Micronutrient supplementation as an interventional therapy for growth faltering in children with environmental enteric dysfunction

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Background
Environmental enteric dysfunction (EED) is a subclinical disorder which affects the small bowel of children, mainly living in developing countries. Zinc acts a major function in intestinal cells proliferation and crypt-villus structure preservation. Omega-3 fatty acids modulate some enzymes implicated in intestinal inflammation.
Objective
This research was performed to assess the effect of receiving both zinc and omega-3 supplements on anthropometric parameters and serum markers levels of EED [high sensitive C-reactive protein (hsCRP), Alpha-1-acid glycoprotein (AGP), tumor necrosis factor alpha (TNF-α), zonulin, and antibody of endotoxin core (EndoCAb)]. In those stunted kids with EED, this evaluation may lead to enhancing the nutritional composition of complementary food introduced to stunted and malnourished children having EED.
Materials and methods
This interventional study included 105 stunted and/or underweight children who were diagnosed as EED patients. They were subdivided into two groups; group I: 55 children receiving zinc sulphate and group II: 50 children receiving omega-3. Quantification of serum markers of EED (hsCRP, AGP, TNF-α, zonulin, antibody of endotoxin core) in addition to serum vitamin D, along with assessment of anthropometric parameters were performed to those children 6 months after zinc and omega-3 supplementation
Results and conclusion
In all subjects postintervention group, anthropometric parameters [height for age z-score (HAZ) score, weight for age z-score (WAZ) score and arm circumference] increased significantly 6 months after supplementation, however serum markers of EED (AGP, hsCRP, TNF-α and zonulin) decreased significantly 6 months after supplementation. Vitamin D level correlated positively with weight for age z-score and height for age z-scores.
Conclusion
Oral zinc sulphate and omega-3 may be added to EED management protocol to improve anthropometric parameters and decrease serum markers of EED.

Keywords:
anthropometric parameters, environmental enteric dysfunction, omga-3 supplementation, serum markers, zinc supplementation

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Introduction

Environmental enteric dysfunction (EED) is a subclinical disorder which affects the small bowel of children, mainly living in developing countries with poor access to safe water and sanitation conditions. It may result from exposure to environmental pathogens and toxins. Faltering growth in EED could be worsen by acute malnutrition episodes due to recurrent gastrointestinal and chest infection [1]. Faltering growth including underweight, stunting and wasting remains a major worldwide health challenge. Globally, both stunting and wasting affect about 165 million children and 52 million children, respectively [2]. Small bowel biopsy through invasive endoscopy is the golden diagnostic method for EED, so group of serum and fecal biomarkers were used to detect intestinal inflammation and increased intestinal leakage involved in EED pathogenesis [3]. Alpha-1-acid glycoprotein (AGP), highly sensitive C-reactive

protein (hsCRP) and tumor necrosis factor alpha (TNF-α) are acute phase reactants proteins. They are produced by liver as a results of both infectious and noninfectious inflammatory conditions. These serum markers were documented to be systemic markers of intestinal inflammation [4]. The antibody of endotoxin core (EndoCAb); a microbial translocation marker which is produced by B cells in response to repeated bacterial intestinal infections. It evaluates the antibodies level produced against lipopolysaccharides (LPs) of gram-negative bacteria translocating through a leaky gut [3]. Zonulin is known to affect tight junctions between intestinal cells, so the increase in its level indicates impaired intestinal permeability [5]. Zinc plays a major function in intestinal cell proliferation and crypt-villus structure preservation. Disturbed intestinal mucosa integrity impairs zinc absorption. Also, zinc deficiency may contribute to EED pathogenesis via several mechanisms such as, impaired intestinal permeability, chronic intestinal inflammation and repeated enteric infections [6]. Omega-3 fatty acids modulate some enzymes implicated in intestinal inflammation. They have protective roles in enhancing the intestinal barrier through partial change in phospholipid structure and reducing inflammatory signaling pathways [7]. Our research was performed to assess the effect of receiving both zinc and omega-3 supplements on anthropometric parameters and serum markers of EED (hsCRP, AGP, TNF-α, zonulin and EndoCAb) in children with EED. This evaluation may lead to enhancing the nutritional composition of complementary food introduced to stunted and malnourished children with EED.

Materials and methods

This current work began in December, 2019 and ended in April, 2021. It is a part of local Project (No. 12060128) which is funded by the National Research Centre (NRC), Cairo, Egypt. This research was conducted at the Medical Scientific Centre of Excellence (MSCE)-NRC.

Our project included 220 children representing both sexes, aged from 1 to 10 years whose weight for age z-score [WAZ] score is less than −2 (underweight kids) and/or kids whose height for age z-score [HAZ] score is less than −2 (stunted kids). They were identified by the World Health Organization Child Growth Standards [8]. Out of these children, 105 kids were detected to have EED by both fecal and serum markers [9,10]. This last group were subjected to an intervention study (pre and post study) in which this group was divided into two subgroups; the first group: 55 children who received oral zinc sulfate supplements (20 mg/day) for 6 months and the second group: 50 children who received oral omega-3 supplements (500 mg/day) for 6 months. Reevaluation of anthropometric parameters, serum and fecal biomarker levels of EED were done for these children.

All kids during this intervention study were subjected to the following:

Careful history taking, stressing on any family history of stunting or wasting, chronic illnesses, such as diabetes mellitus and hypertension, drug usage, and nutritional history. A thorough examination, focusing on anemia and vitamin deficiency symptoms and signs. Anthropometric parameters were assessed based on methods outlined in the Anthropometric Standardization Reference Manual [11,12]. The children weight (in kg) was measured using 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 children left mid-arm circumference was measured using a flexible graded tape, at a location halfway between the humerus and elbow tips. AnthroPlus Pediatric's calculator application calculated the patients' height, weight, and BMI Z-scores [13].

Patients excluded were kids having chronic debilitating illnesses; such as chronic renal problems, congenital heart diseases, neurological affection and developmental delay. Kids with acute diarrheal episode or hematochezia. Kids with parasitic infections discovered by stool examination done during our study. Also, genetic abnormalities and congenital diseases were excluded from our study.

Laboratory investigations

Sample of 3 ml of venous blood was withdrawn from every child. These samples were collected in a vacutainer blood collecting tubes, then samples were subdivided into two parts; the first part was added to a tube containing ethylenediaminetetraacetic acid (EDTA) for complete blood count determination using an automated analyzer (Cel-Dyn.3500; Abbott Diagnostics, Abbott Park, IL). The second part was put in a serum separator tube, then centrifuged for ten minutes at 3000 rpm to separate the serum from remaining components. Serum was preserved in a sterile Eppendorph tubes and stored at −80°C. Quantification of zonulin, EndoCAb, hsCRP, AGP,

and TNF-α levels in serum was assessed by an ELISA method using kits purchased from (SunLong Biotch Co., LTD). Zonulin kit detection range was (30–1500 pg/ml) catalogue number: SL2712Hu. EndoCAb kit detection range was (3–120 pg/ml) catalogue number: SL3521Hu. AGP kit detection range was (1–80 ng/ml) catalogue number: SL1845Hu. TNF-α kit detection range was (20–400 ng/l) catalogue number: SL1761Hu. Vitamin D was determined by ELISA technique using commercially available ELISA kits (SunLong Biotch Co., LTD). Vitamin D detection range was (0.8–50 ng/ml) catalogue number: SL1831Hu.

Ethical approval

The interventional study was a part of local project. This project was 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 for all participants from their guardians after explaining the objectives and methodology of the study.

Statistical analysis

Statistical analysis was performed using statistical package for social sciences (SPSS) version 21 for windows (IBM Corp., Armonk, NY, USA). Categorical data were expressed as frequencies and percentage. Continuous data (anthropometric data and laboratory markers) were expressed as mean ± standard deviation, minimum and maximum. Paired *t*-test was used to compare preinterventional and postinterventional anthropometric and laboratory markers in all groups and in each drug interventional group. Student's *t*-test was used to compare anthropometric data and laboratory markers in postinterventional group according to drug intervention. *P* less than 0.05 was accepted as statistically significant. Pearson's correlation analysis was conducted to evaluate the association between postinterventional anthropometric data and postinterventional laboratory markers in all groups and in each drug interventional group.

Results and discussions

The present study involved 105 children representing both sexes. There were 58 girls (55.2%) and 47 boys (44.8%). The ages of these children ranged between 25 months and 160 months, with (mean±SD = 80±35 months). During this interventional stage of our project, studied kids were classified into two groups; the first group: (55 children who received oral zinc supplements for 6 months) and the second group: (50 children who received omega-3 supplements for 6 months). The characteristics of all kids in our study will be presented in Table 1.

A highly significant difference between pre and postinterventional groups concerning anthropometric parameters (height, HAZ score, weight, WAZ score and arm circumference) was observed (*P* value ≤ 0.001) (Table 2).

Serum biomarkers of EED (AGP, hsCRP, TNF-α, EndoCAb and zonulin) in all subjected children revealed significant decrease in AGP, hsCRP, TNF-α and zonulin levels (*P* values ≤0.001, 0.002, ≤0.001, ≤0.001, respectively), but there was no significant change in EndoCAb level. On the other hand,

Table 1 Characteristics of all post-intervention subjects and both drugs subgroups

Descriptive data	Frequency	Percent (%)	Total
Sex			
Male	47	44.8%	105 (100%)
Female	58	55.2%	
Drug interventional groups			
Zinc group	55	52.4%	105 (100%)
Omega-3 group	50	47.6%	
	Mean±SD	**Range**	
Age (months)	80±35	25–160	

Table 2 Anthropometric parameters before and after supplementation in all interventional subjects

Number of all cases = (105)	Mean±SD	*P*- value
Weight (kg)		
Before supplementation	18.12±5.85	0.000**
After supplementation	20.26±6.31	
Weight Z score		
Before supplementation	−1.99±0.66	0.000**
After supplementation	−1.56±0.85	
Height (cm)		
Before supplementation	108.14 ±16.20	0.000**
After supplementation	113.50 ±15.34	
Height Z score		
Before supplementation	−2.20±0.82	0.000**
After supplementation	−1.74±0.77	
BMI (kg/m^2)		
Before supplementation	15.28±1.35	0.401
After supplementation	15.39±1.46	
BMI Z score		
Before supplementation	− 0.62±1.04	0.406
After supplementation	− 0.54±0.95	
Arm circumference (cm)		
Before supplementation	16.61±1.73	0.000**
After supplementation	17.18 ±2.03	

Paired *t* test. ***P* less than or equal to 0.001 (highly significant), **P* less than or equal to 0.05 (significant).

serum vitamin D level showed significant increase in postinterventional group (*P* value ≤0.001) (Table 3).

The anthropometric measurements (height, HAZ score, weight, WAZ score and BMI score) increased significantly 6 months following receiving oral zinc supplements among the zinc interventional group (*P* value ≤0.001) (Table 4).

Serum markers of EED (AGP, hsCRP, TNF-α and zonulin) decreased significantly after zinc supplementation (*P* values 0.002, 0.001, 0.003, and ≤0.001, respectively), however there was no significant change in EndoCAb level among the zinc interventional group. Vitamin D level increased significantly within the same interventional group (*P* value ≤0.001) (Table 5).

The postinterventional anthropometric parameters (weight, WAZ score, height, HAZ score) increased significantly 6 months after omega-3 supplementation among the omega-3 postinterventional group (*P* value ≤0.001) (Table 6).

Serum markers of EED (AGP, hsCRP and zonulin) decreased significantly after omega-3 supplementation (*P* values 0.012, 0.003, and ≤0.001, respectively), while no significant difference was observed in both TNF-α and EndoCAb levels among the omega-3 postinterventional group. Vitamin D increased significantly in the same group (*P* value ≤0.001) (Table 7).

In all patient's postintervention, vitamin D showed significant positive correlations with both WAZ and HAZ scores (*P* values 0.008 and 0.002, respectively),

Table 3 Vitamin D and environmental enteric dysfunction serum biomarkers before and after supplementation in all studied kids

Number of all cases=(105)	Mean±SD	*P*- Value
Vitamin D (Pg/ml)		
Before supplementation	10.72±8.97	**0.000****
After supplementation	20.53±10.80	
Alpha-1-acid glycoprotein (AGP) (ng/mL)		
Before supplementation	16.61±14.95	**0.001****
After supplementation	11.48±11.21	
Tumor necrosis factor alpha (TNF-α) (ng/l)		
Before supplementation	44.02 ±28.28	**0.001****
After supplementation	30.84±23.008	
Endotoxin core antibody (EndoCAb) (Pg/ml)		
Before supplementation	14.64±14.005	0.810
After supplementation	14.94±9.787	
Zonulin (pg/ml)		
Before supplementation	554.92±128.35	**0.000****
After supplementation	306.50±127.80	
hsCRP(mg/l)		
Before supplementation	4.73±1.2	**0.002***
After supplementation	3.25± 0.9	

Paired *t*â test. ***P* less than or equal to 0.001 (highly significant), **P* less than or equal to 0.05 (significant).

Table 4 Anthropometric parameters in kids receiving zinc before and after its supplements

Kids receiving zinc supplements group (55 cases)	Mean±SD	*P*- value
Weight (kg)		
Before supplementation	19.31±6.22	0.000**
After supplementation	21.65±6.91	
Weight Z score		
Before supplementation	−1.94±0.64	0.001**
After supplementation	−1.57±0.93	
Height (cm)		
Before supplementation	111.86±15.10	0.000**
After supplementation	116.80±14.66	
Height Z score		
Before supplementation	−2.03±0.81	0.001**
After supplementation	−1.71±0.83	
BMI (kg/m^2)		
Before supplementation	15.31±1.46	0.389
After supplementation	15.47±1.83	
BMI Z score		
Before supplementation	−0.83±1.00	0.044*
After supplementation	−0.60±1.06	
Arm circumference (cm)		
Before supplementation	16.91±1.91	0.068
After supplementation	17.37±2.45	

Paired *t*â test. ***P* less than or equal to 0.001 (highly significant), **P* less than or equal to 0.05 (significant).

Table 5 Vitamin D and environmental enteric dysfunction serum biomarkers in kids before and after zinc supplements

Kids receiving zinc supplements group (55 cases)	Mean±SD	*P*- value
Vitamin D (Pg/ml)		
Before supplementation	11.76±9.07	0.000**
After supplementation	22.40±12.46	
Alpha-1-acid glycoprotein (AGP) (ng/mL)		
Before supplementation	17.82±16.79	0.022*
After supplementation	12.35±13.65	
Tumor necrosis factor alpha (TNF-α) (ng/l)		
Before supplementation	45.75±28.32	0.003*
After supplementation	30.65±17.81	
Endotoxin core antiody (EndoCAb) (Pg/ml)		
Before supplementation	16.07±18.12	0.565
After supplementation	14.91±10.02	
Zonulin (pg/ml)		
Before supplementation	567.53±132.20	0.000**
After supplementation	308.94±130.09	
hsCRP (mg/l)		
Before supplementation	4.85±1.3	0.001**
After supplementation	3.46±1.1	

Paired *t*â test. ***P* less than or equal to 0.001 (highly significant), **P* less than or equal to 0.05 (significant).

Table 6 Anthropometric parameters before and after receiving omega-3 supplements in kids receiving omega-3 group

Kids receiving omega-3 Supplements group (50 cases)	Mean±SD	*P*- value
Weight (kg)		
Before supplementation	16.65±5.06	0.000**
After supplementation	18.61±5.10	
Weight Z score		
Before supplementation	−2.06±0.68	0.000**
After supplementation	−1.54±0.75	
Height (cm)		
Before supplementation	103.63±16.53	0.000**
After supplementation	109.47±15.38	
Height Z score		
Before supplementation	−2.40±0.79	0.000**
After supplementation	−1.78±0.70	
BMI (kg/m^2)		
Before supplementation	15.25±1.22	0.800
After supplementation	15.30±0.84	
BMI Z score		
Before supplementation	−0.36±1.05	0.574
After supplementation	−0.46±0.81	
Arm circumference (cm)		
Before supplementation	16.27±1.47	0.000**
After supplementation	16.96±1.45	

Paired *t*â test. ***P* less than or equal to 0.001 (highly significant), **P* less than or equal to 0.05 (significant).

while EndoCAb showed significant negative correlations with both weight and height (*P* values 0.025 and 0.034, respectively). AGP level correlated negatively with HAZ and WAZ scores (*P* value ≤0.001), also zonulin had significant negative correlations with HAZ and WAZ scores (*P* values 0.016, ≤0.001, respectively). There were significant negative correlations between hsCRP and weight, WAZ score, HAZ score, BMI Z score and arm circumference (*P* values 0.002, 0.021, 0.002, 0.004, and 0.003, respectively). (Table 8).

Among zinc postintervention group, there was significant positive correlations between vitamin D and weight and height (*P* values 0.33, 0.32, respectively), while there was significant negative

Table 7 Vitamin D and environmental enteric dysfunction serum biomarkers before and after receiving omega-3 supplements in kids receiving omega-3 group

Kids receiving omega –3 supplements group (50cases)	Mean±SD	*P*- value
Vitamin D (Pg/ml)		
Before supplementation	9.50±8.81	0.000**
After supplementation	18.33±8.03	
Alpha –1-acid glycoprotein (AGP) (ng/ml)		
Before supplementation	15.18±12.50	0.012*
After supplementation	10.46±7.42	
Tumor necrosis factor alpha (TNF-α) (ng/l)		
Before supplementation	41.97±28.48	0.090
After supplementation	31.06±28.20	
Endotoxin core antibody (EndoCAb) (Pg/ml)		
Before supplementation	12.94±6.22	0.126
After supplementation	14.97±9.63	
Zonulin (pg/ml)		
Before supplementation	539.98±123.71	0.000**
After supplementation	303.61±126.70	
hsCRP (mg/l)		
Before supplementation	4.52±1.1	0.003*
After supplementation	3.13±0.8	

Paired *t*â test. ***P* less than or equal to 0.001 (highly significant), **P* less than or equal to 0.05 (significant).

Table 8 Correlation between vitamin D, environmental enteric dysfunction markers and anthropometric parameters in all subjects after supplementation

Vitamin D and EED serum markers after supplementation		Weight (kg)	Weight Z score	Height (cm)	Height Z score	BMI (kg/m2)	BMI Z score	Arm circumference (cm)
Vitamin D (Pg/ml)	***r***	0.228*	0.292**	0.210	0.342**	0.060	0.108	0.145
	P	0.039	0.008	0.058	0.002	0.592	0.332	0.197
Tumor necrosis factor alpha (TNF-α)(ng/l)	***r***	0.091	–0.208	0.134	–0.189	–0.063	–0.111	–0.033
	P	0.416	0.061	0.230	0.089	0.573	0.322	0.772
Endotoxin core antibody (EndoCAb)(Pg/ml)	***r***	–0.247*	–0.051	–0.235*	–0.021	–0.152	–0.010	–0.049
	P	0.025	0.652	0.034	0.851	0.173	0.928	0.665
Alpha –1-acid glycoprotein (AGP) (ng/mL)	***r***	–0.165	–0.455**	–0.140	–0.472**	–0.175	–0.152	–0.154
	P	0.139	0.000	0.211	0.000	0.115	0.174	0.169
Zonulin (pg/ml)	***r***	–0.172	–0.266*	–0.192	–0.430**	–0.027	0.035	–0.148
	P	0.123	0.016	0.083	0.000	0.807	0.752	0.188
hsCRP(mg/l)	***r***	–0.473**	–0.435	–0.138	–0.537**	–0.150	–0.376**	–0.384**
	P	0.002	0.021*	0.160	0.002	0.126	0.004	0.003

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

correlations between EndoCAb level and both weight and height (*P* values 0.033 and 0.044, respectively). AGP level correlated negatively with HAZ and WAZ scores (*P* value 0.002), also zonulin showed significant negative correlations with HAZ and WAZ scores (*P* values 0.005 and 0.044, respectively). HsCRP level correlated negatively with weight, WAZ score, HAZ score, BMI Z score and arm circumference (*P* values 0.003, 0.042, 0.001, 0.005, and 0.004, respectively). (Table 9).

Among omga-3 postintervention group, there was highly significant positive correlations between vitamin D level and both WAZ and HAZ scores (*P* value 0.001), while AGP had highly significant negative correlations with WAZ and HAZ scores (*P* values 0.003 and 0.004, respectively). Zonulin showed high significant negative correlation with HAZ score (*P* value 0.004), also hsCRP showed significant negative correlations with weight, HAZ score, WAZ score, BMI Z score and arm circumference (*P* values 0.03,0.05, 0.005, 0.003, and 0.002, respectively). (Table 10).

Discussion

EED is a structural and functional disorder of the small bowel which primarily spreads among children living in

Table 9 Correlation between vitamin D, environmental enteric dysfunction serum biomarkers and anthropometric parameters in kids after zinc supplementation

Serum Vitamin D and serum markers of EED after zinc supplementation		Weight (kg)	Weight Z score	Height (cm)	Height Z score	BMI (kg/m2)	BMI Z score	Arm circumference (cm)
Vitamin D (Pg/ml)	*r*	0.318‘	0.167	0.319*	0.218	0.109	0.058	−0.219
	P	0.33	0.273	0.032	0.150	−478	0.703	0.153
Tumor necrosis factor alpha (TNF-α)(ng/l)	*r*	−0.059	−0.192	−0.018	−0.223	−0.106	−0.033	−0.031
	P	0.699	0.206	0.907	0.142	0.489	0.830	0.844
Endotoxin core antibody (EndoCAb)(Pg/ml)	*r*	−0.318*	−0.061	−0.301*	0.028	−0.255	−0.036	−0.114
	P	0.033	0.691	0.044	0.857	0.091	0.814	0.462
Alpha-1-acid glycoprotein (AGP) (ng/mL)	*r*	−0.097	−0.455**	−0.053	−0.451**	−0.168	−0.169	−0.148
	P	0.528	0.002	0.729	0.002	0.271	0.267	0.337
Zonulin (pg/ml)	*r*	−0.105	−0.302*	−0.092	−0.410**	−0.093	−0.051	−0.154
	P	0.494	0.044	0.547	0.005	0.545	0.740	0.318
hsCRP (mg/l)	*r*	−0.432**	−0.421*	−0.268	−0.585**	−0.223	−0.366**	−0.392**
	P	0.003	0.042	0.124	0.001	0.172	0.005	0.004

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

Table 10 Correlation between vitamin D, environmental enteric dysfunction serum biomarkers and anthropometric parameters after omega-3 supplementation

Serum Vitamin D and serum markers of EED after omega 3 supplementation		Weight (kg)	Weight Z score	Height (cm)	Height Z score	BMI (kg/m2)	BMI Z score	Arm circumference (cm)
Vitamin D (Pg/ml)	*r*	−0.114	0.516**	−0.108	0.543**	0.067	0.212	−0.007
	P	0.503	0.001	0.526	0.001	0.693	0.207	0.965
Tumor necrosis factor alpha (TNF-α)(ng/l)	*r*	0.334*	−0.236	0.308	−0.147	0.044	−0.249	−0.115
	P	0.044	0.160	0.064	0.386	0.797	0.138	0.497
Endotoxin core antibody (EndoCAb)(Pg/ml)	*r*	−0.143	−0.036	−0.163	−0.092	0.103	0.030	0.080
	P	0.399	0.833	0.336	0.587	0.543	0.861	0.639
Alpha-1-acid glycoprotein (AGP) (ng/mL)	*r*	−0.272	−0.468**	−0.243	−0.514**	−0.197	−0.137	−0.124
	P	0.104	0.003	0.147	0.001	0.243	0.417	0.464
Zonulin (pg/ml)	*r*	−0.261	−0.219	−0.290	−0.458**	0.159	0.163	−0.130
	P	0.119	0.193	0.082	0.004	0.347	0.335	0.444
hsCRP (mg/l)	*r*	−0.396*	−0.411*.	−0.252.	−0.472**.	−0.288.	−0.411**.	−0.432**.
	P	0.03	0.05	0.136	0.005	232.	003.	002.

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

developing countries with poor access to standard quality food and safe water supply [14]. Micronutrients deficiency is usually associated with elevated enteropathy biomarkers, so this may play a major role in EED pathogenesis [15].

In this interventional phase of our research, the studied cases were classified into two groups; the first group which involved 55 kids receiving oral zinc sulphate supplements (20 mg/day) for 6 months and the second group which involved fifty kids receiving omega-3 supplements (500 mg/day) for 6 month, as well.

Zinc deficiency is considered to be a great contributing risk factor for recurrent intestinal infections and impaired growth in under-5 children. This deficiency may lead to increased frequency and duration of intestinal infection by impairing both cell and antibody mediated immunities [16]. Our recent research showed high significant difference between pre and postinterventional studied groups in anthropometric parameters including height, HAZ score, weight and WAZ score. A previous meta-analysis studying the zinc-potentiating effects on children linear growth less than 5 years showed a significant increase in children height, with strong recommendations for zinc sulphate supplementation to decrease stunting incidence in developing countries [17].

During the initial stage of this interventional study, there was a significant decline in zinc level in

malnourished cases compared with controls. Lower zinc level correlated positively with anthropometric parameters and it was the most contributing factor affecting both WAZ and HAZ score [10]. The level of zinc in serum showed significant increase in children receiving zinc therapy than in children receiving omega-3 therapy. There was significant positive correlation between serum zinc level and both HAZ and WAZ scores in all post interventional groups, mainly in omega-3 group [18]. Persistent zinc deficiency aggravates gut enteropathy causing malabsorption and impairing growth during childhood period [19]. Zinc supplementation during infancy showed significant improvement in weight, WAZ score, height without any significant difference in HAZ score six months following zinc supplementation [2], while in another work, HAZ score increased significantly when compared with baseline in children with faltering growth under 5 years of age [20]. Abdolla and colleagues found that oral zinc supplementation for 3 months could affect linear growth improving height and HAZ score with limited effect on weight [21]. These findings are consistent with previous research indicating that zinc supplementation is more likely to affect bones and therefore linear growth than accumulation in muscles or fat mass. Zinc is abundant in bone tissues and is needed to preserve normal bone mineral density and maintain proper bone metabolism [17]. On the contrary, Jongstra and colleagues found no effect for zinc fortified food supplementation on kids having stunted growth [22]. Several studies were done to evaluate the effect of micronutrients and omega-3 therapy in children with EED. In a preceding work done by Smith and colleagues there was a significant increase in children height as well as improvement of environmental enteropathy [23]. This could be explained by omega-3 effect on intestinal microbiota's diversity increasing colonization rate. Moreover, omega-3 fatty acids inhibit pro-inflammatory cytokines action and promote the release of some anti-inflammatory mediators [24]. In another double blinded case-control study, there was a significant improvement in midarm circumference, subscapular skin fold and triceps skin fold in infants aged from 3 to 9 months [25]. On the other hand, a trial showed that there was no effect of long-term supplementation of multivitamins including omega-3 on either growth or micronutrient status in young children. These findings suggested a need to revise the cost effectiveness of widespread supplementation programs [26]. In the initial assessment phase of our study, AGP, hsCRP and EndoCAb increased significantly in the case group in comparison to controls. Zonulin, hsCRP, and AGP had significant negative correlations with both HAZ and WAZ scores [18].

Regarding the interventional phase, assessment of EED serum markers (AGP, hsCRP, TNF-α, zonulin and EndoCAb) revealed significant decrease in AGP, hsCRP, TNF-α and zonulin levels, but there was no significant change in EndoCAb level. AGP, hsCRP, zonulin and TNF-α decreased significantly among zinc interventional group, however AGP, hsCRP and zonulin showed significant decrease in omega-3 interventioal group. Serum vitamin D level increased significantly in all studied case group and both kids receiving zinc and omega-3 subgroups.

The level of AGP showed significant negative correlations with WAZ and HAZ scores in all case postinterventional group and both zinc and omega-3 intervention groups. HsCRP showed significant negative correlations with weight, HAZ score, BMI Z score and arm circumference in all case post interventional group, moreover it showed significant negative correlation with weight, WAZ score, HAZ score, BMI Z score and arm circumference in both zinc and omega-3 intervention groups. Zonulin had significant negative correlations with HAZ and WAZ scores in both all studied case group and zinc receiving subgroup, however it had significant negative correlation only with HAZ score in omega-3 intervention group. EndoCAb had significant negative correlations with weight and height in both all studied case group and zinc receiving subgroup. However, vitamin D level showed significant positive correlation with HAZ and WAZ scores in all studied case group and omega-3 receiving subgroup. Uddin and colleagues found that levels of EndoCAb increased overtime in children with impaired growth and were significantly higher at 24 months in comparison to 3 months. EndoCAb levels are more effective in direct measurement of endotoxins levels in reflecting exposure to endotoxins [27]. Meanwhile, in another study, EndoCAb titres were not correlated with HAZ score, so EndoCAb concentration is not related to linear growth in rural Malawian children [28]. Zonulin level decreased significantly during follow up studies and correlated positively with children stunting [29]. Deficiencies of specific micronutrients including folic acid, zinc, vitamin A, and vitamin B 12 have been shown to inhibit growth and intestinal mucosa turnover so, some of these micronutrients supplementation was found to reduce intestinal inflammation and improve linear growth in children with EED [30]. Systemic inflammatory biomarkers

like hsCRP and AGP had strong negative correlation with linear growth, so systemic inflammation may be a contributing pathway leading to faltering growth in children with EED. There was an increase in both CRP and AGP levels which was accompanied with yearly decrease in HAZ score. These systemic inflammatory markers had significant negative correlation with insulin growth factor-1 (IGF-1) which was in turn correlated positively with linear growth [14,31] Similarly, in another study, AGP and hsCRP serum levels showed significant negative correlation with HAZ score indicating the relationship between systemic inflammation and stunting [32]. Increased CRP and AGP levels were significantly accompanied by increased risk of subsequent stunting [33]. Neither zinc nor multivitamins supplementations ameliorated markers of systemic inflammation such as AGP and hsCRP in a randomized placebo-controlled trial applied on Tanzanian infants [34]. Mokhtar and colleagues observed strong positive correlation between vitamin D level and both HAZ and WAZ scores. Children having low vitamin D levels are more prone to be stunted and underweight [35]. Furthermore, Kremer and colleagues found high significant positive correlation between serum vitamin D level and height, therefore vitamin D could be an important and modifiable factor to prevent stunting [36,37]. Meanwhile, another study stated that, there was a significant relationship between circulating vitamin D level and wasting, but no relationship was found between its level and stunting as well as underweight [38,39].

Conclusion

Oral zinc sulphate and omega-3 may be added to EED management protocol to improve anthropometric parameters and decrease serum markers of EED. Micronutrients deficiency, such as zinc and vitamin D play a major role in EEF pathogenesis and long-term growth impairment in those children. Several researches should be continued on the effect of micronutrient supplementation and the benefits of adding food fortified with specific vitamins to improve growth in those children with faltering growth.

Strengths and limitations

Strengths: This evaluation may lead to enhancing the nutritional composition of complementary food introduced to stunted and malnourished children having EED.

Limitations: The budget was not enough to allow application of this study on lager number of children.

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Conflicts of interest

There are no conflicts of interest.

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