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Zinc Sulfate and Omega-3: Do They Have a Role in Environmental Enteric Dysfunction ?

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Environmental enteric dysfunction (EED) is a subclinical, chronic inflammatory condition of the gut. The purpose of the study: The purpose of this study is to evaluate the effects of zinc sulphate and omega-3 supplementation on anthropometric measurements and faecal EED biomarkers (α-1-antitrypsin (AAT), Neopterin (NEO), and Myeloperoxidase (MPO) in underweight and stunted children as an intervention for EED. An interventional study included 105 underweight and stunted children, divided into two subgroups: one subjected to intervention with zinc supplementation (55 children) and the other subjected to intervention with omega-3 supplementation (50 children) for 6 months. Assessment of anthropometric measurements and faecal EED biomarkers: AAT, NEO, and MPO. Regarding the zinc intervention group, post-intervention weight, weight z score, height, height z score, and BMI z score were highly significantly improved after 6 months of zinc supplementation (p value ≤ 0.001). Serum zinc level was highly significantly increased after supplementation (p value ≤ 0.001), while AAT and NEO were highly significantly and significantly decreased (p value ≤ 0.001) (p value ≤ 0.05) respectively. Regarding the omega-3 intervention group, post-intervention weight, weight z score, height, and height z score were highly significantly improved after 6 months of omega-3 supplementation (p value ≤ 0.001). Meanwhile, no significant change was observed for serum iron and zinc level (p value ≥ 0.05) or EED faecal markers except for AAT, which was highly significant for decreasing after supplementation (p value ≤ 0.001). A significant increase in weight, height, and serum zinc level was observed in the zinc supplementation group more than in the omega-3 supplementation group (p value ≤ 0.05). Alongside no significant difference post intervention in EED fecal markers between the two groups (p value ≥ 0.05). No definite drug intervention or supplementation is documented as appropriate management. Zinc sulphate supplementation is thought to be more beneficial than omega-3 supplementation, as evidenced by the improvement of anthropometric measurements and decrease of EED faecal markers.

Keywords: Environmental Enteric Dysfunction; Omega-3 Fatty Acids; Malnutrition; Stunting; Zinc.

Environmental enteric dysfunction (EED) is a chronic, gut-inflammation-related subclinical disease. It is attributed epidemiologically to low income, poor sanitation, and poor water supply areas, so it is more prevalent in developing countries and low socioeconomic classes [1].

Almost every developing country has defined altered small intestinal structure and

function [2]. It is characterised by moderate to severe crypt hyperplasia, reduced absorption, increased small intestinal permeability, and inflammatory T cell infiltration that causes gut microorganisms and endotoxins to translocate as well as nutrient malabsorption [3,4], This continuous immune and inflammatory response makes the gut sluggish and resistant to repair and healing, leaving the gut vulnerable to additional damage [5]. EED and malnutrition are concomitant with physical and cognitive development problems that impact childhood morbidity and mortality in the long run [2,6].

In low income countries, young children are impacted by micronutrient deficits, impairing their growth and health. The main causes of micronutrient deficiency are poor gut function and malabsorption, in addition to low dietary intake, which appear to cause and result in EED [7].

Zinc is one of the micronutrients responsible for epithelial integrity, mediating immune response, and ensuring gut health [7]. Zinc promotes healthy growth, immunocompetence, and neuro-behavioral development in children. Stunting is linked to a low zinc intake. Low zinc is associated with inflammation. Low zinc level may be accused in entropathy [8]. It is commonly found to coexist with EED and aggravate the condition [9].

Omega-3 is a long-chain polyunsaturated fatty acid; it has been attributed to improving enteric epithelial structure and function. Long-chain polyunsaturated fatty acids have a wide range of anti-oxidant and anti-inflammatory effects [10]. Omega-3 has an antiinflammatory role by mediating the action of different cytokines involved in the inflammatory response [11–13].

Omega-3 has a positive relationship with gut microbiota [14]. It has an antibacterial effect on some gut bacteria. It affects the intestinal bacteria and improves the gut intestinal microenvironment, which improves intestinal mucosa barrier function [15].

EED is commonly subclinical and hidden, and the affected children are frequently exposed to increased incidences of infection, poor oral vaccine responses, and faltering and stunted growth [16,17].

The striking prominence of child health agenda globally is nutrition and gut health. Improving the gut health is a golden target to raise the enteric function during early life that required to face hazards of malnutrition and stunting.

Aim of the study

Improving the nutritional composition of complementary foods for studied cases by supplementation with two compounds, zinc sulfate and omega-3, correlating their effect to fecal EED biomarkers; α-1-antitrypsin (AAT), Neopterin (NEO) and Myeloperoxidase (MPO).

SUBJECTS AND METHODS

This is a case-control interventional study that started in December 2019 and will last until April 2021. It is part of Project No. 12060128 funded locally by the National Research Center (NRC), Cairo, Egypt. The study is carried out in the Medical and Scientific Center of the NRC, at the Child Health Clinic.

The case group involved 105 children of both sexes, aged 1–10 years.

Inclusion criteria

underweight children (weight for age z-score [WAZ] <-2 and/or stunted (height for age z-score [HAZ] <-2), as defined by the World Health Organization Child Growth Standards [18].

Exclusion criteria

children with genetic disorders or congenital abnormalities. children with chronic debilitating illnesses such as chronic renal problems, congenital heart diseases, neurological problems, or developmental disabilities. children with diarrhoea or hemotochezia. children with parasitic infestation revealed by stool analysis at the time of study.

Control group

100 healthy children with WAZ and HAZ > -1, matched by age and sex for the case group.

All children were subjected to the following:

A thorough history is taken, focusing on any family history of short stature or wasting, chronic illnesses such as diabetes and high blood pressure, medication use, and food history. A thorough clinical evaluation focuses on anaemia and vitamin deficiency symptoms. Based on methods outlined in the Anthropometric Standardization Reference Manual [19,20], anthropometric measurements were taken. Children were weighed (in kg) on a calibrated Seca scale (Hamburg, Germany) down to

the nearest 0.1 kg and their heights (in cm) down to the nearest 0.1 cm on a Seca 225 stadiometer. Using a flexible graded tape, the left upper arm's mid-arm circumference was measured at a location halfway between the humorous and elbow tips. AnthroPlus Pediatric's calculator application calculated the subjects' height, weight, and BMI Z-scores [21].

Laboratory investigations

Each youngster had a total of three millilitres of venous blood drawn while fasting for eight hours. A portion was maintained in a tube containing ethylenediaminetetraacetic acid (EDTA) for automated analysis to determine the full blood count (Cel-Dyn.3500; Abbott Diagnostics, Abbott Park, IL). To measure the levels of zinc and iron, the other portion was centrifuged for 10 minutes. at 3000 rpm, then stored at -80 °C. Using the 5-Brom-PAPS colorimetric technique, serum zinc was quantified. The colorimetric CAB method was used to measure the level of serum iron. The Egyptian Company for Biotechnology (S.A.E.) in Obor City Industrial Area, Block 20008, Cairo, Egypt, was where both kits were purchased. Samples of the faeces were taken, fixative-free, and frozen at -70 °C. Using ELISA kits, specimens were tested for MPO, NEO, and AAT (SunLong Biotch Co., LTD). The AAT detection range for the catalogue number was (0.5-40 ng/ml). The SL1230HU catalog's The SL1847Hu MPO detection range was 0.2–10 ng/mL. The NEO detection range, catalogue number: SL2303Hu, was 3-100 pg/mL.

Phase I of this study was a case-control study, involving assessment of anthropometric measurements for the study group and comparing them with those of the control group, adding to the evaluation of selected serum biomarkers of EED in comparison with the control group [22], and evaluation of selected faecal biomarkers of EED in comparison with the control group [23]. Phase II of this study was An interventional study that applied on cases in phase one was concerned with the studied case group as it was divided into two subgroups: group (I) was subjected to intervention with oral zinc sulfate supplementation (20 mg/day), and group (II) was subjected to intervention with oral omega-3 supplementation (500 mg/day), beside providing proper nutritional education followed by reevaluation after six months for anthropometric measurements, serum, and faecal biomarker levels of EED.

Sample size

There were no previous studies comparing interventional techniques in this age group. All the studies found were localised and restricted to certain ages; they were not fully applied and researched, so the calculation of sample size couldn't be accurately measured. This study can be considered a "pilot study" to evaluate the feasibility of some crucial components of future, larger-scale studies.

Ethical approval

This study was submitted under local projects funded by the NRC (Project No. 12060128) and approved by the Medical Ethical Committee of the NRC (19/227). A written informed consent was signed from the gradients of children after explanation of the objectives and methodology of the study.

Statistical analysis

Data was initially collected, verified, and coded, followed by manual entry and processing into an Excel sheet. The data was submitted to the Statistical Package for the Social Sciences (SPSS) version 23 (SSPS Inc., Pennsylvania, USA) for manipulation and analysis. Each piece of numerical and nominal data was described statistically. Comparative tests were done between groups, however, correlation tests were handled between parameters. The p-values were two-tailed and established to determine the statistically significant difference at d" 0.05.

RESULTS

A total 105 children of both sexes, their age ranged from 24-155 months, with Mean ± SD 79.71 ± 34.3 months. In the intervention phase of this study, the children are divided into two groups. Group (I), which included 55 children, was subjected to zinc supplementation for 6 months. group (II), which included 50 children and received omega-3 supplementation for 6 months (Table 1).

There was a highly significant increase in the anthropometric measurements post-intervention when compared with the pre-intervention measurements (p value ≤ 0.001), which appeared clear in weight, weight z score, height, height z score, and arm circumference (Table 2).

Assessment of serum zinc and iron for all the subjected cases revealed highly significant increase in post intervention serum zinc level, while no significant difference was found in serum iron post intervention (p value ≤ 0.001) (p value ≥ 0.05) respectively. On the other hand, assessment of fecal markers of EED showed highly significant and significant decrease in AAT and NEO post intervention (p value ≤ 0.001) (p value ≤ 0.05) respectively. However, no significant difference was found in MPO post-intervention (p value ≥ 0.05) (Table 3).

Regarding the zinc intervention group, post-intervention anthropometric parameters weight, weight z score, height, height z score, and BMI z score) were highly significantly improved after 6 months of zinc supplementation (p value ≤ 0.001) (Table 4). Concurrently serum zinc level was highly significantly increased after supplementation (p value ≤ 0.001) and EED fecal markers AAT and NEO were highly significantly and significantly decreased (p value ≤ 0.001) (p value ≤ 0.05) respectively (Table 5).

Regarding the omega-3 intervention group, post-intervention anthropometric parameters weight, weight z score, height, and height z score were highly significantly improved after 6 months of omega-3 supplementation (p value ≤ 0.001) (Table 6). Meanwhile no significant change for serum iron and zinc level (p value ≥ 0.05) or EED fecal markers were observed except for AAT which was highly significantly reduced after supplementation (p value ≤ 0.001) (Table 7).

The comparison between the zinc intervention group and the omega-3 intervention group showed a significant increase in weight, height, and serum zinc level in the group supplemented with zinc more than the group

Table 1. Descriptive data for the subjects and compared groups:

	Data	Frequency	Percent	Total
Sex	Male	47	44.8%	105 (100%)
	Female	58	55.2%	
Drug intervention groups	Zinc group	55	52.4%	105 (100%)
	Omega-3 group	50	47.6%	
	Mean ± SD	Range		
Age (months)	79.71 ± 34.3	24 -155		

Table 2. Comparison between pre- and post-interventional anthropometric variables in all intervention subjects

	Cases no = (105)	Mean ± SD	t-test	p
Weight (kg)	pre-intervention	18.12 ± 5.85	-12.61	0.000*
	post-intervention	20.26 ± 6.31		
Weight z score	pre-intervention	-1.99 ± 0.66	-5.42	0.000*
	post-intervention	-1.56 ± 0.85		
Height (cm)	pre-intervention	108.14 ±16.20	-17.80	0.000*
	post-intervention	113.50 ±15.34		
Height z score	pre-intervention	-2.20 ± 0.82	-6.10	0.000*
	post-intervention	-1.74 ± 0.77		
BMI (kg/m2)	pre-intervention	15.28 ± 1.35	-0.85	0.401
	post-intervention	15.39 ± 1.46		
BMI z score	pre-intervention	- 0.62 ± 1.04	-0.84	0.406
	post-intervention	- 0.54 ± 0.95		
Arm circumference (cm)	pre-intervention	16.61 ± 1.73	-3.82	0.000*
	post-intervention	17.18 ±2.03		

Independent t test. **p ≤ 0.001 (highly significant), *p ≤ 0.05 (significant).

Table 3. Comparison between pre- and post-interventional markers in all intervention subjects

	Cases no = (105)	Mean ± SD	t-test	p
Zinc (µg/dl)	pre-intervention	82.34 ±31.32	-3.54	0.001*
	post-intervention	99.92 ±37.32		
Iron (µg/dl)	pre-intervention	78.40 ±30.76	-1.59	0.115
	post-intervention	88.150 ±46.99		
α-1-antitrypsin (AAT)(ng/ml)	pre-intervention	10.99 ±6.90	6.16	0.000*
	post-intervention	6.75 ±6.41		
Neopterin (NEO)(pg/ml)	pre-intervention	29.99 ±21.63	2.57	0.012*
	post-intervention	24.52 ±14.88		
Myeloperoxidase (MPO)(ng/ml)	pre-intervention	2.92 ±2.13	0.17	0.866
	post-intervention	2.88 ±2.32		

Independent t test. **p ≤ 0.001 (highly significant), *p ≤ 0.05 (significant).

Table 4. Comparison between pre- and post-intervention anthropometric variables in the zinc intervention group

	Zinc group (55)	Mean ± SD	t-test	p
Weight (kg)	pre-intervention	19.31 ±6.22	-8.61	0.000*
	post-intervention	21.65 ±6.91		
Wt z score	pre-intervention	-1.94 ±0.64	-3.41	0.001*
	post-intervention	-1.57 ±0.93		
Height (cm)	pre-intervention	111.86 ±15.10	-10.981	0.000*
	post-intervention	116.80 ±14.66		
Ht z score	pre-intervention	-2.03 ±0.81	-3.621	0.001*
	post-intervention	-1.71 ±0.83		
BMI (kg/m2)	pre-intervention	15.31 ±1.46	-0.871	0.389
	post-intervention	15.47 ±1.83		
BMI z score	pre-intervention	-0.83 ±1.00	-2.08	0.044*
	post-intervention	-0.60 ±1.06		
Arm circumference (cm)	pre-intervention	16.91 ±1.91	-1.88	0.068
	post-intervention	17.37 ±2.45		

Independent t test. **p ≤ 0.001 (highly significant), *p ≤ 0.05 (significant).

Table 5. Comparison between pre- and post-intervention markers in the zinc intervention group

	Zinc group (55)	Mean ± SD	t-test	p
Zinc (µg/dl)	pre-intervention	82.46 ±26.30	-5.34	0.000*
	post-intervention	109.60 ±34.88		
Iron (µg/dl)	pre-intervention	73.25 ±30.77	-1.71	0.094
	post-intervention	89.59 ±51.12		
α-1-antitrypsin (AAT) (ng/ml)	pre-intervention	11.02 ±7.61	3.87	0.000*
	post-intervention	7.54 ±7.31		
Neopterin (NEO)(pg/ml)	pre-intervention	33.20 ±24.40	2.17	0.035*
	post-intervention	26.23 ±16.21		
Myeloperoxidase (MPO) (ng/ml)	pre-intervention	3.19 ±2.41	0.14	0.892
	post-intervention	3.15 ±2.45		

Independent t test. **p ≤ 0.001 (highly significant), *p ≤ 0.05 (significant).

supplemented with omega-3 (p value ≤ 0.05) (Table 8). Alongside no significant difference post intervention in EED fecal markers between the two groups (p value ≥ 0.05) (Table 9).

Serum zinc levels after intervention were significantly positively correlated with both the weight z score and the height z score, according to a study on the relationship between laboratory markers and anthropometric parameters. EED faecal markers NEO and AAT showed highly significant and significant negative correlations with weight z score, height z score, BMI, and BMI z score (p value ≤ 0.001) (p value ≤ 0.05) respectively) (Table 10).

As laboratory markers and anthropometric measurements were correlated, it was shown that the faecal markers NEO and AAT from EED showed highly significant and substantial negative correlations with the z scores for weight, height, BMI, and BMI, respectively (p value ≤ 0.001) (p value ≤ 0.05). (Table 11). On the opposite side no significant correlation was found between EED faecal markers and anthropometric parameters in the omega-3 intervention group (p value ≥ 0.05); however, serum zinc level was significantly positively correlated with weight z score and height z score (p value ≤ 0.05) (Table 12).

Table 6. Comparison between pre- and post-intervention anthropometric variables in the omega-3 intervention group

	Omega-3 (50)	Mean ± SD	t-test	p
Weight (kg)	pre-intervention	16.65 ±5.06	-10.41	0.000*
	post-intervention	18.61 ±5.10		
Weight z score	pre-intervention	-2.06 ±0.68	-4.30	0.000*
	post-intervention	-1.54 ±0.75		
Height (cm)	pre-intervention	103.63 ±16.53	-15.79	0.000*
	post-intervention	109.47 ±15.38		
Height z score	pre-intervention	-2.40 ±0.79	-5.10	0.000*
	post-intervention	-1.78 ±0.70		
BMI (kg/m2)	pre-intervention	15.25 ±1.22	-0.26	0.800
	post-intervention	15.30 ±0.84		
BMI z score	pre-intervention	-0.36 ±1.05	0.57	0.574
	post-intervention	-0.46 ±0.81		
Arm circumference (cm)	pre-intervention	16.27 ±1.47	-4.25	0.000*
	post-intervention	16.96 ±1.45		

Independent t test. **p ≤ 0.001 (highly significant), *p ≤ 0.05 (significant).

Table 7. Comparison between pre- and post-intervention markers in the omega-3 intervention group

	Omega-3 (50)	Mean ± SD	t-test	p
Zinc (µg/dl)	pre-intervention	82.19 ±36.77	-0.72	0.479
	post-intervention	88.45 ±37.30		
Iron (µg/dl)	pre-intervention	84.50 ±30.01	-0.28	0.783
	post-intervention	86.450 ±42.18		
α-1-antitrypsin (AAT)(ng/ml)	pre-intervention	10.95 ±6.017	4.88	0.000*
	post-intervention	5.79 ±5.05		
Neopterin (NEO)(pg/ml)	pre-intervention	26.18 ±17.36	1.37	0.179
	post-intervention	22.50 ±13.06		
Myeloperoxidase (MPO) (ng/ml)	pre-intervention	2.59 ±1.72	0.10	0.922
	post-intervention	2.57 ±2.15		

Independent t test. **p ≤ 0.001 (highly significant), *p ≤ 0.05 (significant).

DISSCUSSION

A high-priority research domain in EED is the identification of a panel of biomarkers that can be obtained easily without excessive labour or cost for the diagnosis of this condition and provide a valuable indication in follow-up treatment. Micronutrient deficiencies may contribute to EED pathophysiology as they are associated with abnormal entropathy biomarkers[24].

In the intervention phase of this study, the cases were divided into two subgroups, and each was subjected to a different supplementation for 6 months: group I was subjected to oral zinc sulphate supplementation (20 mg/day) and involved 55 children; group II was subjected to oral omega-3 supplementation (500 mg/day) and involved 50 children.

The serum zinc and iron levels in the early stages of our study showed a considerable decrease

Table 8. Comparison of post-intervention anthropometric variables according to drug intervention

Anthropometric variables post-intervention	Supplementation intervention groups	Mean ± SD	t-test	p
Weight (kg)	Zinc group	21.65 ±6.91	2.22	0.029*
	Omega-3 group	18.61 ±5.10		
Weight z score	Zinc group	-1.57 ±0.93	-0.19	0.846
	Omega-3 group	-1.54 ±0.75		
Height (cm)	Zinc group	116.80 ±14.66	2.20	0.030*
	Omega-3 group	109.47 ±15.38		
Height z score	Zinc group	-1.71 ±0.83	0.40	0.694
	Omega-3 group	-1.78 ±0.70		
BMI (kg/m2)	Zinc group	15.47 ±1.83	0.55	0.585
	Omega-3 group	15.29 ±0.84		
BMI z score	Zinc group	-0.60 ±1.06	-0.69	0.492
	Omega-3 group	-0.46 ±0.81		
Arm circumference (cm)	Zinc group	17.37 ±2.45	0.88	0.382
	Omega-3 group	16.96 ±1.45		

Independent t test. **p ≤ 0.001 (highly significant), *p ≤ 0.05 (significant).

Table 9. Comparison between post-intervention markers according to drug intervention

Post-intervention markers	Supplementation intervention groups	Mean ± SD	t-test	p
Zinc (µg/dl)	Zinc group	109.60 ±34.88	2.67	0.009*
	Omega3 group	88.45 ±37.30		
Iron (µg/dl)	Zinc group	89.59 ±51.12	0.30	0.764
	Omega3 group	86.45 ±42.18		
Myeloperoxidase (MPO.ng/ml)	Zinc group	3.15 ±2.45	1.15	0.254
	Omega-3 group	2.57 ±2.15		
Neopterin (NEO)(pg/ml)	Zinc group	26.23 ±16.21	1.14	0.258
	Omega-3 group	22.50 ±13.06		
α-1-antitrypsin (AAT)(ng/ml))	Zinc group	7.54 ±7.31	1.24	0.220
	Omega-3 group	5.79 ±5.05		

Independent t test. **p ≤ 0.001 (highly significant), *p ≤ 0.05 (significant).

in cases compared to controls. The connection between serum zinc and the WAZ and HAZ scores was favourable. Serum zinc has been identified as a contributing factor to HAZ and WAZ [23]. After drug intervention, the serum iron showed no significant difference in level between the pre- and post-intervention groups, neither for the zinc nor the omega-3 intervention groups. The serum zinc level was significantly higher in the zinc intervention group than in the omega-3 intervention group. A significant positive correlation was observed between serum zinc level and each of Weight z score and Height z score for all cases group post drug intervention and particularly omega-3 intervention group.

Zinc is an essential intracellular trace element. It acts as a catalyst, structural element of cells, gene expression regulator, and modelator ion for the metabolic process. Zinc deficiency results in lymphopenia, thymic atrophy, and impaired cell- and antibody-mediated immunological responses, which increase the frequency and duration of infections [25].

Zinc is involved in intestinal epithelial hemostasis, it plays a role in the proliferative function of enterocytes, renewal of intestinal

Table 10. Correlation between post-intervention anthropometric variables and post-intervention markers in all subjects

Post-intervention		Weight (kg)	Weightz score	Height	Heightz score	BMI (kg/m2)	BMIz score	Arm circumference (cm)
Zinc (µg/dl)	r	-.102	.338**	-.070	.529**	-.090	-.009	.009
	p	.362	.002	.530	.000	.420	.937	.939
Iron (µg/dl)	r	-.216	.158	-.227*	.064	-.004	.186	.009
	p	.051	.157	.040	.568	.972	.094	.937
Myeloperoxidase (MPO) (ng/ml)	r	-.035	.006	-.029	.025	-.060	-.038	-.003
	p	.752	.957	.795	.821	.595	.733	.982
Neopterin (NEO) (pg/ml)	r	-.161	-.387**	-.085	-.301**	-.264*	-.220*	-.151
	p	.149	.000	.447	.006	.017	.047	.178
α-1-antitrypsin (AAT) (ng/ml)	r	-.153	-.338**	-.078	-.303**	-.259*	-.095	-.094
	p	.170	.002	.486	.006	.019	.398	.406

Pearson's coefficient correlation test. **p ≤ 0.001 (highly significant), *p ≤ 0.05 (significant), P > 0.05 (insignificant).

Table 11. Correlation between post-intervention anthropometric variables and post-intervention markers in the zinc intervention group

Post-intervention zinc intervention Group		Weight (kg)	Weightz score	Height	Heightz score	BMI (kg/m2)	BMIz score	Arm circumference (cm)
Zinc (µg/dl)	r	.033	.273	.032	.150	.478	.703	.153
	p	45	45	45	45	45	45	44
Iron (µg/dl)	r	-.102	.267	-.017	.563**	-.188	-.157	.071
	p	.506	.076	.914	.000	.216	.304	.647
Myeloperoxidase (MPO) (ng/ml)	r	-.060	-.006	-.061	-.021	-.065	.003	-.056
	p	.697	.968	.692	.892	.672	.983	.720
Neopterin (NEO) (pg/ml)	r	-.199	-.476**	-.057	-.304*	-.361*	-.299*	-.133
	p	.191	.001	.709	.043	.015	.046	.390
α-1-antitrypsin (AAT) (ng/ml)	r	-.168	-.473**	-.055	-.465**	-.313*	-.131	-.047
	p	.270	.001	.720	.001	.036	.390	.761

Pearson's coefficient correlation test. **p ≤ 0.001 (highly significant), *p ≤ 0.05 (significant), P > 0.05 (insignificant).

Table 12. Correlation between post intervention anthropometric variables and post intervention markers in the omega-3 intervention group

Post-intervention omega-3 intervention Group		Weight (kg)	Weightz score	Height	Heightz score	BMI (kg/m2)	BMIz score	Arm circumference (cm)
Zinc (µg/dl)	r	-.182	.454**	-.183	.495**	.089	.218	-.148
	P	.281	.005	.279	.002	.601	.194	.382
Iron (µg/dl)	r	-.231	-.175	-.220	-.271	-.047	.062	-.023
	P	.169	.299	.191	.105	.783	.715	.892
Myeloperoxidase (MPO) (ng/ml)	r	-.115	.038	-.087	.084	-.100	-.080	.032
	P	.496	.825	.608	.620	.556	.636	.851
Neopterin (NEO) (pg/ml)	r	-.124	-.253	-.132	-.300	-.060	-.095	-.204
	P	.465	.130	.435	.071	.723	.575	.227
α-1-antitrypsin (AAT) (ng/ml)	r	-.156	-.143	-.118	-.078	-.184	-.037	-.205
	P	.355	.400	.485	.645	.277	.827	.223

Pearson's coefficient correlation test. **$p \leq 0.001$ (highly significant), *$p \leq 0.05$ (significant), $P > 0.05$ (insignificant).

epithelial cells, and maintaining crypt–villus axial structure. Moreover zinc controls the tight junction between intestinal cells and affects the intracellular connection between the intestinal cells. Zinc modulates cells producing lysozyme that defense against intestinal cells. Zinc impacts goblet cells responsible for mucin production. zinc affect the microbial balance in the intestine as it controls the immune pathway against microbes meanwhile, zinc impacts the microbes growth and reproduction [26].

Zinc homeostasis has been shown to be impaired in EED in a vicious circle. Disturbed intestinal structure reduces the absorptive capacity of zinc, while zinc deficiency exacerbates numerous pathways contributing to environmental entropathy, such as intestinal permeability, enteric infection, and chronic inflammation. Persistent zinc deficiency propagates the adverse outcomes of gut entropathy, which mediates malabsorption and impaired growth and development [27].

Previous results assessed for zinc and iron levels in poor rural borderlands areas showed lower serum levels in children less than 5 years old, suggesting increased demand and decreased intake due to malnutrition [28].

A meta-analysis study comparing food supplementation with zinc alone and supplementation with zinc and other micronutrients found significant improvements in zinc levels either alone or in combination with other micronutrients, as well as a decrease in the prevalence of zinc deficiency, a significant increase in child weight and a decrease in the incidence of diarrhea [29].

A meta-analysis of studies evaluating the zinc-enhancing effect on linear growth in children under the age of five revealed actual increases in linear child length, with strong recommendations for use as a supplement to reduce stunting in developing countries [30].

Zinc supplementation during infancy (6months-2years) showed beneficial results as it was associated with a significant increase in the average length difference after 6 months of zinc supplementation [31].

A previous study that determined supplementation with zinc sulphate for school children and respective assessments for weight, height, and upper arm span in comparison to a placebo group after 6 months revealed a significant increase in height growth in the zinc sulphate group when compared to the placebo group, but no significant difference was found regarding increase in weight [32]. Oppositely, WAZ score and serum zinc levels were significantly increased after 6 months of supplementation for children with failure to thrive under 6 years old, while HAZ score showed an improvement when compared with the baseline [33].

On the other hand, Lauer et al., provided an insufficient link between Zinc and multivitamin supplementation with EED [34]. Also, no effect was found for zinc-fortified food supplementation on zinc-deficient stunted children[35].

Meanwhile, Hinnouho et al. provided zinc had no impact on growth and EED, nor NEO or MPO faecal markers [36]. Also, none of the faecal markers of intestinal inflammation were associated with zinc absorption when controlling for dietary zinc [37].

Enterocytes, immune cells, mucus, microbiota, and antimicrobial factors are a constellation of contributing factors that control the harmony and optimum function of the intestinal gut barrier. Omega-3 fatty acids' protective mechanism to enhance the intestinal gut barrier function involves a partial change in phospholipid cellular structure, reduced inflammatory signalling pathways, inhibition of cellular activation and production of inflammatory cytokines, and promotion of protective mediators. Moreover, Omega-3 modulates some enzymes involved in the inflammation process, interrupting it [10].

Omega-3 can affect the gut microbiota's diversity, quantity, and rate of bacterial growth. It inhibits proinflammatory mediators and enhances the production of anti-inflammatory mediators [15].

Some other studies tested trial therapy for ameliorating the condition of EED in children 1-3 years old who were subjected to multiple micronutrients and omega-3 supplementations; the results were favourable and an improvement of the environmental entopathy condition was declared [38]. The study done in 2020 noted that omega-3 has a beneficial role in increasing children's height who suffer from stunting after two months of supplementation [39].

Likewise, Supplementation of omega-3 for rural infants aged 3months with follow up at 9 months in another double blinded case control study showed improvement of mid arm circumference, triceps skinfold thickness, and subscapular skinfold thickness lacking of other anthropometric measurements which may be due to the young age group subjected to the study [5]. However, another study found that wasting was not associated with any polyunsaturated fatty acids [40].

In the primary assessment phase of our study, AAT was significantly higher in the case group as compared to the control group, moreover In the pre-intervention stage of the trial, it had a substantial negative correlation with weight, height, WAZ, and HAZ scores. AAT has been declared an associative factor affecting HAZ and WAZ [23]. Regarding the intervention phase, the AAT had a significant level decrease in all case groups post-supplementation intervention as compared to the pre-intervention level, and that appeared clear in each zinc and omega-3 intervention group. The AAT also had a highly significant and significant negative correlation with weight z score, height z score, and BMI for all case groups post intervention, and particularly the zinc intervention group. Nevertheless, no significant correlation was found between it and any anthropometric measurements in the omega-3 intervention group.

The pre-intervention phase of the study for NEO declared a significant difference between cases and controls; additionally, there was a definite inverse relationship between NEO level and both the WAZ and HAZ scores. NEO has been declared an associative factor affecting WAZ [23]. In the intervention phase, the NEO had a significant decrease in all case groups post-supplementation intervention as compared to its pre-intervention level, and that appeared particularly clear in the zinc intervention group. For all case groups after supplementation intervention, a significant negative correlation was observed between NEO level and each of weight z score, height z score, BMI, and BMI z score, particularly in the zinc intervention group.

In the preliminary phase of the study, myeloperoxidase (MPO) did not show any significant difference in its level as compared to the control healthy group. However, it is negatively correlated with anthropometric parameters [23]. Similarly, the post-intervention results showed no significant difference between pre- and post-drug intervention in either the zinc or omega-3 intervention groups, and furthermore, no meaningful relationships between it and any post-drug intervention anthropometric parameters were discovered.

A different study declared that greater micronutrient intake was negatively associated with EED. MPO, a faecal marker, was linked to anaemia and high transferrin receptors, whereas AAT was linked to low ferritin. AAT had a lower

risk of low plasma zinc. Inverse associations between nutrient densities and micronutrient deficiency largely disappeared after adjustment for EED, suggesting that EED mediates these associations [7].

Perin et al.'s study stated anthropometric measurements were significantly lower and levels of AAT, NEO, and MPO were significantly higher in children with malnutrition when compared to controls [41]. Also, Kosek et al. attributed high levels of AAT, NEO, and MPO to impaired linear growth [42].

A Bangladeshi study carried out on children in the first two years of life showed elevated MPO levels, but not NEO or AAT levels, were associated with decreases in short-term linear growth during the second year of life, supporting previous data suggesting the relevance of MPO as a marker of EED [43].

On comparing both subgroups in the drug intervention phase of our study, it was found that there was a significantly greater increase in the mean SD of weight and height in the zinc intervention group than in the omega-3 intervention group. On the other side, only the serum zinc level was a significantly higher biomarker in the zinc intervention group than in the omega-3 intervention group. That may support the observation that using zinc supplementation as intervention management in EED may have had better results than using omega-3.

Meta-analyses of randomised controlled zinc supplementation intervention trials revealed that zinc supplementation resulted in highly significant positive increases in height and weight measurements; additionally, zinc supplementation resulted in a significant increase in the children's serum zinc concentrations; and growth responses were greater in children with low initial weight and height z scores [44].

Other studies revealed that vitamin A-treated children had a more rapid improvement in gut integrity than others but did not reach normalised standards, as vitamin A deficiency may influence gut integrity [45]. As well, a greater risk of multiple micronutrient deficiencies was associated with lower vitamin C intake and increased faecal concentrations of MPO [46].

Several other nutritional approaches and trial interventions have been proposed to manage EED, but the results have been mixed. Some trials involved antibiotic administration [47], other trials provided gut probiotics to enhance the gut microbiome [48], others suggested vitamin A supplementation [45], alanyl-L-glutamine [49], albendazole and zinc [50], long-chain polyunsaturated fatty acids [5], multiple micronutrient supplements [38], lactoferrin and lysozyme [38].

Suggesting treatment with antibiotics to treat travellers' diarrhoea and bacterial overgrowth provided insufficient evidence as the basic line of EED treatment [47]. The same was found in another study that found treatment with probiotic Lactobacillus to be inefficient [48].

Costa et al., used seven 24-hour dietary recalls for infants aged 9 to 15 months to examine usual dietary intake from complementary feeding in relation to EED faecal biomarkers. At 15 months of age, faecal biomarker concentrations of AAT, MPO, and NEO were associated with elements of complementary food intake, indicating that they can help with EED [51].

Another study looked at interventions for improving poor water, sanitation, and hygiene environmental factors, as well as their impact on anthropometric measurements and the EED faecal biomarkers AAT, MPO, and NEO. The intervention was inversely associated with AAT level and had an inverse association between MPO and HAZ [1].

CONCLUSION

To summarise the EED management map strategy, it entails a constellation of different strategies; no specific drug intervention or supplementation is documented as appropriate management. Micronutrient deficiencies are believed to contribute to EED pathophysiology. Zinc sulphate supplementation is thought to be more beneficial than omega-3 supplementation, as evidenced by the improvement of anthropometric measurements and decrease of EED faecal markers. However, EED seemed to be clinically silent and asymptomatic, but its effect on growth is massive, and its full effect on the child cannot be measured because it persists all through the child's life Thus, the problem and trials of optimum management should continue to be explored for the benefit of future generations.

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Authors' contributions

All authors contributed to the intellectual content of the manuscript and approved its submission.

Conflicts of Interest

There is no conflict of interest.

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