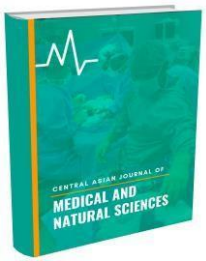

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Article

Hematological and Biochemical Study in Children Infected with Entamoeba histolytica in Al Diwaniyah Province/Iraq

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Abstract: Intestinal parasitic infections, especially Entamoeba histolytica, are a serious public health problem worldwide, especially in developing countries, particularly Iraq. The current study aimed to estimate the correlation between E. histolytica infection in children and some hematological and biochemical parameters. Ninety clinically ill patients presented to the Women and Children's Hospital with contaminated diarrhea between September 2024 and March 2025. Patients' ages ranged from 5 to 13 years. Stool samples were collected from 90 individuals for direct microscopic examination. Stool samples were obtained from 90 participants for direct microscopic analysis. Out of these, 60 individuals were diagnosed with E. histolytica infection, while the remaining 30 healthy individuals formed the control group. Body mass index (BMI) and hematological markers, including erythrocytes, hemoglobin level, packed cell volume (PCV), white blood cell count (WBC), neutrophils, and lymphocytes, were assessed using a Sysmex analyzer. The results showed a significant decrease ($P < 0.05$) in body mass index (BMI), the red blood cell (RBCs), hemoglobin level, and packed cell volume (PCV) in patients diagnosed with an E. histolytica infection compared to the healthy control. The study revealed that patients infected with E. histolytica showed a marked increase ($P<0.05$) in total white blood cell (WBC) count, neutrophil count, and lymphocyte count when compared to the healthy group. Biochemical parameters, including C-reactive protein (CRP) and kidney function (urea and creatinine), were significantly elevated ($P<0.05$) in the E. histolytica infected group compared with the healthy control, In contrast, lipid profile, cholesterol, and triglyceride levels were significantly lower ($P<0.05$) in comparison to the healthy group. The study concluded that there is an correlation between E. histolytica infected and changes in hematological and biochemical parameters, leading to anemia and immune system disorders. The kidneys and liver are also affected, resulting in impaired lipid metabolism and digestion.

Keywords: Entamoeba Histolytica, Hematological Parameters, Biochemical Parameters, Children Infection, Al Diwaniyah Province

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1. Introduction

Protozoan Entamoeba histolytica is the organism that due to intestinal amebiasis as well as symptoms that are seen outside of the intestines. According to [1], amoebic dysentery is an infection that is caused by the parasite of Entamoeba histolytica. The most majority of infections do not cause any symptoms; nevertheless, invasive intestinal disease can manifest itself with symptoms such as abdominal pain, cramps, diarrhea that is either watery or bloody, and weight loss that lasts for several weeks. Several examples of disseminated extra intestinal diseases, including liver abscess, pneumonia, and cerebral

amoebiasis, have been documented in the medical literature. It is believed that over fifty million people are infected with E. histolytica at any given time, with the bulk of infections occurring in countries that are considered to be undeveloped. This infection is responsible for more than one hundred thousand deaths each year. Transmission normally takes place through the consumption of contaminated water or food that is the result of cysts being excreted in the feces. Additionally, fecal-oral transmission can take place inside households and during male homosexual behaviors. An anti-inflammatory immune response is triggered when E. histolytica invades the colon and liver. This immune response has the potential to successfully decrease the immunological responses [2]. E. histolytica lives primarily in the colon as a harmless parasite, but it is capable of causing colitis, dysentery and devastating liver abscesses.

The factors that initiate the transition to a pathogenic phenotype and the commencement of disease remain unidentified. We are becoming increasingly aware of Mortimer [1]. Clinical amoebiasis results from the spread of parasites that normally live in and outside the colon wall; The publishing of the genome of E. histolytica represented a significant advancement in comprehending this intricate, multi-faceted process.

Entamoeba histolytica ranks as the third major cause of mortality attributed to parasites globally. Entamoeba histolytica infection typically remains asymptomatic; yet, the parasite possesses pathogenic potential.

This study aims to assess the correlation between anemia and intestinal parasite infections caused by E. histolytica, along with an specific of hematological and biochemical parameters in children living in Diwaniyah city.

2. Materials and Methods

Sample Collection

Samples were collected from December 2024 to February 2025 in various areas of Al-diwaniyah governorate.

The study participants were categorized into two groups following a general stool microscopic examination. The first group, consisting of (60) children, was diagnosed as having E. histolytica. In contrast, the stool samples of the second group, comprising (30) children, revealed no pathogens, and this group was deemed healthy group. Blood samples from all participants were collected and analyzed in the laboratory, while the nutritional status of each child was also evaluated.

Body Mass index (BMI)

Parents of the children were interviewed using a pre-designed questionnaire to collect data on their basic characteristics, as well as their past and current diseases. Body weight and height were measured using standardized procedures and recorded as the midpoint of repeated measurements. Children's weights were measured using a special electronic scale (India Mart, India). The children wore simple clothing. Body mass index (BMI) was used to assess the children's nutritional status, which was categorized as severe malnutrition (BMI < 20.7 kg/m^2) or normal malnutrition (BMI > 25 kg/m^2). The nutritional status of children was assessed between the ages of 5 and 13 years.

Stool examinations

Collecting a stool samples

Stool samples were collected from each patient and placed in clean, dry, tightly sealed containers. They were examined within half an hour in the parasitology laboratory, and Entamoeba histolytica was found in the samples.

Macroscopic examination stool samples

The presence of the parasite was noted by the gross consistency of stool samples, with mucus, blood and other materials present.

Direct Stool Microscopy Method

This procedure involved examining two smears from each stool sample, utilizing both normal saline and Lugol's iodine solution. To begin, two clean, dry microscope slides were prepared, one for each solution. A clean wooden stick was used to collect portions of the stool sample from various areas, ensuring any streaks of blood or pus would be detected. Each solution—normal saline and Lugol's iodine—was carefully mixed with the specimen on the corresponding slides. Coverslips were then placed over the prepared smears, and both slides were examined under a microscope using low (x10) and high (x40) magnification to evaluate the samples thoroughly.

Collection of blood

For each person, 5 ml of humeral venous blood was drawn in type of plastic tubes, where a portion of the blood was placed in a tube containing the EDTA inhibitor for conducting hematological parameters.

Studies standards

Hematological test

Hematological parameters (RBCs, HG, PCV) were measured using the Nsysmex – kx21 (Auto B).

Biochemical measurements

a. Kidney functions :

Kidney function was measured using a spectrophotometer, and the number of ready analyzes was used for each of the studied parameters (urea, creatinine) by the French company (BIOLABO).

b. Measuring C-reactive protein Levels

The percentage of C-reactive protein was determined using the ELISA device using Atlas Medical equipment.

c. Lipid profiles levels

Lipid profile was measured using a colorimetric enzymatic method using the STANDARD LipidoCare device.

Statistical analysis

The results were expressed as mean ± SE.The data were analyzed by using T- test (MS – works version 1.5) and taking ($P<0.05$) as the lowest limit of significance.

3. Results

A. Body mass index (BMI)

Results showed that those infected with E. histolytica had severe malnutrition (BMI) compared to the healthy control group with the percentages reaching(25.87) 58% and (18.79)42%, respectively, as shown in Figure 1.

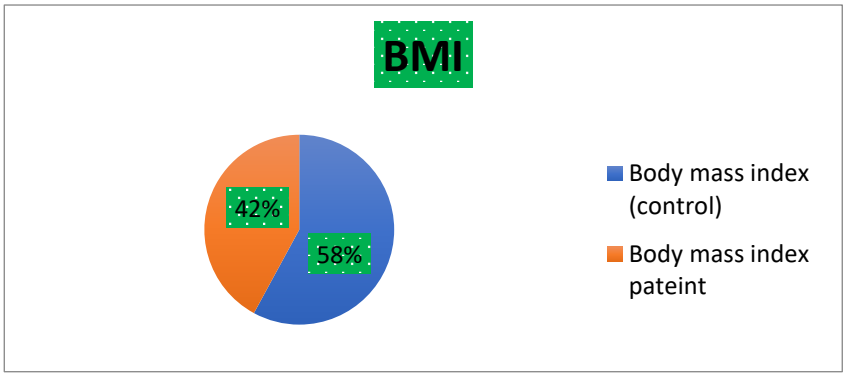

Figure 1. Body mass index for patients diagnosed with an E. histolytica infection and healthy control.

B. Hematological parameters

In the current study, (60) patients were confirmed with E. histolytica infection, and (30) healthy individuals free of parasites were used as the control group. Table 1 shows a significant difference (P ≥ 0.05) between patients and the healthy group in red blood cells (RBCs), hemoglobin (Hb) level, and packed cell volume (PCV), which showed a significant decrease (P ≥ 0.05) when compared to the healthy group, as shown in Figure 2. The study results also showed a significant increase (P ≥ 0.05) in the total white blood cell count (WBC), neutrophil count, and lymphocyte count when compared to the healthy group in patients diagnosed with E. histolytica infection, as shown in Figure 2.

Table 1. Show hematological parameters in patient group and control group.

Parameter	Control Healthy n=30	Patients n = 60
RBCs (X106/mm³)	* 4.00 0.079	2.971 ±0.012
HB g/dl	* 12.91±0.732	8.512±0.181
PCV (%)	* 38.24±0.652	30.98 ± 0.765
WBCs (X103/mm3)	6.891±0.568	* 8.123 ± 0.213
Neutrophil	48.448±0.231	*59.589 ± 0.789
Lymphocyte	22.587±0.055	*25.232 ± 0.215

* This highlights a statistically significant difference (P≤0.05) between the patient group and the healthy group.

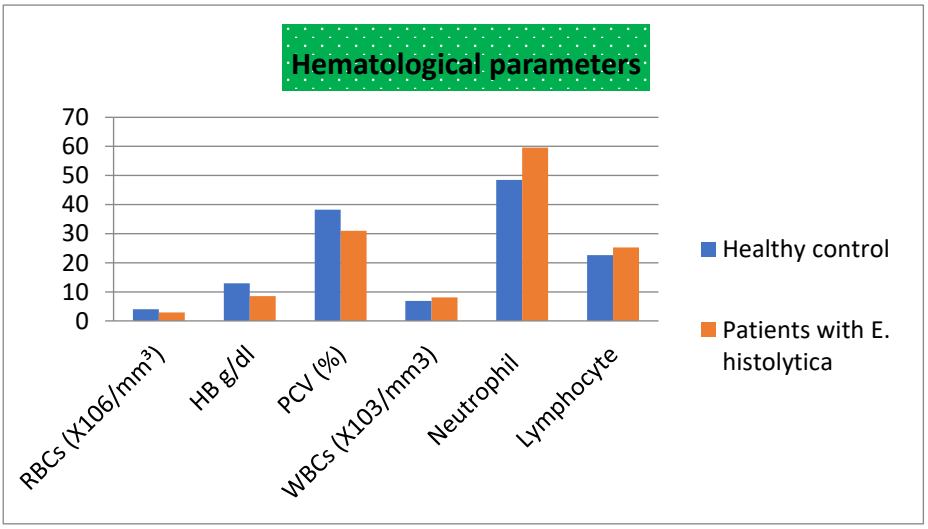

Figure 2. Show hematological parameters for patients infected with E. histolytica and healthy control.

C. Biochemical parameters

Kidney functions

Regarding creatinine and urea levels, the results of the current study in Table 2 showed a slight significant higher (P>0.05) in urea and creatinine levels in patients diagnosed with an E. histolytica infection compared to the healthy group, as shown in Figure 3.

Lipid profiles parameters

The findings of the present study in Table 2 showed significant differences (P>0.05) in the cholesterol level in patients infected with the E. histolytica parasite compared to the healthy group, while the results of triglycerides showed a significant decrease (P<0.05) in patients diagnosed with the E. histolytica parasite infection compared to the healthy controls as in Figure 3.

C-reactive protein

Showed result in table 2 increase significant (P>0.05) in C-reactive protein concentration in patients with parasites E. histolytica infection compared to healthy control as in figure 3.

Table 2. Show Biochemical parameters in patient group and control group.

Parameters	Control Healthy n=(30)	Patient n=(60)
Creatinine (mg/dl)	1.01±0.05	*** 1.49±0.25**
Blood urea (mg/dl)	23.1±3.55	*** 44.2±2.29**
CRP (mg/dl)	1.21±3.25	*** 15.54±2.33**
Cholesterol	165.19±51.3	*** 145.8±77.2**
Triglyceride	**155.43±45.2**	*** 136.72±62.4**

* This highlights a statistically significant difference (P≤0.05) between the patient group and the healthy group.

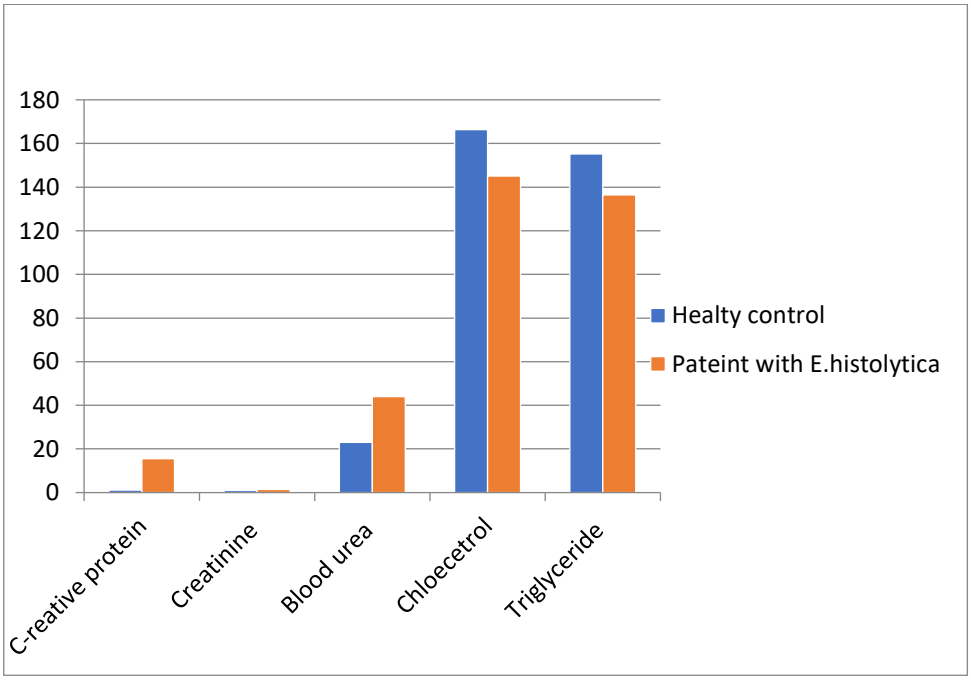

Figure 3. Show Biochemical parameters for patients infected with E. histolytica and healthy group.

4. Discussion

Amoebiasis constitutes a significant health issue in underdeveloped nations, particularly affecting youngsters. The incidence and prevalence of amoebiasis are rising

due to heightened travel and migration from endemic regions to wealthier countries. Due to the fact that the vast majority of parents do not display any symptoms, the process of identification and treatment presents difficulties for clinicians, which may lead to the continued transmission of the disease among the population. When certain demographic parameters (such as gender, ethnicity, and travel history) are present, E. histolytica should be considered as a differential diagnosis for specific intestinal symptoms, including colitis [3]. This is especially true when certain demographic factors are present.

Findings indicated that 56.9% of children infected with E. histolytica exhibited severe malnutrition (body mass index <31.9 kg/m^2), in contrast to healthy controls who demonstrated lower levels of malnutrition [4]. It is likely that the pathogenicity of the parasite and the development of amebic colitis, which is distinguished by diarrhea that may be bloody and watery, coupled with stomach discomfort and weight loss, are to blame for the greater prevalence of acute malnutrition in children who are infected with E. histolytica [5].

This finding is consistent with other previous studies that have shown that malnutrition is associated with a lower weight in those infected with E. histolytica, as measured by weight and height (WFH). This finding is consistent with the research that suggests that children who are smaller in both height and weight are more likely to be infected with intestinal parasites than children who have larger anthropometric measurements [6].

According to the presented results, patients diagnosed with an E. histolytica infection had significantly lower levels of hemoglobin (Hb), and red blood cell (RBC) counts and packed cell volume (PCV), compared to the healthy control. This study is consistent with [7] but differs from [8]. This result may be explained by the fact that the parasite causes digestive disturbances and releases a motile feeding stage that attaches to the intestinal villi and absorbs nutrients from them. Proteolytic enzymes are secreted, which are responsible for the decomposition of host tissues and cells, while also consuming red blood cells [9]. There are also marked variations in serum iron concentrations between patients infected and uninfected with some intestinal parasites. E. histolytica requires high iron concentrations to survive, and this protozoan can extract iron from host proteins. Therefore, it is possible that a severe infection with E. histolytica leads to low host iron levels. Study data indicated a significant increase in the white blood cell count, likely attributed to elevated neutrophil and lymphocyte counts. Following infection with pathogenic E. histolytica, the immune system mounts a strong response, ultimately leading to protective immunity [10].

E. histolytica interacts with the mucosal layer of the small intestine, resulting in varying degrees of villous atrophy. Villous atrophy is accompanied by inflammatory infiltration and hyperplasia of enterocytes. As a result of these activities, enterocytes are weakened, and bile acid metabolism is altered, affecting the absorption of essential nutrients required for physiological function. These nutrients include vitamins such as folic acid, iron, and zinc [10].

According to the findings of the study, there were significant differences ($P>0.05$) in the levels of cholesterol between patients who were infected with E. histolytica and healthy controls. When comparing the levels of triglycerides between patients who were infected with E. histolytica and the control group, it was observed that there were significant differences ($P<0.05$) in all of the values. The results demonstrated slight absorption of cholesterol from lipids during E. histolytica infection. This study is consistent with [11], which indicated that the parasite utilizes lipids for growth within the host. No notable variation in cholesterol levels was observed. A notable disparity in triglyceride levels has been seen in patients with amoebiasis. E. histolytica acquires lipids and cholesterol from the upper gut, and its mechanisms of action remain ambiguous. The virulence and pathogenicity of its adhesion's on epithelial cells may also be a contributing factor in this phenomenon [12]. This parasite, responsible for amoebiasis, functions by diminishing

cholesterol absorption in liver abscesses, resulting in increased serum cholesterol and triglyceride concentrations [13]. Figures indicate altered lipid levels in most E. histolytica patients, especially in inflammatory conditions [14]. This is because the parasite utilizes cholesterol due to E. histolytica's inability to synthesize it itself [11]. Triglyceride levels exhibited considerable fluctuations within the same patient, as they are contingent upon cholesterol levels. The findings of this study aligned with those of [14] and [15].

Regarding urea concentration, the results showed an increase in urea concentration. This finding differed from a study that recorded a decrease in urea concentration in people infected with the parasite. The current investigation revealed a minor reduction in creatinine levels, aligning with the findings of Pamanekar et al.

The study demonstrated an elevation in C-reactive protein levels, corroborating the findings of Seo and Shin, indicating that people who are infected with parasites have higher quantities of C-reactive protein.

C-reactive protein (CRP) plays a fundamental and important role in fighting infections by binding to neutrophils, which are known for their effective role in fighting infection. Therefore, C-reactive protein stimulates the immune response and eliminates dead cells and pathogens. Other studies have shown that C-reactive protein plays an important role in cellular immunity when it binds to and activates phagocytic receptors.

5. Conclusion

The study showed an association between E. histolytica infection and changes in blood parameters, leading to anemia and immune system disturbances. Kidney and liver function were also affected, leading to impaired fat metabolism and digestion in the body.

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