

focusing on prevention, treatment, and improved living conditions.

2. Continued research is needed to understand the complex interplay between EE, nutrition, and stunting.
3. Further research is needed to develop a combination of biomarkers that can better identify EED than any single biomarker.

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Environmental enteric dysfunction (EED) is a syndrome characterized by impairments of digestion and absorption and intestinal barrier failure in people living in insanitary or tropical environments. (Hodges, et.al, 2021)

Stunting is defined as height below 3rd centile or less than two standard deviations (SDs) below the median height for that age and sex according to the population standard. Height- for- age (HA) is a stunting indicator of chronic malnutrition based on the principle that an individual has an expected height for his age. It is an indicator of shortness/ tallness and low score is evidence of chronic under nutrition. (Al-Mansoob and Masood, 2018)

Fecal CP as biomarker of inflammatory diseases in the gut before the possibility to detect CP in the stool in 1992, clinicians relied on serological markers to assess the possibility (or severity) of gut inflammation. However, erythrocyte sedimentation rate and serum C-reactive protein (CRP) are elevated in response to various non-inflammatory processes and poorly correlate with patient symptoms and intestinal disease activity. (Jukic, et.al, 2021)

Similary, Humphrey, (2009) suspected also that many of these infections in children are subclinical and that diarrhea only accounts for a small proportion of EE.

Lefebo', et.al. (2023) study revealed that gut inflammation was associated with stunting. On the other hand, children with reported diarrhea within the last week were 6 times more likely for the probability of being stunted.

Our results revealed that Calprotectin were higher in cases with stool analysis infections with a statistically significant difference.

In line with our results; Mahmoud, et.al. (2021) cross- sectional study recruited patients whom presented with either upper or lower G.I.T symptoms and proved with inflammatory, infectious condition by histopathological examination of the G.I.T and fecal calprotectin level measurement for each patient, concluded that FC is a useful marker in the diagnosis of G.I.T inflammatory, infectious and malignant conditions.

Majority of cases in the study were having 3 meals per day with lunch was the main meal, however with no statistically significant relationship to gender. Most children who never or rarely had breakfast were females, while 61.7% of males always had breakfast. Among the total number of cases, the intake of water, fat, fibers were significantly inadequate. The intake of proteins was significantly higher than RDA. However, the intake of total calories, carbohydrates were adequate.

Among the total number of cases, the intake of potassium, calcium, magnesium, iron, vitamin A, vitamin C and vitamin B1 were significantly lower than recommended dietary allowance (RDA). The intake of sodium was significantly higher than RDA. On the other side, the intake of copper, zinc, phosphorus and vitamin B2 were adequate, compared to RDA. There was a positive statistically significant relationship between daily protein and carbohydrate intake with repeated gastroenteritis. On the other hand, there was a negative statistically significant correlation between daily intake of fresh fruits and vegetables with fecal calprotectin

level and another negative correlation between daily intake of dairy products to MCHC. Finally, there was a positive statistically significant correlation between weekly intake of carbohydrate and history of repeated attacks of diarrhea during first 2 years of life. There was no statistically significant correlation between weekly intake of other food groups to anthropometric measurements or laboratory investigation results.

This was in accordance with; Kelly (2021) EE is a very widespread alteration in gut structure and function which predisposes disadvantaged populations to the consequences of inflammation, leakiness and reduced surface area. These include micronutrient (deficiencies) through maldigestion, malabsorption, wastage of plasma constituents into the gut and possibly through inflammation- induced anorexia.

Similary, Fahim, et.al. (2022) this study reports the relationship between inflammatory biomarkers of EED and Multiple Micronutrient Deficiencies among young children. It also demonstrates that increased intake of vitamin C from complementary foods may reduce the risk of MMD. This finding emphasizes the necessity of increasing fresh fruit and vegetable intake during the early years of life. The results also imply the importance of effective strategies to prevent and control EED and improve nutrient intake in order to reduce MMD during the early years of life.

Vitamins A is belonging to fat- soluble group of vitamins. Vitamin A enhances gut mucosal lining and control permeability leading to defendant barrier against microbial infestation as reported by Thurnham, et.al (2000) and Loechl (2015). In our study vit A not meet RDA this adding factor to enhance EE. In addition, vitamin A has a modulating role in T lymphocytes production, which plays a role controlling the chronic condition of EED. Moreover, de Medeir, et.al (2018) reported that vitamin A deficiency may alter the intestinal microbiota which may compromise enteric pathogen clearance. El-Shahee, et.al (2019) also reported that the main site of Vitamins A absorption is small intestine which is the place of EED pathology.

Applied interventional study conducted by Loechl (2015) have shown that micronutrients, vitamins, and mineral supplementation improve intestinal morphology, reduce intestinal permeability, ameliorate gut functionality, and enhance linear growth.

Conclusion:

Calprotectin can be used as a non- invasive and inexpensive biomarker to discriminate short stature children with EE from those with non-inflammatory disease of the gut.

Acknowledgments:

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Conflict of interest:

None declared.

Recommendations:

From our study we recommend that:

1. Addressing EE and stunting requires a multi- faceted approach

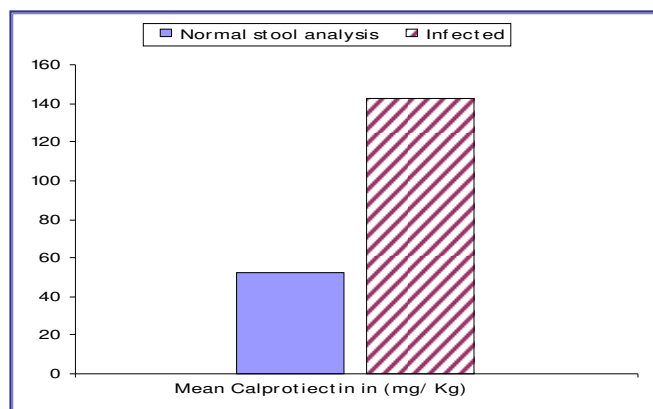


Figure (2) Bar chart showing mean calprotectin among cases with normal and infected stool analysis

Table (5) Comparison between males and females as regards main meal and frequency of having breakfast

Variables			Gender		P Value
			Male	Female	
Main Meal	Breakfast	Count	20	16	0.57
		% Within Gender	42.6%	42.1%	
	Lunch	Count	27	22	
		% Within Gender	57.4%	57.9%	
Breakfast Intake Frequency	Always	Count	29	24	0.006*
		% Within Gender	61.7%	63.2%	
	Sometimes	Count	12	1	
		% Within Gender	25.5%	2.6%	
	Rarely	Count	0	2	
		% Within Gender	0.0%	5.3%	
	Never	Count	6	11	
		% Within Gender	12.8%	28.9%	

Majority of cases in the study were having 3 meals per day with lunch was the main meal, however with no statistically significant relationship to gender of the study group. Most children who never or rarely had breakfast were females, while 61.7% of males always had breakfast, with a statistically significant difference.

Table (6) The mean intake of (macronutrients) compared to recommended dietary allowance (RDA)

Variables	Mean	SD	P Value
Water (mL/d)	1029.2822	135.97860	0.000*
RDA	1173.53	106.790	
Total calories (Kcal/d)	974.1929	426.29688	0.399
RDA	934.47	124.855	
CHO (gm/d)	120.0874	46.48189	0.053
RDA	130.00	0.000	
Proteins (gm/d)	32.4504	15.33798	0.000*
RDA	12.4959	1.70832	
Fat	39.8509	26.76621	0.001*
Fat RDA	49.53	1.469	
Fiber (gm/d)	3.1266	1.60585	0.000*
RDA	17.096	2.4065	

Among the studied cases, the intake of water, fat, fibers were significantly inadequate; lower than recommended dietary allowance (RDA). The intake of proteins was significantly higher than RDA. However, the intake of total calories, carbohydrates were comparable to RD.

Table (7) Comparison between males and females as regards macronutrients intake

Variables	Males			Females		
	Mean	SD	P Value	Mean	SD	P Value
Water (mL/d)	1013.95	122.17	0.000*	1048.25	150.84	0.005*
RDA	1209.57	109.04		1128.95	86.12	
Total calories (Kcal/d)	981.99	446.28	0.845	964.55	405.94	0.29
RDA	969.47	125.22		891.18	111.43	
CHO (gm/d)	117.36	37.26	0.024*	123.46	56.19	0.48
RDA	130	0		130	0	
Proteins (gm/d)	32.81	17.78	0.000*	32.001	11.86	0.000*
RDA	13.05	1.76		11.81	1.37	
Fat	41.29	31.37	0.073	38.07	19.93	0.001*
Fat RDA	49.68	1.24		49.34	1.71	
Fiber (gm/d)	3.12	1.64	0.000*	3.14	1.58	0.000*
RDA	18.29	2.39		15.621	1.4	

Water intake deficiency (compared to RDA) was more evident among males, while fat intake deficiency was more evident among females, with a statistically significant difference. While fiber intake was higher (compared to RDA) among males than the female group, with a statistically significant difference.

Table (8) The mean intake of different micronutrients among males and females compared to (RDA)

Variables	Males			Females		
	Mean	SD	P Value	Mean	SD	P Value
Na (mg/d)	1330.16	729.88	0.001*	1294.25	739.39	0.004*
RDA	953.19	85.59		915.79	100.07	
K (mg/d)	1296.36	717.44	0.000*	1355.62	749.99	0.000*
RDA	2229.79	128.39		2173.68	150.11	
Ca (mg/d)	418.91	360.63	0.000*	388.51	249.58	0.000*
RDA	929.79	128.39		873.68	150.11	
Ph (mg/d)	473.13	320.35	0.708	428.42	203.65	0.1
RDA	490.64	17.12		483.16	20.01	
Mg (mg/d)	65.12	33.77	0.000*	64.51	37.49	0.000*
RDA	118.3	21.39		108.95	25.02	
Fe (mg/d)	5.64	2.26	0.000*	5.93	3.04	0.000*
RDA	9.3	1.28		8.74	1.5	
Zn (mg/d)	4.16	2.017	0.210	3.78	1.75	0.28
RDA	4.53	0.86		4.16	1.001	
Cu (mg/d)	0.47	0.47	0.410	0.59	0.7	0.1
RDA	0.42	0.043		0.39	0.05	
VitA (mg/d)	108.77	126.84	0.000*	191.73	357.47	0.007*
RDA	376.6	42.79		357.89	50.04	
VitC (mg/d)	15.85	23.27	0.051	12.88	11.04	0.000*
RDA	22.66	4.28		20.79	5.004	
VitB1 (mg/d)	0.32	0.18	0.000*	0.31	0.19	0.000*
RDA	0.58	0.04		0.56	0.05	
VitB2 (mg/d)	0.52	0.34	0.274	0.5053	0.37651	0.39
RDA	0.58	0.04		0.558	0.0500	

Potassium, magnesium, vitamin A and vitamin B1 intake deficiency, (compared to RDA), were more evident among males, while calcium intake deficiency was more evident among females, with a statistically significant difference. While sodium and iron intake were higher (compared to RDA) among males, with a statistically significant difference.

Discussion:

The current cross- section study aimed to investigate the association between fecal calprotectin levels and EE in short stature children.

1. Children (3- 6) years (male or female) with nutritional stunting.
2. (Defined as height- for- age> 2 standard deviations below the mean height of same age and sex).

✧ Exclusion Criteria:

1. Stunting other than nutritional cause, Examples:
2. Children less than 3 years and more than 6 years.

✧ Pathological causes of short stature: Undernutrition, Glucocorticoid therapy, Rheumatologic disease, Chronic kidney disease, Pulmonary disease, Cardiac disease, Immunologic disease, Endocrinal causes of short stature, Syndromes with short stature, Diagnosed inflammatory bowel diseases.

✧ Ethical Considerations:

1. Patient information and informed consent: Before being enrolled into the study, the patient's parents (or guardians) consented to participate after the nature, scope and possible consequences of the clinical study had been explained in a form understandable to them.
2. Confidentiality and protocol approval: Before the beginning of the study and any accordance with the local regulation followed, the protocol and all the corresponding documents were declared for ethical and research approval by the council of Medical Studies department, Faculty of Postgraduate Childhood Studies, Ain Shams University, and the Ethical Committee at the National Research Centre and National Nutrition Institute.
3. Concerning safety and efficacy: No evidence of harmful effects of study interventions. Patients' blood samples were discarded after performing the required investigations and were not used for any other purposes.

✧ Limitations of the study: Refusal of some parents or guardians to enroll their child in the study.

Statistical Analysis:

The data was collected, revised, coded, and entered into IBM SPSS version 23. Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean, standard deviations and ranges. The comparison between groups with qualitative data were done by using Chi- square test. The comparison between more than two groups with quantitative data and parametric distribution were done by using One Way ANOVA test. Spearman correlation coefficients were used to assess the correlation between two quantitative parameters in the same group.

Results:

Table (1) Age and anthropometric measurements among our cases (N= 85)

Variables	Mean±SD	Range
Age (M)	54.58±12.133	36- 72
Weight (KG)	13.471±2.1358	10.0- 18.5
Height (Cm)	93.551±8.1341	74.0- 110.9
BMI	15.3601±1.5680	11.38- 20.34
Mid upper arm circumference (MUAC)	13.686±1.0336	11.5- 17.6
Waist Circumference (Wc)	54.515±2.3047	50.0- 58.6
Wc Percentiles	70.08±13.544	20- 95

Among our cases, mean age was 54.58± 12.133 with (min.- max.) of (36- 72) months, mean height was 93.551± 8.1341 cm and mean weight was 93.551± 8.1341 kg table (1).

Table (2) laboratory investigations of our cases

Variables	Mean± Sd	Range
Hb level (g/ dl)	11.4932±1.15460	9.20- 13.80
MCV	77.269±7.0307	55.0- 99.0
MCH	25.415±2.5367	17.0- 34.0
MCHC	33.109±1.7410	29.6- 37.2
Calprotectin (mg/kg)	113.39±160.6	0- 801.1

Table (3) History of repeated attacks of diarrhea during first 2 years of life

Variables	Gender		P Value
	Male Mean±SD Range	Female Mean±SD Range	
History of repeated attacks of diarrhea during first 2 years of life	4.06± 2.09 (1- 9)	3.66±2.08 (1- 8)	0.38

Among our patients, history of repeated attacks of diarrhea during the first 2 years of life ranged from 1 to 9 times among patients, without any statistically significant difference between male and female groups.

Frequency of infected stool analysis results more among total patients than normal stool analysis.

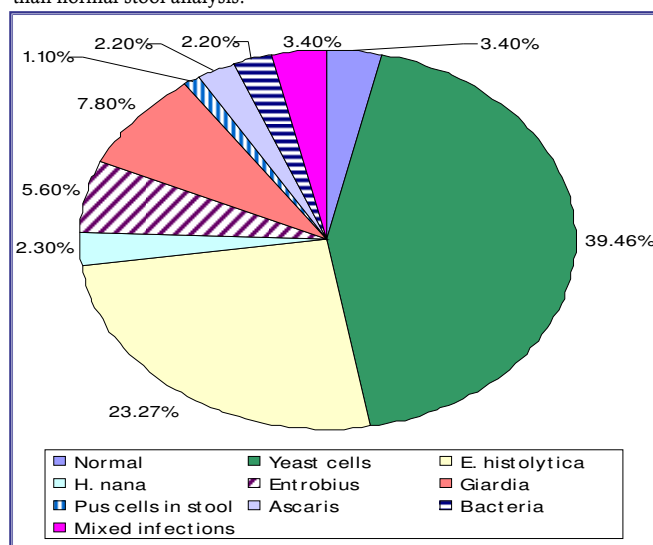


Figure (1) Pie chart showing frequency distribution and percentage of results of stool analysis

Table (4) Comparison between cases with normal and those with infected stool as regards fecal calprotectin

Variables	Stool Analysis Results		P Value
	Normal Mean±SD Range	Infected Mean±SD Range	
Calprotectin (mg/kg)	75.790±112.6314 (0.4- 410.4)	145.280±187.534 2 (0- 801.1)	0.046*

Calprotectin was significantly higher in cases with stool analysis infections in comparison to those with normal ones.

Introduction:

Stunted growth in developing countries may occur as a result of chronic nutrient deficiencies, recurrent infections, chronic inflammatory disorders including environmental enteric dysfunction (EED), as well as other hormonal and metabolic disorders. (Regassa, et.al, 2024)

Environmental enteropathy (EE) It is characterized by intestinal inflammation, maldigestion and malabsorption, gut permeability, translocation of microbes or microbial products^s and a systemic inflammatory response. (Hodges, et.al, 2021)

EED typically starts in infancy and continues into adulthood. Growing evidence has demonstrated that EED has a strong association with stunting which contributes to more than one million deaths among children under 5 years old every year. (Liu, et.al, 2022)

Environmental enteropathy primarily affects children living in low- and middle- income countries (LMICs), children living in these countries were found to have enteric pathogens related to EE in their systems throughout much of their early childhoods. (Tickell, et.al, 2019)

People living in poor sanitary conditions are continuously exposed to intestinal bacteria, viruses and parasites by ingestion of food and water contaminated with feces. Ingested bacteria may stimulate the small intestine, resulting in the chronic gut inflammation and subsequent increased gut permeability resulting in the malnutrition. (Kelly, et.al, 2022)

Chronic exposure to fecal pathogens is hypothesized to cause inflammation and resultant structural changes in the small bowel, which result in functional changes, include gut barrier disruption, carbohydrate malabsorption and chronic inflammation, and are hypothesized to contribute to impaired gut immune function, oral vaccine failure, ultimately growth faltering and impaired child development. (Lin, et.al, 2013)

Under physiological conditions, the intestinal barrier must ensure the right balance between the selective permeability of dietary nutrients from the intestinal lumen to the systemic circulation and internal environment, and the protection of the body from the penetration of pathogens and harmful components of the external environment. Selective absorption of nutrients occurs through intercellular or transcellular transport, while harmful and waste substances are removed from the gastrointestinal tract through the feces. (Di Vincenzo, et.al, 2024)

Calprotectin is a cytoplasmic protein prominently found in neutrophils that accounts for more than 40% of the cytosolic proteins in neutrophils, and to a lesser extent in monocytes and macrophages. Calprotectin is released to extracellular environment during inflammatory responses upon neutrophil activation or necrosis and induces neutrophil chemotaxis and adhesion. (Liu, et.al, 2020)

Before the possibility to detect CP in the stool in 1992, clinicians relied on serological markers to assess the possibility (or severity) of gut inflammation. However, erythrocyte sedimentation rate and serum C-reactive protein (CRP) are elevated in response to various non-

inflammatory processes and poorly correlate with patient symptoms and intestinal disease activity. NOW clinicians relied on fecal CP as biomarker to assess the possibility (or severity) of gut inflammatory diseases in the gut. (Jukic, et.al, 2021)

Calprotectin values depend also on patient's age. In newborns, in the first week of life, calprotectin levels are the highest and decrease with age. (Lezyk- Ciemniak, et.al, 2021)

Aim of the Study:

Study association of EE in short stature children using CP as a biomarkers.

Methods:

This cross- section study was conducted at National Research Center and National Nutrition Institute. The study was conducted on 85 stunted children aged for 3 years to 6 years. Care givers of children were informed about the nature and aims of the study. All studied children enrolled in this study were subjected to full history taking according to a predesigned questionnaire with stress on dietary history, history of repeated gastroenteritis, anthropometric measurements and putting them on growth charts for age, full clinical examination and laboratory investigations either radiological using X- ray left hand for bone age and biochemical evaluation as CBC and fecal calprotectin.

Nutritional assessment: To obtain both qualitative and quantitative information about the different items of food and beverage consumed by every child. Food frequency questionnaires (FFQ) are an alternative assessment to the 24- h recall and the extended food record. Utilization involves patients noting the frequency with which they consume representative foods from the major food groups within specific periods of time (Vijay, et.al, 2020). The 24- hours dietary recall method was used, in which recording of food intake for three scattered days (3 days recall sheet), including 2 regular days and one weekend, then taking their mean (Huang, et.al, 2020). Dietary assessment was done by asking the caregiver to remember in detail all the food and drink they consumed during a period of time in the recent past (the previous 24 hours). The description and preparation methods of foods were clarified according to (Van Staveren and Ocke, 2006). Food and drinks consumption are quantified by estimating portion sizes, using household measures (Thompson and Subar, 2017). The conversion of household measures to grams was achieved through the use of a prepared list of commonly used household measures in Egypt. Data of the dietary history was computed using the Nutrition Institute's (Egypt) food consumption tables (National Nutrition Institute Egypt Food Composition Table, 2006), in order to calculate the average daily intake of each child of total calories, macronutrients (calories, protein, fat and carbohydrates) (Faizan and Rouster, 2020) and micronutrients (minerals as sodium, potassium, calcium, phosphorus and iron). These nutrients were calculated as percentage of recommended dietary daily allowances (RDA) for age and sex. (Taylor and Stallings, 2009)

✎ Inclusion Criteria:

Fecal Calprotectin (CP) as a Diagnostic Biomarker of Environmental Enteropathy (EE) in Short Stature Children

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Summary

Background: Environmental enteropathy is a subclinical intestinal disorder linked to growth faltering in children from low- and middle- income countries. Fecal calprotectin; a neutrophil- derived protein, has been explored as a potential biomarker for intestinal inflammation.

Aims: Study the association of EE in short stature children using CP as a biomarkers.

Methodology: cross- sectional study was conducted on 85 stunted children aged 3 years to 6 years. All studied children enrolled in this study were subjected to full history taking according to a predesigned questionnaire with stress on nutritional assessment was performed to obtain both qualitative and quantitative information about the different items of food consumed by every child, by 24- hours dietary recall method and food frequency questionnaire, history of repeated gastroenteritis. Anthropometric measurements; putting them on growth charts for age, and full clinical examination. Laboratory investigations like; CBC& fecal calprotectin and radiological; using X- ray left hand, for bone age.

Results: Among the included subjects, the intake of water, fat, fibers were significantly inadequate; lower than recommended dietary allowance (RDA). The intake of proteins was significantly higher than RDA. However, the intake of total calories, carbohydrates were adequate, compared to RDA, whereas the intake of potassium, calcium, magnesium, iron, vitamin A, vitamin C and vitamin B1 was significantly lower than (RDA). The intake of sodium were significantly higher than RDA. On the other side, the intake of copper, zinc, phosphorus and vitamin B2 were adequate, compared to RDA. Mean calprotectin was statistically higher in cases with stool analysis infections or infestations than normal stool analysis cases (145.280 ± 187.5342 , 75.790 ± 112.6314) respectively.

Conclusion: Elevated fecal calprotectin levels are associated with EE in short stature children. This finding suggests that fecal calprotectin could be a valuable biomarker for diagnosing EE and monitoring its impact on growth. Further longitudinal studies are needed to establish the causal relationship between calprotectin and growth faltering.

KeyWords: Environmental Enteropathy, Fecal Calprotectin, Short Stature.

الكالبروتكتين البرازي كدلالة تشخيصية لاعتلال الأمعاء البيئي لدى الأطفال قصار القامة

الخلفية: اعتلال الأمعاء البيئي هو اضطراب معوي تحت سريري مرتبط بتأخر النمو عند الأطفال في البلدان ذات الدخل المنخفض والمتوسط. تم استكشاف الكالبروتكتين البرازي، وهو بروتين مشتق من الخلايا المتعادلة، كعلامة بيولوجية محتملة للالتهاب المعوي.

الأهداف: دراسة ارتباط اعتلال الأمعاء البيئي مع قصر القامة لدى الأطفال باستخدام الكالبروتكتين كعلامة حيوية.

الطريقة: أجريت دراسة مقطعية في معهد التغذية القومي في عيادة قصر القامة وتحليل المختبر في المركز القومي للبحوث. أجريت على ٨٥ طفلاً يعانون من قصر القامة تتراوح أعمارهم بين (٣-٦) سنوات. تم إخضاع جميع الأطفال المشاركين في هذه الدراسة لأخذ التاريخ المرضي الكامل وفقاً لاستبيان محدد مسبقاً مع التركيز على تقييم التغذية الذي تم إجراؤه للحصول على معلومات نوعية وكمية حول مختلف العناصر الغذائية التي يستهلكها كل طفل عن طريق طريقة استرجاع النظام الغذائي لمدة ٢٤ ساعة. تاريخ الإصابة بالتهاب المعدة والأمعاء المتكرر، والقياسات الأنثروبومترية ووضعها على مخططات النمو حسب العمر، والفحص السريري الكامل والتحقيقات المخبرية باستخدام الأشعة التشخيصية لتصوير اليد اليسرى لعمر العظام والتقييم البيوكيميائي مثل صورة الدم الكامل والكالبروتكتين البرازي.

النتائج: بين الموضوعات المشمولة، كان تناول الماء والدهون والألياف غير كاف بشكل ملحوظ؛ أقل من الكمية الموصى بها. كان تناول البروتينات أعلى بكثير من الكمية الموصى بها. ومع ذلك، كان تناول السعرات الحرارية الكلية والكربوهيدرات كافياً مقارنة بالكمية الموصى بها، وكان تناول البوتاسيوم، الكالسيوم، المغنيسيوم، الحديد، فيتامين أ، فيتامين ج وفيتامين ب ١ غير كاف بشكل ملحوظ؛ أقل من الكمية الموصى بها. كان تناول الصوديوم أعلى بكثير من الكمية الموصى بها. من ناحية أخرى، كان تناول النحاس والزنك والفوسفور وفيتامين ب ٢ كافياً مقارنة بالكمية الموصى بها. كان متوسط الكالبروتكتين أعلى في الحالات المصابة بعدوى تحليل البراز مع فرق إحصائي ذي دلالة 145.280 ± 187.5342 مقارنة بالحالات الطبيعية لتحليل البراز 75.790 ± 112.6314 .

الاستنتاج: ترتبط مستويات الكالبروتكتين البرازية المرتفعة باعتلال الأمعاء البيئي عند الأطفال قصار القامة. يشير هذا الاكتشاف إلى أن الكالبروتكتين البرازي يمكن أن يكون علامة بيولوجية قيمة لتشخيص اعتلال الأمعاء البيئي ومراقبة تأثيره على النمو. هناك حاجة إلى مزيد من الدراسات الطولية لتحديد العلاقة السببية بين الكالبروتكتين وتأخر النمو.

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