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Gut–brain Axis: Impact of Intestinal Inflammation and Micronutrient Deficiency on Psychomotor Development and Cognitive Functions in Egyptian Children with Undernutrition

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Abstract

BACKGROUND: Cognitive impairment, growth faltering, and stunting are pervasive in many countries. Many times, cause is unknown. Suspecting the role of intestinal inflammation in such cases should be minded, especially in countries with low and middle income.

AIM: The study objective is to assess the impact of malnutrition and macronutrient deficiency that might occur secondary to gut inflammation on cognitive functions in children.

METHODS: We investigated serum markers of inflammation, fecal markers of intestinal inflammation, and serum micronutrients in cases with an age range from 1 to 10 years who suffer from moderate or severe malnutrition "having weight-for-age z-score and height-for-age z-score (WAZ or HAZ) less than −2 SD". Cognitive abilities were assessed using the Wechsler Intelligence Scale for Preschool and School Children and Bayley Scale III. 55.6% of preschool cases were below average or had mild or moderate intelligence retardation, while 24.5% of school cases and 5% of children below 2 years were below average regarding cognitive functions.

RESULTS: Cases showed a statistically significant reduction of Vitamin D, zinc, and iron as compared to controls. Endo Cab Serum markers of inflammation such as alpha-1-acid glycoprotein (α1-AGP) and endotoxin-core antibody (EndoCAB) and fecal markers of intestinal inflammation such as alpha-1 antitrypsin (α1-AT) and neopterin (NEOP) showed a statistically significant increase in cases than in controls. Schoolchildren showed a negative correlation between processing functions and α1-AGP and a positive correlation between perceptual reasoning and serum Vitamin A. Children below 2 years showed negative correlations between motor function and α1-AT, α1-AGP, tumor necrosis factor-alpha (TNF-α), and Endo Cab and a positive correlation between language and serum zinc.

CONCLUSION: The study showed impaired neurocognitive and psychomotor functions in malnourished stunted children. Furthermore, vitamin and mineral deficiency and increased markers of intestinal inflammation were observed in cases compared to healthy controls indicating the role of intestinal inflammation in psychomotor development and cognitive functions in children with undernutrition.

Introduction

It is a challenge in developing countries to trace the reasons that can affect the mental and physical health of children, especially in our country in view of sustainable development. Researches have shown that intestinal inflammation could be a cause of growth impairment and affection of cognitive functions and psychomotor development [1], [2].

The gut–brain axis (GBA) is a connection between central nervous system (emotional and cognitive centers of the brain) and enteric nervous system (peripheral intestinal functions). A growing body of research has indicated that emotions significantly affect the colonic response and that intestinal inflammation and undernutrition affect psychomotor development and cognitive functions [3].

Intestinal inflammation is caused by different reasons; it can be due to infection with bacteria, viruses, or parasites or due to inflammatory bowel diseases. Furthermore, it can be allergic or drug induced. Environmental enteric dysfunction (EED), which occurs in middle- and low-income countries, is another cause of intestinal inflammation; recurrent exposure to pathogens in areas of poor sanitation and hygiene leads to histopathological intestinal changes [4], [5]. Researchers still investigate the exact pathway by which intestinal inflammation affects cognition and psychomotor development. Cytokine imbalance, which has many neurological consequences, can be the pathway. Besides, patients with intestinal inflammation are often malnourished, having multiple nutrient deficiencies which can possibly impair cognitive and psychomotor functions [6].

Micronutrients, vitamins, and minerals play a vital role in healthy development, disease prevention,

and well-being. They are not produced in the body and must be derived from the diet except for Vitamin D, which is provided from sun exposure, so consuming the recommended amount is important. Micronutrient deficiency leads to growth arrest, cognitive impairment, and multiple different disorders. Approximately half of children worldwide younger than 5 years of age suffer from vitamin and mineral deficiencies [7].

Cognition is a term referring to a group of mental processes involved in gaining knowledge and comprehension. These mental processes include problem-solving, thinking, knowing, judging, remembering, imagination, language, planning, and perception. Psychomotor development is the progressive acquisition of different skills involving mental, motor, and social activities starting from intrauterine life till adolescent stage [8].

The study objective is to assess the impact of malnutrition and macronutrient deficiency that might occur secondary to gut inflammation on cognitive functions in children.

Subjects and Methods

This work is a part of ongoing in-house project in National Research Centre with approval number 12060128. The current case–control study was conducted on 105 children aged 1–10 years from those attending the Immune Nutrition Clinic in Medical Research Centre of Excellence in National Research Centre, Egypt, in the period from October 2019 to December 2020.

Children from both sexes with moderate or severe malnutrition "having weight-for-age z-score and height-for-age z-score (WAZ or HAZ) ranging from <−2 SD were enrolled in the study. Children with congenital abnormalities, genetic disorders, and any chronic debilitating illness were excluded from the study. Children with a recent or present history of diarrhea or heavy parasitic infection were also excluded from the study. One hundred age and gender group-matched "non-malnourished healthy children" having a WAZ better than –1 were included as a control group.

The study had the ethical approval of the Medical Research Committee at the National Research Centre, having number 19/227. Furthermore, signed informed consent was collected from caregivers of the children enrolled in the study prior to participation.

All children included in the study were subjected to full history taking, including age, name, sex, and dietetic history. All anthropometric measurements were assessed according to described techniques in the Anthropometric Standardization Reference Manual [9]. Body mass index-for-age z-score (BMI.z-score) was recorded according to WHO standards using AnthroPlus software for personal computers.

Wechsler Intelligence Scale for Children Fourth Edition (WISC-IV), Arabic translation, published by Anglo-Egyptian library 2012, was applied to assess the overall cognitive and intellectual abilities of children from 6 to 16 years. It is an individually administered intelligence test which contains four main subscales: Verbal Comprehension, Working Memory, Perceptual Reasoning, and Processing Speed (PS). Together, the 4 subscales provide the overall level of intelligence or full-scale IQ. The full scale consists of 15 subtests; the main 10 scales were applied [10].

Wechsler Preschool and Primary Scale of Intelligence–Third Edition (WPPSI-III) Arabic translation, published by Anglo-Egyptian library 2012, was used in assessment of cognition and intellectual abilities for children between the ages of 2 years, 6 months (2:6 years), and 7:3 years; the scale yields three IQs: a verbal subscale IQ, a performance subscale IQ, and a full-scale IQ. Within the scale, there are 10 mandatory subtests. The verbal subscale included five mandatory tests: information, similarities, arithmetic, vocabulary, and comprehension. The performance subscale included five mandatory tests: object assembly, coding, block design, picture arrangement, and picture completion, and the full scale is the sum of the verbal and performance subscales [11].

The Bayley Scales of Infant and Toddler Development, Third Edition is an individually administered scale designed to assess the developmental functioning in children aged 1–42 months. The scale provides coverage of the five subscales: cognitive, language, motor, adaptive, and social-emotional development. In the current study, three domains only were assessed: cognitive, language, and motor [12].

Laboratory assessment

Three milliliters of venous samples were withdrawn from each child, put in a serum separator, then centrifuged, and stored at −8°C for assessment of alpha-one-acid glycoprotein (α1-AGP), alpha-1 antitrypsin (α1-AT), tumor necrosis factor-alpha (TNF-α), endotoxin-core antibody (Endo Cab), Vitamin A (vit. A), and Vitamin D active form (vit. D) using commercially available ELISA kits, SunLong Biotech Co., Ltd. Serum zinc and iron were measured by colorimetric method.

Stool samples were collected without fixative and frozen at −7°C prior to testing. Specimens were processed at dilution of 1:500 and then were evaluated for myeloperoxidase (MPO), neopterin (NEOP), and alpha-1-antitrypsin (α1-AT) using commercially available ELISA kits, SunLong Biotech Co., Ltd.

Statistical methods

All test data was converted and manipulated by using SPSS software program version 25.0. (IBM,

Chicago, IL, USA. The data was analyzed, mean and standard deviation were calculated regarding quantitative data (such as age, anthropometric measures, birth weight, and laboratory results), while qualitative data such as sex was presented by number and percent. Comparisons between cases and controls and between patients with different IQ levels were done according to anthropometric measures and laboratory results. The quantitative data were compared and *t*-test or ANOVA was applied for normally distributed data, Mann–Whitney or Kruskal–Wallis test was applied for non-parametric data, and p value was established to determine the statistically significant difference between the two groups. Chi-square was calculated among groups as regards qualitative data. Pearson's correlation among IQ scores and anthropometric and laboratory data were done and Pearson's correlation coefficient was computed and p-value was established. The difference between groups was considered statistically significant when $p < 0.05$ and considered highly statistically significant when $p < 0.01$.

Results

Table 1 shows a significant difference between cases and controls as regards weight, weight z score, height, height z score, BMI, and BMI z score.

Table 1: Comparison between cases and controls

Variable	Cases (*n* = 105)	Controls (*n* = 100)	p
Age (months), mean ± SD	79.71 ± 34.3	79.33 ± 39.5	0.85
Sex, *n* (%)			
Male	47 (44.8)	46 (46.0)	0.859
Female	58 (55.2)	54 (54.0)	
Height (cm)	107.94 ± 15.9	119.91 ± 21.3	<0.001**
Height z score (HAZ)	−2.18 ± 0.8	0.44 ± 0.4	<0.001**
Weight (kg)	17.88 ± 5.7	25.18 ± 11.0	<0.001**
Weight z score (WAZ)	−2.08 ± 0.8	0.4 ± 0.4	<0.001**
Arm circumference (cm)	16.56 ± 1.8	17.73 ± 2.0	<0.001**
BMI	15.14 ± 1.5	16.71 ± 1.5	<0.001**
BMI z score (BMI z)	−0.74 ± 1.2	0.34 ± 0.4	<0.001**

**p ≤ 0.001 (highly significant), *p ≤ 0.05 (significant). WAZ: Weight-for-age z-score, HAZ: Height-for-age z-score, BMI z: Body mass index z score, SD: Standard deviation.

When applying ANOVA test between different total IQ levels regarding anthropometric measurements, a significant difference was found between three groups in weight, height, and height z-scores; birth weight; and arm circumference, as shown in Table 2. When applying ANOVA test for comparison between different WPPSI IQ levels regarding anthropometric data, we found no significant difference apart from birth weight.

Table 3 shows a highly significant difference between cases and controls regarding micronutrient serum level (Vitamin D, zinc, and iron) and a significant difference between cases and controls regarding serum α1-AGP, fecal α1-AT, and NEOP.

Table 3: Comparison between cases and controls according to laboratory results

Variable	Mean ± SD		p
	Cases (*n* = 105)	Controls (*n* = 100)	
Serum markers			
α1-AGP	17.11 ± 14.0	12.48 ± 8.6	0.024*
TNF-α	44.45 ± 26.3	39.2 ± 15.8	0.567
Endo Cb	15.19 ± 13.0	10.48 ± 3.3	0.05
Micronutrients			
Zinc	86.02 ± 33.5	107.74 ± 27.1	<0.001**
Vitamin A	63.26 ± 22.4	68.02 ± 32.7	0.884
Vitamin D	10.19 ± 8.8	13.5 ± 5.9	<0.001**
Iron	78.24 ± 31.7	104.78 ± 54.2	<0.001**
Fecal markers			
α1-AT	11.7 ± 7.1	8.82 ± 5.9	0.012*
NEOP	30.95 ± 21.0	22.06 ± 7.3	0.021*
MPO	3.03 ± 2.2	2.4 ± 1.4	0.276

**p ≤ 0.001 (highly significant), *p ≤ 0.05 (significant). α1-AGP: Alpha-1-glycoprotein, TNF-α: Tumor necrosis factor-alpha, Endo Cab: Endotoxin-core antibody, α1-AT: Alpha-1 acid, NEOP: Neopterin, MPO: Myeloperoxidase, SD: Standard deviation.

When applying ANOVA between three groups in Table 4, we found a significant difference between three groups in serum Vitamin A, α1-AT, and MPO.

As shown in Table 5, we found a significant positive correlation between birth weight and different Bayley subscale scores and a significant positive correlation between zinc and language subscale score of Bayley, while significant negative correlations between motor subscale Endo Cab score of Bayley and α1-AGP, MPO, TNF-α, EndoCAB, α1-AT, and NEOP.

As shown in Table 6, we found a significant positive correlation between WPPSI verbal scores and Endo Cab and a significant negative correlation between WPPSI total scores and birth weight.

Table 7 shows a significant positive correlation between Wechsler verbal, perceptual, and total scores and fecal MPO.

Table 8 shows a significant positive correlation between Wechsler verbal, non-verbal, memory, processing, and total scores and HAZ. Furthermore, it shows a significant positive correlation between Wechsler performance subscale score and Vitamin A serum level.

Discussion

The current study was conducted to assess the impact of intestinal inflammation on serum

Table 2: Comparison between different intelligence quotient levels as regards anthropometric measures

Lab test	IQ level, mean ± SD			p	p1	p2	p3
	Below average (*n* = 33)	Average (*n* = 59)	Above average (*n* = 13)				
Weight	18.17 ± 6.5	16.93 ± 5.3	21.46 ± 3.8	0.015*	0.463	0.04	0.003
WAZ	−2.16 ± 0.8	−2.09 ± 0.8	−1.78 ± 0.6	0.318	0.662	0.135	0.197
Height	106.98 ± 14.9	105.88 ± 16.3	119.69 ± 11.8	0.021*	0.619	0.012	0.009
HAZ	−2.5 ± 0.8	−2.08 ± 0.8	−1.78 ± 0.6	0.004*	0.015	0.003	0.107
BMI	15.42 ± 1.6	14.99 ± 1.6	15.09 ± 1.0	0.434	0.199	0.514	0.829
BMI z score	−0.53 ± 0.9	−0.92 ± 1.4	−0.41 ± 1.1	0.192	0.136	0.758	0.166
Birth weight	2.66 ± 0.2	2.93 ± 0.7	2.97 ± 0.3	0.009*	0.004	0.811	0.08
Arm circumference	16.41 ± 2.0	16.41 ± 1.7	17.62 ± 1.2	0.021*	0.793	0.011	0.008

*p ≤ 0.05 (significant). *P* value: *P* value between the 3 groups, p1 value: *P* value between below average and average, p2 value: *P* value between below average and above average, p3 value: *P* value between average and above average. IQ: Intelligence quotient, WAZ: Weight-for-age z-score, HAZ: Height-for-age z-score, BMI z: Body mass index z score, SD: Standard deviation.

Table 4: Comparison between different intelligence quotient levels as regards serum and fecal markers and serum micronutrients

Lab test	IQ level, mean ± SD			p	p1	p2	p3
	Below average (*n* = 33)	Average (*n* = 59)	Above average (*n* = 13)				
α1-AGP (ng/ml)	17.12 ± 14.0	18.71 ± 15.0	9.85 ± 4.1	0.119	0.599	0.112	0.039*
TNF-α (ng/l)	48.42 ± 39.6	42.58 ± 17.2	42.58 ± 17.9	0.581	0.311	0.52	0.973
Zinc (μg/dl)	80.09 ± 34.4	91.53 ± 33.7	75.79 ± 26.7	0.143	0.115	0.685	0.121
Vitamin A (pg/ml)	55.18 ± 20.5	68.19 ± 23.3	61.39 ± 16.4	0.025*	0.007*	0.386	0.31
Iron (μg/dl)	75.56 ± 36.5	81.21 ± 30.7	71.77 ± 21.4	0.532	0.415	0.725	0.34
Endo Cab (pg/ml)	11.14 ± 5.1	17.68 ± 16.2	13.58 ± 6.3	0.055	0.018*	0.5580	0.287
α1-AT (ng/ml)	9.12 ± 5.7	13.15 ± 7.9	11.55 ± 5.3	0.031*	0.009*	0.327	0.398
NEOP (pmol/ml)	28.42 ± 21.1	32.05 ± 20.2	32.39 ± 25.3	0.709	0.432	0.5690	0.959
MPO (ng/ml)	1.93 ± 1.1	3.65 ± 2.4	3.23 ± 1.8	0.001*	<0.001*	0.055	0.562
Vitamin D	9.35 ± 8.1	10.95 ± 9.3	9.08 ± 8.5	0.626	0.412	0.93	0.499

*p ≤ 0.05 (significant). *P* value: *P* value between the 3 groups, p1 value: *P* value between below average and average, p2 value: *P* value between below average and above average, p3 value: *P* value between average and above average. IQ: Intelligence quotient, α1-AGP: Alpha-1-acid glycoprotein, TNF-α: Tumor necrosis factor-alpha, Endo Cab: Endotoxin-core antibody, α1-AT: Alpha-1 antitrypsin, NEOP: Neopterin, MPO: Myeloperoxidase, SD: Standard deviation.

micronutrients level and the cognitive functions in children aged from 1 to 10 years suffering from moderate to severe malnutrition. In addition, the study assessed serum and fecal markers of intestinal inflammation.

The current study showed a highly significant statistical difference between cases and controls regarding weight, weight z score, height, height z-score, BMI, and BMI z score.

The studied sample showed a reduction of Vitamin D, zinc, and iron serum levels in cases as compared to controls. On the contrary, cases showed higher levels of serum markers of inflammation such as alpha-1-glycoprotein (α1-AGP), endotoxin-core antibody Endo Cab, and TNF-α and higher levels of fecal markers of intestinal inflammation (alpha-1 antitrypsin (a1-AT), neopterin (NEOP), and MPO.

Cognitive abilities were assessed using the Wechsler Intelligence Scale for Preschool and School Children and Bayley Scale III. Results came as follows: 55.6% of preschool cases were below average or had mild to moderate intellectual disabilities, while 24.5% of school cases and 5% of children below 2 years scored below average in cognitive functions.

The current study aimed to review evidences generated about the theory that unexplained growth faltering and stunting with impaired cognitive functions and psychomotor development could be attributed to intestinal inflammation and micronutrient deficiencies.

In this study, dietary history of cases confirmed no deficient intake for nutrients. Examination and history showed no physical, psychiatric, chromosomal disorders or worm infestation that can explain developmental impairment.

The GBA is a bidirectional communication between the central and enteric nervous systems; in other words, it could be said that the function of the intestine is influenced by the cognitive and emotional brain centers and vice versa [3].

The current study showed a highly significant statistical difference between cases and controls regarding all anthropometric measures "in Concordance with of high serum level of intestinal inflammatory markers," this supports the hypothesis that environmental enteropathy could be a cause of growth faltering. In addition, deficiency in serum micronutrients such as zinc, iron, and Vitamin D in the absence of low intake could explain reduced anthropometric measures in the studied cases. Similarly, a study of Lin *et al*., 2013, in rural Bangladesh reported that young children living in environmentally clean households had improved measures of gut function and improved growth compared with similar children living in contaminated environments [13]. The same context study of Guerrant *et al*., 2016, addressed the systemic effects of intestinal barrier disruption and local inflammation on growth [14]. Thurstan *et al*., 2022, reported that wasting and stunting are interconnected processes, explaining that they are frequently coexisted [15].

Table 5: Correlations between different Bayley subscale scores and serum markers, fecal markers, serum micronutrients, and anthropometric measurements

Cognitive assessment test	α1-AGP	TNF-α	Zinc	Vitamin A	iron	Endo Cab	α1-AT	NEOP	MPO	Vitamin D	HAZ	WAZ	BMI z	Birth weight
Bayley language scores														
r	−0.411	−0.304	0.466*	−0.299	0.216	−0.412	−0.044	−0.325	0.059	0.228	0.233	0.402	0.088	0.670**
p	0.072	0.192	0.038	0.200	0.361	0.071	0.853	0.162	0.806	0.335	0.323	0.079	0.714	0.001
n	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Bayley cognition scores														
r	−0.111	0.034	0.394	−0.139	0.062	0.037	−0.066	0.065	0.200	−0.017	0.389	0.428	0.165	0.500*
p	0.641	0.887	0.085	0.559	0.795	0.876	0.782	0.785	0.398	0.943	0.090	0.060	0.487	0.025
n	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Bayley motor scores														
r	−0.462*	−0.713**	0.324	−0.149	−0.012	−0.794**	−0.606**	−0.656**	−0.548*	0.062	0.332	0.280	0.260	0.507*
p	0.040	0.000	0.164	0.532	0.960	0.000	0.005	0.002	0.012	0.794	0.153	0.232	0.267	0.023
n	20	20	20	20	20	20	20	20	20	20	20	20	20	20

**p ≤ 0.001 (highly significant), *p ≤ 0.05 (significant). α1-AGP: Alpha-1-glycoprotein, TNF-α: Tumor necrosis factor-alpha, Endo Cab: Endotoxin-core antibody, α1-AT: Alpha-1 antitrypsin, NEOP: Neopterin, MPO: Myeloperoxidase, WAZ: Weight-for-age z-score, HAZ: Height-for-age z-score, BMI z: Body mass index z score.

Table 6: Correlations between different WPPSI subscale scores, serum markers, fecal markers, serum micronutrients, and anthropometric measurements

Cognitive assessment test	α1-AGP	TNF-α	Zinc	Vitamin A	Iron	Endo Cab	α1-AT	NEOP	MPO	Vitamin D	HAZ	WAZ	BMI z	Birth weight
WPPSI verbal scores														
r	−0.030	−0.257	0.214	−0.023	0.199	0.347*	0.149	0.189	0.047	0.128	0.147	−0.204	−0.206	−0.323
p	0.864	0.130	0.211	0.896	0.245	0.038	0.387	0.271	0.784	0.456	0.392	0.232	0.228	0.055
n	36	36	36	36	36	36	36	36	36	36	36	36	36	36
WPPSI non-verbal scores														
r	−0.113	0.045	0.222	0.218	0.001	0.030	0.026	0.117	0.017	0.184	0.238	0.428	−0.069	−0.279
p	0.511	0.794	0.193	0.200	0.995	0.861	0.880	0.495	0.920	0.282	0.162	0.060	0.691	0.100
n	36	36	36	36	36	36	36	36	36	36	36	36	36	36
WPPSI total scores														
r	−0.066	−0.124	0.260	0.061	0.084	0.223	0.121	0.188	0.042	0.139	0.245	−0.001	−0.116	−0.370*
p	0.704	0.470	0.126	0.724	0.624	0.191	0.481	0.272	0.809	0.417	0.149	0.998	0.500	0.026
n	36	36	36	36	36	36	36	36	36	36	36	36	36	36

*p ≤ 0.05 (significant). α1-AGP: Alpha-1-glycoprotein, TNF-α: Tumor necrosis factor-alpha, Endo Cab: Endotoxin-core antibody, α1-AT: Alpha-1 antitrypsin, NEOP: Neopterin, MPO: Myeloperoxidase, WAZ: Weight-for-age z-score, HAZ: Height-for-age z-score, BMI z: Body mass index z score, WPPSI: Wechsler preschool & primary scale for intelligence.

The current study concluded a significant correlation between HAZ and different subscales of Wechsler intelligence test for schoolchildren (verbal, non-verbal, perceptual, memory, and processing) in the studied sample, while non-significant correlation between child WAZ and different neurocognitive domains. This could be explained by the impact of chronic malnutrition "represented by stunting" on the development of higher cognitive processes during childhood leading to long-lasting cognitive impairments, added to the associated mild inflammatory response and release of inflammatory cytokines and consequent structural and functional brain pathology, which, in turn, affect multiple cognitive processes [16]. This was in line with a study of Kroupina *et al*., 2015, which showed that child's height influences his non-verbal IQ score positively [17]. A study of Poh *et al*., 2013, came with different results as it documented that height and weight both influence performance IQ [18].

In the current study, it was found that birth weight had a significant positive correlation with Bayley Scale score "total, cognitive, and language scores" and there was a significant difference in birth weight between cases with below-average IQ and average IQ (p = 0.004). This could be explained by the impact of birth weight on brain volume, cortical surface area, and consequently, the complexity of neurological networks. In the same context study of Gu *et al*., 2017, approved that low birth weight had a negative association with IQ and the lower birth weight categories had approximately 10–11 points' lower IQs than normal birth weight individuals [19]. There are notable similarities between the current findings and other prior researches that reported higher IQ with greater size at birth using Wechsler Primary and Preschool Scales of Intelligence [20], [21].

Table 7: Correlations between Wechsler subscale scores, serum markers, and fecal markers

Cognitive assessment test	α1-AGP	TNF-α	Endo Cab	α1-AT	NEOP	MPO
Wechsler verbal scores						
r	−0.027	0.316*	0.348*	0.295*	−0.095	0.430**
p	0.851	0.027	0.014	0.039	0.518	0.002
n	49	49	49	49	49	49
Wechsler non-verbal scores						
r	−0.096	0.082	0.018	0.128	−0.058	0.214
p	0.510	0.573	0.900	0.380	0.690	0.140
n	49	49	49	49	49	49
Wechsler perceptual scores						
r	0.132	0.163	0.241	0.258	−0.090	0.424**
p	0.366	0.263	0.095	0.074	0.538	0.002
n	49	49	49	49	49	49
Wechsler memory scores						
r	−0.003	−0.081	−0.006	0.142	−0.012	0.204
p	0.986	0.582	0.970	0.330	0.933	0.160
n	49	49	49	49	49	49
Wechsler processing scores						
r	−0.369**	−0.017	−0.225	−0.120	0.03	−0.213
p	0.008	0.907	0.117	0.405	0.830	0.137
n	50	50	50	50	50	50
Wechsler total						
r	−0.07	0.17	0.21	0.24	−0.15	0.421**
p	0.61	0.22	0.13	0.08	0.28	0.00
n	49	49	49	49	49	49

**p ≤ 0.001 (highly significant), *p ≤ 0.05 (significant). α1-AGP: Alpha-1-acid glycoprotein, TNF-α: Tumor necrosis factor-alpha, Endo Cab: Endotoxin-core antibody, α1-AT: Alpha-1 antitrypsin, NEOP: Neopterin, MPO: Myeloperoxidase.

The mean serum iron of cases in the current study was 78.24 ± 31.7 μg/dL compared to 104.78 ± 54.2 μg/dL in controls, p < 0.001**, with no significant correlation between serum iron levels and any subscale of Intelligence tests. The current study suggested that cognitive deficit in studied cases seems to be not associated with iron deficiency but could be attributed to effect of inflammatory mediators and cytokines resulted from intestinal inflammation, which, in turn, affect communication networks in brain.

The current study showed a statistically significant difference between cases and controls regarding serum Vitamin D level; although, the study found no significant relationship between Vitamin D level and different neurocognitive domains in the studied sample. This could be explained by the long time needed for neurodegenerative changes of Vitamin D deficiency to manifest and influence the cognitive scale results. In the same context, cognitive, motor, language, and behavior development in infants and adolescents were assessed using Bayley-III and Raven's Standard Progressive Matrices test. They did not find any association of Vitamin D status of infants and adolescents with their cognitive or motor scores [22], [23].

Mentioned findings were not matched with other studies which reported a significant correlation between low levels of Vitamin D and reduced cognitive function, which could be explained by different roles of Vitamin D in neuroprotection such as calcium influx regulation, homeostasis, and anti-inflammatory signaling [24].

Serum Vitamin A levels in the current study did not show a statistical difference between cases and controls, but there was a high significant statistical difference in serum Vitamin A between cases with below-average IQ and cases with average IQ (p = 0.007) with a positive correlation between perceptual scores in Wechsler Scale for schoolchildren and serum Vitamin A

Table 8: Correlations between Wechsler subscale scores, serum micronutrients, and anthropometric measurements

Cognitive assessment test	Zinc	Vitamin A	Iron	Vitamin D	HAZ	WAZ	BMI z	Birth weight
Wechsler verbal scores								
r	−0.264	0.270	0.066	0.054	0.317*	0.131	−0.026	0.044
p	0.067	0.060	0.650	0.715	0.027	0.368	0.861	0.762
n	49	49	49	49	49	49	49	49
Wechsler non-verbal scores								
r	−0.169	0.106	0.141	−0.010	0.457**	0.007	−0.166	−0.080
p	0.249	0.468	0.333	0.944	0.001	0.963	0.255	0.586
n	49	49	49	49	49	49	49	49
Wechsler perceptual scores								
r	−0.148	0.320*	0.129	−0.001	0.422**	−0.043	−0.176	−0.225
p	0.309	0.025	0.378	0.992	0.003	0.767	0.228	0.120
n	49	49	49	49	49	49	49	49
Wechsler memory scores								
r	−0.079	0.060	0.200	0.075	0.372*	0.124	−0.047	−0.068
p	0.589	0.680	0.168	0.606	0.009	0.397	0.749	0.120
n	49	49	49	49	49	49	49	49
Wechsler processing scores								
r	−0.052	−0.231	−0.011	−0.061	0.248	−0.173	−0.272	0.166
p	0.722	0.107	0.938	0.670	0.083	0.229	0.056	0.249
n	50	50	50	50	50	50	50	50
Wechsler total scores								
r	−0.11	0.25	0.11	−0.03	0.529**	0.09	−0.14	−0.08
p	0.41	0.07	0.42	0.80	0.00	0.52	0.33	0.56
n	49	49	49	49	49	49	49	49

**p ≤ 0.001 (highly significant), *p ≤ 0.05 (significant). WAZ: Weight-for-age z-score, HAZ: Height-for-age z-score, BMI z: Body mass index z score.

level (r = 0.320, p = 0.025). The current study explained this difference by the important actions of Vitamin A on brain physiology and its requirement for the storage and recovery of memory [25] added to its effect as antioxidant and neuroprotective [26]. A study of Huang *et al*., 2018, came with the same results but in elders [27].

Mean serum zinc in cases of the study was statistically significantly lower than in controls, p < 0.001**. Serum zinc in cases with below-average total IQ was 80.09 ± 34.4, while in cases with an average total IQ score, it was 91.53 ± 33.7 (p = 0.115). There was a positive correlation between language score of Bayley Scale III in children under 2 years and serum zinc level (p = 0.038). The current results were in agreement with previous results which report significant correlation between zinc level and verbal, non verbal communication and symptoms severity in autistic patients [28], [29]. The current finding could be explained by the vital role of zinc in axonal and synaptic transmission added to its Zn effect on neuronal differentiation and white matter grow [30].

The study results showed that the mean serum α1-AGP in cases was 17.11 ± 14.0, while in controls, it was 12.48 ± 8.6 with *p*-value 0.024*. α-1 acid glycoprotein is one of the major acute phase proteins in humans, rats, and other species. As most of acute phase proteins, its serum level increases in response to inflammation or tissue trauma [31]. There was a strong negative correlation between α1-AGP and processing functions in schoolchildren (p = 0.008) and motor function in children under two years (p = 0.04); the study suggested that low-degree chronic inflammation with increase in serum α1-AGP level is considered an important risk factor for child neurodevelopment. Current results were not matching with study of Mancuso et al which showed no association between serum α 1-AGP concentration and cognitive performance in diabetic patients [32].

In addition, the current study reported a significant statistical difference in serum (EndoCab) between cases and controls and a negative correlation between motor function in children <2 years and EndoCab (p < 0.01). This significant difference in the presence of (Endo Cab) raises the possibility of gastrointestinal tract infection as the circulating endotoxin originates from luminal gastrointestinal tract bacteria, leading to malabsorption and growth faltering with motor delay. Similarly, Brown 2017 in his study about infants below 2 years living in unhygienic environment, documented that faltering growth is directly related to environmental enteropathy [33].

There was no statistically significant difference in serum TNF-α between cases and controls (p = 0.567), but there was a negative correlation between motor function in children under 2 years and TNF-α (p < 0.01). The current study suggested that persistent high level of this inflammatory marker could be considered a risk factor for normal development. Conversely, a study of Zareen *et al*., 2020, found an association between high TNF-β levels and low gross motor scores on developmental assessment in a sample of schoolchildren with past history of neonatal encephalopathy [34].

Similarly, individuals with post-traumatic stress disorder show significantly high levels of pro-inflammatory markers, such as interleukin-1β, interleukin-6, TNF-α, and C-reactive protein. A key feature of this disorder is neurocognitive function impairment, mainly in attention and PS, executive function, and verbal learning and memory [35], [36].

Recurrent enteric infections in the early life years cause enteric barrier leakage and dysbiosis; this, in turn, facilitates translocation of bacteria to the blood leading to low-grade systemic inflammation. Added to this, the impact of circulating bacterial products that affect the endothelial cells "which form the blood–brain barrier" to release inflammatory cytokines and mediators, thus activating microglial cells. If this subclinical neuroinflammatory state occurs in early life, it affects cognitive development in children. That can explain the negative correlations between Bayley motor subscale and inflammatory markers in the study [37].

In alignment with increase in serum inflammatory markers, fecal markers also were

increased, as the current study showed a significant increase in fecal (α1-AT) (p = 0.012*) and NEOP concentrations (p = 0.021*) in cases compared to controls, while the increase in α1-AT level did not reach the significant level. Moreover, a negative correlation between motor function and α1-AT (p = 0.05) was found in children under 2 years. The study suggested that the chronic inflammatory condition with consequent increase in inflammatory markers and cytokines could be the cause behind impaired motor development.

MPO, α1-AT, and NEO are three important biomarkers that can be assessed and measured easily in fecal and blood samples; they have been considered biomarkers of environmental enteric enteropathy [38]. A study of Kosek *et al*., 2013, combined the absolute measured concentrations of α1-AT, MPO, and NEO into a single metric and formed a composite EED score; the study investigated the link between this score and infants' linear growth and concluded an association between higher composite EED scores and faltering growth [39]. In addition, excretion of α1-AT in stool is an indicator of albumin leakage or exudation into the intestinal lumen [40].

Cases of the study with below-average IQ showed a statistically significant increase in MPO, EndoCab, and α1-AT than cases with average IQ. The increase in the level of α1-AT in CSF correlated directly with disturbances in the blood–brain barrier, with a higher risk for cognitive impairment [41].

Overall, the decrease in serum micronutrients, mainly zinc, Vitamin D, and iron, with elevation of serum and fecal markers of intestinal inflammation in cases of the current study added to the concomitant growth faltering and cognitive affection indicates that there is a vicious circle linking intestinal inflammation, micronutrient deficiencies, growth impairment, and cognitive affection.

Study limitations

The current study did not address socioeconomic standard of the studied sample and its correlation with micronutrient deficiency, faltering growth, and cognitive impairment. This is considered an important limitation to the study. Another limitation is the lack of similar researches in our country and the Middle East region "up to our knowledge" which presents the need for further studies in this field.

Conclusion

Our study showed impaired neurocognitive and psychomotor functions in malnourished, stunted children. Furthermore, there were observed vitamin and mineral deficiency and increased markers of intestinal inflammation in cases compared to healthy controls indicating role of intestinal inflammation in psychomotor development and cognitive functions in children with undernutrition.

To our knowledge, this is the first study in Egypt that searches for possible causes of unexplained growth and cognitive impairment. The potential developmental consequences of enteropathy or stunting can be devastating to the full physical and neurocognitive development in impoverished areas. Thus, early recognition and intervention in those at greatest risk is of paramount importance.

Assessment of fecal concentration of MPO, α1-AT and NEO helps in early detection of environmental enteropathy, and consequently early intervention. They provide promising biomarkers early detection and intervention.

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