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Original Article

Assessing the relationship between intestinal protozoal infection and nutritional status among children under 5 years of age

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Abstract

Intestinal protozoan infections are common, particularly among children under 5 years, who are more vulnerable due to their developing immune systems. This cross-sectional study aimed to assess the relationship between protozoal infections and nutritional status in 260 children under 5 years, conducted from 1/9/2024 to 1/2/2025. Parasitological diagnosis was done using direct microscopy (saline and iodine wet mounts) and modified Ziehl-Neelsen staining for coccidian parasites. Body mass index (BMI) was evaluated based on WHO standards to compare infected and non-infected groups. Entamoeba histolytica was the most prevalent protozoan, with a 56.25% infection rate. Underweight status was notably common among children aged 4-5 years. Findings indicated a strong association between protozoal infection and malnutrition, likely resulting from nutrient malabsorption, chronic diarrhea, and reduced appetite due to infection.

Keywords: Intestinal protozoa, Infection, Nutritional status, 5 years of age.

Introduction

An estimated 3.5 billion individuals are infected annually by intestinal protozoal infections, that are among the most prevalent infectious disorders. These infections can lead to severe health issues such as diarrhea, abdominal pain, undernutrition, general malaise, weakness, and impaired growth and physical development [1,2]. According to their weakened immune systems, children are particularly susceptible to these diseases and frequently contract them again [3,4].

Acute and chronic undernutrition are separated by their etiology. Yet, there is an aberrant nutrient loss, excessive energy expenditure, or decreased food intake which are usually the causes of secondary acute undernutrition in children. Primary acute undernutrition is caused by inadequate food intake [5,6], otherwise secondary acute undernutrition, which results in nutritional loss and decreased food intake, is primarily caused by chronic infectious conditions [7].

The main sign of acute undernutrition is wasting, which is caused by a deficiency of nutrients at particular points in early childhood, which inhibits both cognitive and physical development [8]. The most prevalent and visible sign of chronic undernutrition is stunting [9]. In addition to limiting healing, undernourishment raises the risk of infection and its severity. In addition, the immune system and undernutrition are related as immune system development that can be fatally disrupted by undernutrition, and vice versa. For example, the child is more susceptible to parasite infection when their immune system is compromised [10,11].

Two billion people, or about one-third of the world's population are deficient in some micronutrients [12], which leads to be infected with different protozoa in severe cases. Children's growth is affected by environmental factors such as sickness and nutrition, that are influenced by infection [13]. In particular, weight and height remain the only anthropometric measurements that are consistently verified and authorized for assessing nutritional status, particularly in children, and predicting performance [14].

Aim of the study:

The current study aimed to assess the relationship between protozoal infection and nutritional status among children under 5 years.

Methodology

Study subjects:

Ethical approval: Official aproval was obtained from Kirkuk Health Directrate before starting the study. A written agreement was taken from the parents for children who are under 5 years old which is included in the current study.

Study population: A total of 260 children under 5 years of age were included in the study after receiving an agreement from their parents before starting the current study and across sectional between September 2024 till end of month February 2025.

Stool samples: a representative sampling was taken from Pediatric Hospital, General Kirkuk Hospital, Azadi Teaching Hospital. A stool sampls were taken to detect protozoal infection by conducting the following tests**:**

Wet preparation: A drop of physiological saline (0.85%) was added to fresh feces samples (2 mg) then placed on a slide using a wooden applicator stick. For semi-solid and diarrheal samples, the stool was emulsified. Iodine had been used for formed stools. A cover slide was then placed over them and examined under a microscope with 10x and 40x objectives lenses [15].

Modified Ziehl Neelsen Technique (hot technique): After scooping and smearing stool samples on a sterile glass slide, they were fixed with 96% pure methanol for two to five minutes and allowed to dry at room temperature. They were fixed in flame for a short time. The smear was then stained with carbol fuchsin, left to stand for 20 to 30 minutes, and then cleaned with fresh tap water.

The smear was differentiated with $H_2SO_{4\ for}$ (20-60 seconds) (concentrations from 0.25 to 10%), rinse with water and counterstained with weight per volume malachite green for 5 minutes. Again, the smear was removed using fresh tap water and allowed to air dry. After that, the slide was examined under a microscope to check for oocysts [16].

Nutritional status: nutritional status **was** estimated by determining the body weight using the ordinary weight management balance leaving the heavy metal and the length using the ordinary stadiometer.

BMI (body mass index) was detected by the following formula:

= weight(kg) /square of height (m^2) [17]

This estimates the nutritional status of subject by comparing the value of BMI (body mass index) in both positive and negative groups.

BMI in children was classified due to WHO [18]:

BMI	*Nutritional status*
Below 16.5	Underweight
16.5 – 23.0	Normal weight
23.0-28.0	Over weight
More than 28.0	Obese

Statistical analysis: Statistical analysis: descriptive statics was used to determine the frequency and percent %.

Chi-test was used to indicate the relation between the variable and p-value which is regarded as significant at a level of (0.05).

Results and Discussion

Total number of participants sample were (260). The rate of intestinal protozoa prevalence among children under 5 years old was *Entamoeba histolytica* 56,25%, *Blastocystis hominis* 22,5%, *Cryptosporidium parvum* 15% and last *Giardia lambilia* 6,25% . The examined stool samples are shown in Table (1):

Table 1. Total number of participants

Total number of participants	*260*
male	115
femal	145
Total no. Of stool samples	260
Positive for intestinal protozoa	160
Negative for intestinal protozoa	100

Distribution of the studied samples due to age, sex, type of water and type of nutrition:

The results have shown in Table (2) that the most intestinal protozoa prevalence among children under 5 years old is *E.histolytica* 56,25% ,*B.hominis* 22,5%, *C.parvum* 15% and last *G.lambilia* 6,25%. The ages of the patients infected with *Entamoeba histolytica* were distributed under 5 years of 160 samples collected, (4-5) years 50%,(2-3) years 33%, (0-1) % 16,6%. Prevalence of intestinal protozoa in female is more than that in male except in *Cryptosporidium parvum* in male 62% in female 37%.

According to type of nutrition, the results showed that children depended on feeding (bulky food) were more infected and infants that depending on bottle feeding especially who are used tap water not sterilized more infected than infants depending on breast feeding as shown in the table (2).

The present study found that children infected with Gastrointestinalis and other intestinal parasites exhibited considerably greater levels of psychomotor development delay and growth retardation compared to children who were not infected. Growth delay was up to 2.9 times, general development delay was up to 1.9 times, language–cognitive development delay was up to 2.2 times, and fine motor development delay was up to 2.9 times worse in children with parasite illnesses than in children without any parasitic infections. Significant risk variables for intestinal protozoa were parental education, financial hardship, household size, hand washing, soil play, and a family history of parasite infections [19].

Intestinal parasitic infections (IPI) are more common in African and Asian cultures, but less is known about their incidence in European children or the risk factors that may contribute to parasite transmission [20]. The prevalence rate for all IPIs in children living in European nations was 5.9% overall. The parasite that was most frequently found, *Blastocystis hominis,* had a prevalence rate of 10.7% [21].

Table 2. Demographic characteristics of positive study subjects (160) examined by wet preparation and modified ziehl neelsen stain (M.Z.N) hot technique:

Demographic parameters		*Intestinal protozoa*			
		E.histolytica	*B.homins*	*C. parvum*	*G.lambilia*
Ages	(0-1)years	15(9.3%)	3(1.8%)	0(0%)	0(0%)
	(2-3)years	30(18.75%)	8(5%)	6(3.75%)	2(1.25%)
	(4-5)years	45(28.12%)	25(15.6%)	18(11.3%)	8(5%)
Sex	Male	35(21.8%)	16(10%)	15(9.37%)	2(1.25%)
	Femal	55(34.3%)	20(12.5%)	9(5.6%)	8(5%)
Type Of Water	Tap water	85(53%)	33(20,6%)	20(12,5%)	10(6,25%)
	Filtered water	5(3,12%)	3(1,8%)	2(1,25%)	0(0%)
Type Of Nutrition	Feeding (bulky food)	55(34,3%)	30(18,8%)	20(12,5%)	10(6,25%)
	Milk(breast feeding)	2(1,25%)	1(0,63%)	0(0%)	0(0%)
	Milk (bottle feeding)	33(20,6%)	5(3,12%)	4(2,5%)	0(0%)
Mean ±SD		30±12.25	12 ± 9,4	8 ± 7,5	3,33 ± 3,4

Relationship between BMI and the type of protozoal infection:

The results of the current study indicate that *E. histolytica* is the most prevalent protozoan, affecting 87 of 160 cases (84.75%). It is most common in the underweight category (66 cases, 64%), followed by the normal BMI group (15 cases, 53.5%), and the least in the stunting category (6 cases, 20.7%). *G. lamblia* is present in 18 cases (11.25%), with the highest percentage in the underweight category (12 cases, 11.56%). *C. parvum* affects 34 cases (21.25%), showing a higher prevalence in the stunting group (8 cases, 27.6%), followed by underweight (21 cases, 20.4%), and then normal BMI (5 cases, 17.8%).*B. hominis* is found in 21 cases (13.13%), with the highest percentage in the stunting category (11 cases, 37.9%), followed by normal BMI (6 cases, 21.4%), and the least in underweight (4 cases, 3.8%) as shown in the table 3.

The findings suggest that protozoan infections may contribute to malnutrition, particularly underweight and stunting, due to their impact on nutrient absorption and gastrointestinal health. Further research and intervention strategies should focus on improving hygiene, nutrition, and treatment for parasitic infections to reduce their impact on malnutrition [22].

According to weight-for-age, height-for-age, and weight-for-height, the prevalence of malnutrition was 6.7, 5.8, and 7.7%, respectively, in other research. A third (35.1%) of children were infected with parasites, of which 35.5% were infected with oxyuris, 22.8% were pathogenic, and 26.4% were non-pathogenic. The prevalence of parasite infection in the boys (51.1%) was significantly higher than the girls (42.3%).

Additionally, there was a substantial correlation ($P<0.05$) between parasitic illnesses and malnutrition (height-for-age). According to the study's findings, about one-third of Shahroud kindergarten students had at least one intestinal parasite infection, which can lead to malnutrition in preschoolers [23].

Table 3. Relationship between BMI and the type of protozoal infection

Type of protozoa	*BMI (body mass index)*			*Total*	*p-value*
	Normal	*underweight*	*stunting*		
Mean± SD	7 ±4	25.75±24.003	7.25±2.25		
E.histolytica	15(53.5%)	66(64%)	6(20.7%)	87(84.75%)	0.925
G.lambilia	2(7.14%)	12(11,56%)	4(13.8%)	18(11.25%)	0.484
C.parvum	5(17.8%)	21(20.4%)	8(27.6%)	34(21.25%)	0.799
B.hominis	6(21.4%)	4(3.8%)	11(37.9%)	21(13.13%)	0.000
Total	28(17.5%)	103(64.4%)	29(18.2%)	106(100%)	

Relationship between BMI and age of children in this study:

Children aged 4-5 years have the highest percentage of cases (97 of 160, 60.6%). They also have the highest prevalence of underweight (53 cases, 76.8%) and stunting (9 cases, 45%), indicating a greater risk of malnutrition in this age group. Children aged 2-3 years represent 21.8% (35 cases) of the total. This group has a moderate percentage of stunting (6 cases, 30%) but a relatively low percentage of underweight (6 cases, 8.7%) compared to the 4-5 years group as shown in the table (4).

Children aged 0-1 year make up the smallest portion (28 cases, 17.5%). They have a higher proportion of stunting (5 cases, 25%) compared to their total number but lower underweight cases (10 cases, 14.5%) than older age groups.

The highest prevalence of malnutrition (underweight and stunting) is in children aged 4-5 years, suggesting that nutritional deficiencies accumulate as the child grows. Underweight is most common in the 4-5 years group, indicating possible long-term inadequate nutrition, recurrent infections, or poor feeding practices [24]. Stunting is more frequent in younger age groups (0-3 years) but worsens in the 4-5 age group, suggesting a chronic issue related to prolonged nutritional deficiencies [25]. Intervention strategies should focus on early childhood nutrition, proper feeding practices, and controlling infections to prevent malnutrition from worsening as children grow.

Intestinal parasite infections are widespread and can hinder growth and physical development, due to a comprehensive analysis of the association between intestinal parasite infections and undernutrition in children under five. According to their immature immune systems and habits that increase their exposure to parasites, children under five are especially at risk [22].

Protozoan infections in children under five are associated with adverse effects on growth and nutritional status. The prevalence of these infections tends to increase with age within this group, potentially leading to growth delays and undernutrition. Implementing preventive measures and timely interventions is crucial to mitigate the impact of protozoan infections on children's health and development [22]

prevalence of protozoal infection among the studied sample

= No. of Protozoal Infection/Total * 1000

Most intestinal protozoa prevalence among children under 5 years old is *E.histolytica* 56,25%, *B.hominis* 22,5%, *C.parvum* 15% and last *G.lambilia* 6,25.

Table 4. Relationship between BMI and age of children in this study

Ages	*BMI (body mass index)* *Normal*	*underweight*	*stunting*	*Total*	*p-value*
Mean± SD	23.67±8.9	23±21.3	6.67±1.7		
(0-1) years	13 (18.3?%)	10(14.5%)	5(25%)	28(17.5%)	0.809
(2-3) years	23(32.4%)	6(8.7%)	6(30%)	35(21.8%)	0.004
(4-5) years	35(49.3%)	53(76.8%)	9(45%)	97(60.6%)	0.008
Total	71(44.4%)	69(43.13%)	20(12.5%)	160(100%)	

Conclusion

The most effective intestinal protozoa are *Entamoeba histolytica*. In addition, the mest effected ages related to BMI relationship is (4-5) years.

Recommendations

Further advances of nutritional education program regarding the risks group must be employed. Scientific large population study to involve large groups from different ages is also recommended. Public health implications findings should guide group-related prevention such as improving sanitation and health education.

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