which can cause gastrointestinal, skeletal, immune, reproductive, central nervous system disorders [7-9], diarrhea [10,11], pneumonia [12] and acrodermatitis Enteropathica [13]. Deficiency of zinc in humans was initially declared in 1961 in patients presenting with growth stunting, dissymmetric gonads, skin lesions, and mental dizziness [14,15]. While its deficiency is generally due to inadequate diet or bio-available zinc content [16], increased requirements (depending on age groups), malabsorption, increased losses and impaired utilization also contribute to the maintenance of plasma zinc levels. It is more common is areas where cereal is high in diet as compared to animal food because red meat is a good source to bio available zinc content [17]. Cross-sectional studies conducted in Pakistan suggest zinc deficiency in our diet and implicate its effects on health [18]. The 2011 National Nutrition Survey of Pakistan reports zinc deficiency in 39% children (39.3% urban and 39.1% rural). Punjab (38.4%), Sindh (38.6%), KP (45.4%), 39.5% Baluchistan (39.5%), AJK (47.2%) and in Gilgit Balitistan (32.6%) showed Provincial data statistics of the zinc deficiency at our population [19]. Keeping in view deficiency states of zinc in our population, interventional strategies have been employed such as fortification of cereals, food products, and zinc preparations in suspension forms.

Although, cereals diets such as wheat contains inhibitors such as phytate [20] which reduces the availability of absorbable zinc, its fortification is a better option as wheat flour is most common and easily accessed food in resource poor settings compared to other fortification methods. Although main source of zinc is red meat and other animal products, wheat contributes 50% of daily zinc intake in Pakistani population due its frequent use. Studies also suggest that dietary modification such as genetically modified plants can reduce phytate content [21]. Also certain techniques (i.e. sourdough) are helpful in reducing harmful concentration of phytate with the help of enzyme phytase that hydrolyze phytate and release inorganic phosphorous [22,23]. Besides, zinc in suspension form has more bio-available content than solids (tablets), but transportation, availability and proper measurement may be an issue [24]. Human gut has a very complex diversity of gut microbes existing throughout the intestinal system. Gut bacteria constitute genome that is 10 times more than that of humans' own genome. They serve several important functions such as generation of Short chain fatty acids (SCFAs), vitamins, development of immunity and protection against allergens [25]. The role of zinc may mostly have addressed by prevention and treatment of diarrhea in children, changes in gut microbial diversity with zinc intervention are anticipated. Relationship between gut bacteria, healthy and malnourished children have been indicated [26]. One recent study on chicks have shown phylum levels difference in gut microbiota composition between normal and chronic zinc deficient chicks [27]. Little is known about changes in gut microbiota such as Enterobacteria with zinc supplementation. Investigating these changes in humans especially children, therefore needs further exploration. Original articles and systematic review papers exploring the relationship of the gut microbiome with zinc status published between year 2000 to 2016, were retrieved for the purpose of literature review using PubMed and Google scholar.

Mechanisms Suggesting the Effect of Zinc on Gut Microbiota and its Interaction with Gut Epithelium

Intracellular zinc is an essential promoter of normal intestinal barrier functions and the regeneration of impaired epithelium [28]. Animal studies suggest that intracellular zinc (IZ) stimulates the release of ghrelin in the stomach of pigs. Ghrelin is a polypeptide hormone required for the regulation of appetite [29]. Besides, IZ

reduces the inflammation of intestinal mucosa [30]. Zinc also regulates intestinal permeability through occludin proteolysis and occludin transcription and thus protects intestines from the invading molecular ions and pathogens [31]. Under zinc deficiency states, the intestinal tight junction and membrane function was impaired and also induced the migration of large number of neutrophils that leads to mucosal inflammation [32]. However, this study was performed *in-vitro* using specific cells (Caco-2 cells) and may not reciprocate to humans or in-vivo models [32]. In addition, it has been shown that zinc adequately restrict the loss of barrier function under malnutrition states [1], alcoholic liver disease [2], chronic inflammatory bowel disease or crohn's diseases [3], and infectious diarrhea [28]. There is also some evidence that zinc oxide (ZnO) protects against intestinal diseases such as infectious diarrhoea. Although ZnO has antibacterial effects, the mechanisms of this protective effect have not yet been elucidated [33].

In nature, the bioavailability of essential trace elements such as zinc are less, therefore bacterial species have evolved themselves through high affinity ligand binding proteins and transporter systems [34]. It has been demonstrated that *Campylobacter* survival is linked through ZnuABC transporter system while this setup is not being seen during limited microbiota as compared to normal flora of gut microbes [35]. Less attention has been paid to the mechanism of zinc transporters such as Zn transportation in free form or in chelates across the epithelium membrane [36]. As the gut mucosa acts as an intestinal barrier against pathogenic microbes and foreign invading bacteria [37], zinc has an ultimate effect on its normal function and permeability to invading pathogens and microbes. How gut can accomplish prevention to specific molecular ions and integral membrane proteins as well as pathogens and their relationship with gut diversity under the influence of dietary zinc is yet another area of study which needs further investigation.

Animal Studies Suggesting Relationship of Zinc in Relation to Gut Microbiota

There is much evidence about animal studies which is based on supplementations and dietary modification/fortifications of zinc. Zinc given in the pre-weaning stage influences growth, consumption of feed intake by an animal, weight gain and improve overall health of the gut by increasing the counts of beneficial bacteria and reducing Enterotoxigenic bacteria's such as *Salmonella typhimurium*. However, this effect was not seen in post weaning stages [38]. Other study on *Salmonella* infection suggested a reduction in growth and feed intake of an animal beside changes in its cecal microbial community [4,5]. Zinc has a two-pronged effect on gut as it not only enhances gut health but also affects immune system during attachment of certain virulence factors [39]. However, Sometimes high doses of zinc may need to be avoided after weaning as some studies also suggest that post-weaning zinc supplementation has no effect on average daily food intake (ADFI), average daily live weight gain (ADG) and food conversion ratio (FCR) of piglets [40].

Study Ref	Study Design	Participants	Intervention	Main Outcomes	Additional Remarks
Shao [39]	Randomized trial	180, 1-day-old Male broiler chicks	Unchallenged, *S. Typhimurium*-challenged, and *S. Typhimurium*-challenged were treated with 120 mg/kg of zinc supplementation in the diet.	Zinc supplementation enhanced growth, intestinal shape, and intestinal microbiota in *S. Typhimurium*-challenged broilers.	
Reed [27]	Randomized trial	12 chicks	2 treatment groups: 1) Zn(+): 42 µg/g zinc. 2) Zn(−): 2.5 µg/g zinc	Phylum level difference in gut microbiota composition between normal and chronic zinc deficient chicks	Further research needed in zinc clinical biomarker.
David [39]		Male Mice, 28 days old	Challenged with EAEC	EAEC pathogens count were increased in zinc-deficient mouse	
Broom [40]	2 × 2 factorial experiment	208 piglets	3100 mg ZnO/kg feed), and *E. faecium* SF68 supplementation 1.4 × 10^9 CFU/kg feed	ZnO and *E. faecium* SF68 are found to be not effective in the trial conditions.	Further research is needed to study the immunomodulatory role of zinc
Molist [41]	2 × 2 factorial experiment	64 piglets	WB (0 *vs.* 40 g/kg) and ZnO (0 *vs.* 3 g/kg) in the diet	ZnO shown good results in feed intake, growth and reduced the incidence of diarrhea, the negative impact was correlated with wheat bran (WB) as it increases *E. coli* count.	Does not provide evidence that how ZnO blockage causes increase of *E. coli*
Vahjen [42]		336 piglets	57 (low), 164 (intermediate) or 2425 (high) mg kg^{-1} analytical grade ZnO	Dietary zinc are suggested to be avoided after 2 weeks of post-weaning in pigs, as due to the possible increase of antibiotic resistance in Gram-negative bacteria	
Herrero-Fresno [43]		4 pigs	Repetitive extragenic palindromic-PCR (REP-PCR)	Correlation was not observed between REP-profiles, ST-types and resistance/virulence patterns	This is the first study analyzing in depth the genetic variability of commensal *E. coli* from pigs

Table 1: Animal studies suggesting role of in modifying gut microbiota; [ZnCP=Zinc-bearing Clinoptilolite; EAEC=Enteroaggregative *Escherichia coli*; dZD=Zinc deficient diet; ST=Sequence type; ZnO=Zinc oxide, REP=Repetitive extragenic palindromic; CFU=Colony forming unit].

Furthermore, increase in the concentration of phytase enzyme is important in dietary supplementation of post-weaning piglets as its absence or reduced concentration is linked to increased *E. coli* numbers in wheat bran (WB) [41]. However, no evidence regarding mechanism of increase in *E. coli* after ZnO blockage is not clear. It may be that zinc oxide increases resistance to Gram negative bacteria [42], the mechanism of which needs further investigation [43,44].

Studies on chicks have suggested that there may have a decrease of cecal zinc concentration at germ free chicks when compared to their counterparts. Beside alteration occur at the phylum levels in gut microbiota composition between normal and chronic zinc deficient chicks [27]. Evidence (Table 1) shows overall summary of listed studies.

Human Studies Suggesting Role of Zinc in Modifying Gut Microbiota

In humans, zinc medicines are mainly used for the prevention and treatment of loose bowels [45], and less often in connection with the improvement of immune response [46], and metabolic and epithelial permeability [3].

In developing countries [47,48] such as Bangladesh [45] Pakistan [49] and India [50] zinc is inexpensive, simple and affordable strategy to overcome diarrhea [51].

Study	Study type	Participants	Treatment	Main Outcomes
Roya [45]	Randomized double blind controlled trial	111 children, 3 to 24 months' old	Treatment Group: 20 mg zinc/day Control Group: Zinc-free diet	Weight gain in children with the treatment of diarrheal complication through zinc intervention
Sazawal [50]	Double-blind, randomized, Controlled trial	937 children, 6 to 35 Months of age	20 mg zinc/day	For infants and young children Zinc supplementation reduce duration and the severity of diarrhoea.

Table 2: Humans studies suggesting role of zinc supplementation on gut microbiota.

Limited evidence is available on the effect of zinc on the richness and diversity of gut microbiota in humans. Some evidence suggests that zinc supplementation have a negative impact on the counts of diarrhoea-causing agents such as *E. coli* [46]. Moreover it has also been seen that beneficial bacteria e.g. *Lactobacillus* (probiotic) and *Streptococcus* [52] in gut are increased with zinc supplementation. Evidence (Table 2) shows overall summary of listed studies.

Conclusion

In conclusion, this literature review provides potential mechanisms that could explain the possible relationship of zinc supplementation on the gut microbiota and its interaction with gut epithelium, reduction of inflammation of intestinal mucosa and improvement in host immune system. Although the impact of zinc supplementation on potentially pathogenic gut microbiota in humans such as *Campylobacter jejuni* is limited.

Acknowledgment

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