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Original Article

The Impact of *Entamoeba histolytica* Infection on Vitamin B_{12} and Ferritin Levels in Children: A Study in Al-Muthanna City, Iraq

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Abstract

Introduction: *Entamoeba histolytica* is an invasive and pathogenic protozoan responsible for amoebiasis and significantly contributes to diarrheal disease in developing countries. This research aims to evaluate the association of *Entamoeba histolytica* infection with some blood parameters, especially its effect on vitamin B12 absorption and ferritin levels in children.

Methods: A total of 25 stool specimens and blood were obtained from pediatric patients (aged 1–6 years, both male and female) presenting with moderate to severe diarrhea, and 25 others from healthy children's blood as a control group. These samples were collected from November 2023 to February 2024 from Al-Hussain Educational General Hospital and selected private diagnostic laboratories in Al-Muthanna City. All cases were microscopically confirmed to be positive for *E. histolytica* infection. This process involves using fresh stool samples for wet mounts, permanently stained slides, and concentrates prepared with iodine staining.

Results: Statistical analysis revealed a highly significant difference in serum vitamin B12 levels between the infected patients and healthy controls. The results demonstrated a marked reduction in vitamin B12 concentrations among children infected with *E. histolytica* compared to their healthy counterparts regarding serum ferritin levels. Statistical analysis revealed a significant difference between infected and healthy children, with a *P* value of 0.001 at the 0.05 significance level. The data demonstrated a pronounced decrease in ferritin concentrations among children infected with *E. histolytica* compared to the healthy control group.

Conclusion: The results showed that ferritin and vitamin B12 levels were significantly lower ($P<0.05$) in children infected with *E. histolytica* compared to the control group.

Keywords: *Entamoeba histolytica*, Vitamin B12 and ferritin, Children, Al-Muthanna city, Iraq

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Introduction

Entamoeba histolytica is an invasive and pathogenic parasite responsible for amoebiasis and is a significant contributor to diarrheal disease in developing countries. The life cycle of *E. histolytica* includes two distinct stages: the cyst and the trophozoite. The cyst is infective, non-motile, excreted in the feces, and can survive for several weeks in the external environment (1, 2). The parasite is responsible for amoebic dysentery and amoebic hepatitis. Amoebiasis ranks third in global incidence among tissue-invasive protozoan infections, following malaria and schistosomiasis, with epidemiological data estimating that approximately 50 million individuals are infected (3). Parasitic infections can negatively impact nutritional status by disrupting food intake, digestion, and nutrient absorption. In children, this often results in malnutrition, anemia, growth retardation, cognitive impairment, and increased vulnerability to other infections. Protein malnutrition is widespread in low-income settings with poor sanitation, where damage to the intestinal mucosa leads to malabsorption, increased permeability, and diarrhea (4, 5). The association between *E. histolytica* infection and anemia has recently received increased attention (6, 7). Vitamin B12 (cobalamin) is an essential cofactor involved in DNA synthesis, purine metabolism, and carbohydrate-lipid metabolic pathways, and plays a critical role in maintaining normal neurometabolic and hematologic functions (8, 9). Vitamin B12 deficiency may result from inadequate dietary intake, maternal deficiency, the use of H2 receptor antagonists or proton pump inhibitors, malabsorptive conditions such as atrophic gastritis, pernicious anemia, and celiac disease, as well as from defects in intracellular cobalamin metabolism (10). Ferritin is a key iron-storage protein that maintains iron homeostasis, protects against oxidative stress, and supports metabolism. Abnormal ferritin levels are linked to conditions such as anaemia, neurodegeneration, cardiovascular disease, and cancer, emphasizing the need to better understand its regulation (11, 4). So it's essential to study the levels of these parameters in patients' serum. This study aimed to evaluate the impact of the parasite on

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the serum levels of vitamin B12 and ferritin in children infected with Entamoeba histolytica.

Materials and Methods

Study Design

Twenty-five samples were collected from November 2023 to February 2024 from Al-Hussain Educational General Hospital and some private diagnostic laboratories in Al-Muthanna City. The study population consisted of pediatric patients presenting with abdominal pain and bloody diarrhea, from whom stool samples were collected for parasitological analysis.

The study design involved the initial diagnosis of *E. histolytica* infection through direct microscopic examination of fresh stool samples using saline and iodine wet mount preparations. However, Figures 1 and 2 display the parasite's cyst form; they represent different magnifications (10x and 40x, respectively). Since samples were not collected on the same day, they were preserved in 10% formalin solution to maintain the parasite's structural integrity for accurate microscopic analysis.

After diagnostic confirmation, venous blood samples were collected from each patient and subsequently subjected to serological tests and biochemical analysis. The results were compared with another 25 blood samples collected from healthy children in the same age group. These analyses were performed using the AFIAS,cobas® c 111 analyzer, and cobas® e 411 analyzer to obtain the relevant clinical and immunological parameters required for the study.

Stool Examination

Stool specimens were examined using the direct smear technique. A drop of physiological saline was placed on a clean glass slide, and a small portion of feces was collected from multiple areas of the sample using a sterile wooden applicator. The fecal material was thoroughly mixed with the saline, and a coverslip was carefully applied to avoid air bubble formation. Particular attention was given to sampling regions containing mucus or blood, as these are commonly associated with *E. histolytica* trophozoites. The preparation was then examined under a light microscope (Optika B-350, Italy) to detect motile trophozoites.

Ferritin Test

The AFIAS-6® (© Boditech Med Inc, Korea) device was used for a ferritin test following these steps: Insert a cartridge into the cartridge holder, then insert a tip into the cartridge's tip hole. Next, select 'General mode' on the instrument for AFIAS tests. Using a pipette, take 100 µL of the sample (serum, plasma, or control) and dispense it into the sample well of the cartridge. Tap the 'Start' button on the screen. The test result will be displayed on the screen after 10 minutes. The cobas® e 411 analyzers (© F. Hoffmann-La Roche Ltd, Switzerland) were used for B12 measurement (fully automated).

ELISA Analysis of Vitamin B12

Serum levels of vitamin B12 were measured using a human-specific Enzyme-Linked Immunosorbent Assay (ELISA) kit (Sunlong Biotech Co., China), based on the sandwich ELISA principle, and following the manufacturer's protocol. In this method, the wells of the microplate are pre-coated with antibodies specific to vitamin B12. When the sample is added, vitamin B12 present in the serum binds to these capture antibodies. A secondary enzyme-linked antibody is then added, which binds to the captured antigen. A color change occurs after adding a substrate solution due to the enzymatic reaction. The intensity of the resulting color, directly proportional to the concentration of vitamin B12 in the sample, was measured using a microplate ELISA reader at 450 nm. Concentrations were calculated by comparing the absorbance values to a standard curve generated from known concentrations of vitamin B12.

Statistical Analysis

Chi-square and Student's *t*-tests were used to analyze

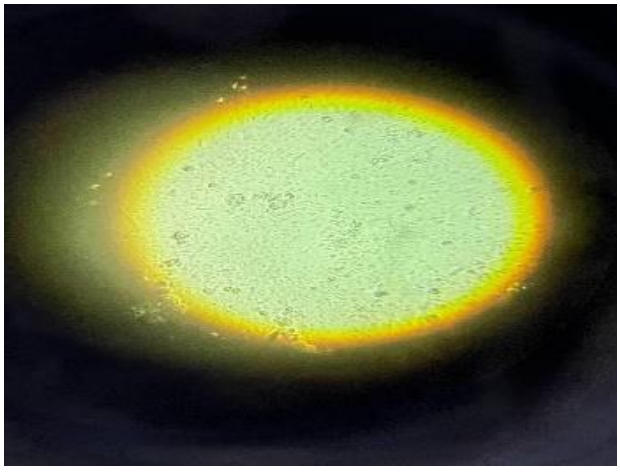

Figure 1. *E.histolytica* cyst under the light microscope X10

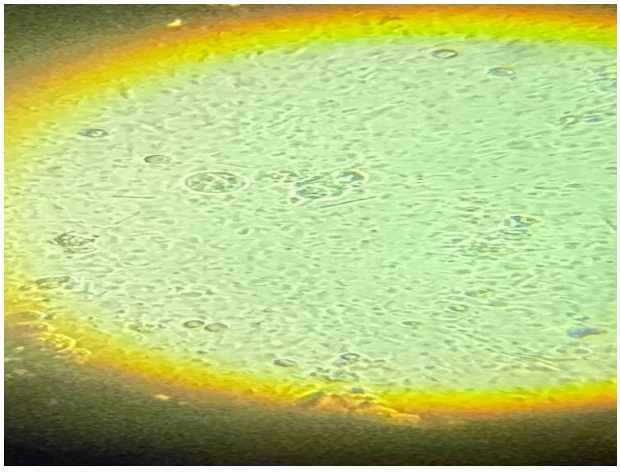

Figure 2. *E.histolytica* cyst under the light microscope x 40

blood parameter data between patients with *E. histolytica* infection and the control group. Differences were considered statistically significant at $P < 0.05$.

Results

The present study was conducted in Al-Muthanna Governorate, specifically at Al-Hussein Teaching Hospital and selected private laboratories within the city. Stool and blood samples were obtained from 25 pediatric patients (aged 1–6 years, both male and female) diagnosed with *E. histolytica* infection.

All infected children presented with abdominal pain and diarrhea, with some also experiencing episodes of vomiting. Blood test results from these patients were compared to those from a control group of 25 healthy children of similar age and sex.

Statistical analysis (Table 1) revealed a significant difference in serum vitamin B12 levels between the infected patients and healthy controls, with a *P*-value of 0.001, indicating strong statistical significance at the 0.05 probability level. The results demonstrated a marked reduction in vitamin B12 concentrations among children infected with *E. histolytica* (Table 2) compared to their healthy counterparts. Specifically, the mean vitamin B12 level in the control group was 617.04 pg/mL with a standard deviation of 184.082. In contrast, the infected group had a substantially lower mean value of 201.52 pg/mL and a standard deviation of 133.249. These findings suggest a potential link between *E. histolytica* infection and impaired vitamin B12 status.

Regarding serum ferritin levels, statistical analysis revealed a highly significant difference between infected and healthy children, with a *P* value of 0.001 at the 0.05 significance level. The data demonstrated a pronounced decrease in ferritin concentrations among children infected with *E. histolytica* compared to the healthy control group. Specifically, the mean ferritin level in healthy children was 65.76 ng/mL (SD = 28.661), whereas the infected group exhibited a markedly lower mean of 9.88 ng/mL (SD = 3.700), as detailed in Table 2.

The statistical analysis revealed a highly significant reduction in the serum levels of vitamin B12 and ferritin among children infected with Entamoeba histolytica compared to the healthy control group. As shown in Table 3, the mean vitamin B12 concentration in the infected group was 201.52 pg/mL, substantially lower than the control group mean of 617.04 pg/mL, with a standard deviation of 133.25 and 184.08, respectively. Similarly, the mean ferritin level in infected children was 9.88 ng/mL compared to 65.76 ng/mL in controls, with standard deviations of 3.70 and 28.66, respectively. Both comparisons showed a p-value of 0.001, indicating strong statistical significance ($P < 0.05$). These findings confirm a significant association between *E. histolytica* infection and deficient key micronutrients in pediatric patients.

Table 1. Statistical analysis of the results of blood tests (vitamin B12) for children infected with *E. histolytica*, compared to healthy children. Group Statistics for Vitamin B12

Group	N	Mean	Std. Deviation	Std. Error Mean
patients	25	201.52	133.249	26.650
control	25	617.04	184.082	36.816

Table 2. Statistical analysis of the results of blood tests (ferritin) for children infected with *E. histolytica*, compared to healthy children

Group	N	Mean	Std. Deviation	Std. Error Mean
patients	25	9.88	3.700	0.740
control	25	65.76	28.661	5.732

Discussion

E. histolytica is a protozoan parasite responsible for amoebiasis, a disease that primarily affects the colon and can also involve the liver. It is distributed globally, with higher prevalence in regions with inadequate sanitation. The parasite requires elevated levels of iron for survival and proliferation. Iron plays a crucial role in modulating the expression of key virulence factors, including hemoglobinases, hemoglobin-binding proteins, cysteine proteases, and proteins associated with the amoebic cytoskeleton (12, 6).

The results demonstrated reduced vitamin B12 and ferritin levels in children infected with *E. histolytica* compared to healthy controls. These results align with a study conducted in Baghdad, which reported a high prevalence of anemia among infected children; however, that study did not observe significant differences in ferritin levels between infected and non-infected groups (12).

In contrast, the present findings are consistent with a study conducted in Kirkuk involving children aged 8 months to 4 years, which revealed a significant reduction in serum ferritin levels among those infected with *E. histolytica* (13, 8). Similar results were reported in a study from Najaf, where ferritin levels were notably lower in the serum of infected individuals (14, 2).

Furthermore, a correlation was demonstrated between low serum vitamin B12 levels and infections caused by intestinal parasites, including E. histolytica (7). This observation is supported by a study conducted in Najaf, which found reduced vitamin B12 levels in adult patients infected with the parasite (3). These findings are consistent with international research. For instance,

Table 3. Statistical Relationship between *Entamoeba histolytica* Infection and Serum Levels of Vitamin B12 (pg/mL) and Ferritin (ng/mL) in Children

Parameter	Mean (Infected)	Mean (Control)	Std. Deviation (Infected)	Std. Deviation (Control)	*P* Value
Vitamin B12 (pg/mL)	201.52	617.04	133.25	184.08	0.001
Ferritin (ng/mL)	9.88	65.76	3.70	28.66	0.001

a strong association between severe acute malnutrition and vitamin B12 deficiency among Indian children (1). Similarly, a cross-sectional study in southern Madagascar confirmed that undernutrition, anemia, and micronutrient deficiencies, especially vitamin B12, are prevalent in school-aged children with high intestinal parasite burden (15). These global observations reinforce the consistent link between intestinal parasitic infections and disrupted vitamin B12 and ferritin status across diverse settings.

When *E.histolytica* infects the intestine, it secretes the enzyme histolysin, which contributes to the formation of ulcers in the intestinal mucosa and leads to gastrointestinal bleeding (16, 17). This mucosal damage can impair the absorption of essential nutrients such as ferritin and vitamin B12, which play critical roles in hematopoiesis (18-20). Additionally, the disruption of the gut environment negatively affects iron absorption. Since ferritin indicates iron storage, impaired absorption can lead to a decline in ferritin levels, contributing to iron deficiency and anemia in infected individuals (21).

The infection is widespread among children, primarily due to inadequate personal hygiene and frequent consumption of contaminated or unhealthy food (22, 23). These factors collectively contribute to nutrient malabsorption and subsequent iron and vitamin B12 deficiencies.

Conclusion

Our current study also demonstrated the significant association of E. histolytica infection with lower vitamin B12 and ferritin levels in children. These shortcomings can lead to anemia and compromise children's growth, immunity, and cognitive development. These data stress the clinical relevance of tracking micronutrient levels in children with intestinal parasitic infections, particularly where there is poor sanitation and limited access to healthcare.

Recommendations

- Routine surveillance: Incorporate stool and blood testing into routine checks for children from endemic areas to facilitate the early detection of parasitosis and associated nutrient deficiencies.
- Nutrition: Implement vitamin and iron supplementation programs in at-risk children, especially amoebic children.
- Public Health Education: Conduct community-based health education to raise awareness of hygiene and safe food practices in families to minimize transmission routes.
- Sanitation Enhancement: Work with the regional governments to help provide clean water access and sanitation facilities to low-income citizens to prevent amoebiasis.
- Combined Treatment Strategies: Combine antiparasitic treatment and nutritional rehabilitation in clinical protocols for infected children.

Authors' Contribution

Conceptualization: Mohammed H. Dakhil AL-Ghazzi.
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Methodology: Mohammed H. Dakhil AL-Ghazzi.
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Resources: Khaldoun M. Al-Rishawi.
Software: Khaldoun M. Al-Rishawi.
Supervision: Mohammed H. Dakhil AL-Ghazzi.
Validation: Khaldoun M. Al-Rishawi.
Visualization: Mohammed H. Dakhil AL-Ghazzi.
Writing–original draft: Khaldoun M. Al-Rishawi.
Writing–review & editing: Mohammed H. Dakhil AL-Ghazzi.

Competing Interests

The authors declare no conflict of interest.

Ethical Approval

Not applicable.

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