NTU Journal of Pure Sciences
Double Blind Peer Review Journal
ISSN: 2789-1089 EISSN: 2789-1097
Available Online: https://journals.ntu.edu.iq/index.php/NTU-JPS
Volume 5, No. 1, 2026

Original Article

Estimation of Some Hematological, Biochemical and Some Electrolytes Parameters in Children Infected with Entamoeba histolytica in Kirkuk city

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Received: 26 May 2024
Accepted: 19 August 2024
Published: 12 April 2026
DOI: https://doi.org/10.56286/hp27j930
Article ID: 992

Abstract

The current investigation aimed to estimate some parameters in child infected with E. histolytica. A total of 240 children were included in this study which was carried out in the laboratory of the Pediatric Hospital, Azadi Teaching Hospital, Maternity children Hospital and Kirkuk Teaching Hospital, in Kirkuk City. The results found that the highest infection rate was due to *E. histolytica*, which reached 89 (68.9%) using the direct examination method, while the ELISA method showed that 48(37.2%) of the total children had diarrhea. Hemoglobin concentration, PCV, ferritin and some electrolytes (potassium, sodium and chloride) showed that some inflammatory parameters (WBC, CRP) significantly reduced($P \leq 0.05$) and others significantly elevated($P \leq 0.05$) in samples. Also, the creatinine demonstrated non-significant ($P \leq 0.05$) differences in serum of patients with *E. histolytica* compared with the controls. It is concluded that *E. histolytica* led to anemia in children and caused a high immune response in patients.

Keywords: E. histolytica, Hemoglobin, Urea, ferritin.

Introduction

Amebiasis is a parasite infection that can cause a variety of clinical manifestations, including non-intestinal amebiasis and severe fulminant colitis, as well as asymptomatic colonization by the protozoan *Entamoeba histolytica* in humans [1]. *Entamoeba histolytica* has a rather straightforward life cycle that consists of two stages: the vegetative trophozoite and the dormant cyst [2]. Entamoeba histolytica is an intestinal parasite that causes amebiasis. According to several studies, it infects 50 million people worldwide and kills 40,000–100,000 people annually. Amebiasis is one of the leading causes of death from parasitic infections, along with schistosomiasis and malaria [3]. Amebiasis is one of the most significant health issues in underdeveloped nations [4]. Two dysentery phenotypes that

are suggestive of a symptomatic amoebic infection are blood and mucus in the feces. Sometimes, the parasite enters the liver and causes abscesses, which might appear months or years after visiting or residing in a region where amoebis is endemic [5]. Enzyme-linked immunosorbent assay (ELISA) has been developed by several researchers to identify antigens in fecal samples. These antigen detection techniques are quick to conduct and offer a sensitivity that is comparable to stool culture. Monoclonal antibodies against the Gal/GalNAc-specific lectin of E. histolytica are used in antigen-based ELISA kits that are specific for the species [6]. Children who have amoebic dysentery have dehydration and an imbalance in electrolytes. Children with acute diarrhea die at a significantly higher rate when several forms of electrolyte disturbances are present [7]. So, the current study aims to estimate some hematological, kidney function and inflammatory parameters in children infected with *E. histolytica*.

Materials & Methods

Patients

A total of 240 children with ages ranging from 1 week to 5 years were included in this study. The present work was carried out in laboratory the Pediatric Hospital, Azadi Teaching Hospital, Maternity children Hospital and Kirkuk Teaching Hospital, all located in Kirkuk City. The samples were obtained from children in the hospital (of different sexes and age under 5 years) during the period from September 2023 to January 2024.

Stool Collecting

A stool sample (2 mg) from each one was collected in a sterile container and each specimen was examined microscopically. Then, two direct smears were done, one in normal saline and the other one in lugal's iodine. The examination was done under 10x and 40x objective lens were performed to demonstrate the presence of protozoan trophozoites or cysts. The second smear was stained with iodine which is used to examine the cystic stages of the parasites. A part of stool sample for each patient was put in sterile screw cap containers and kept at -20°C until being examined by ELISA. And dates were collected from patients by using questionnaires assessing the contrasts in prevalence among genders, age groups, and months.

Blood Collection

Blood samples were taken from (48) children who had *E. histolytica*. Each patient was given a disposable plastic syringe, and blood was drawn via vein puncture. 5ml of venous blood were drawn from a sick and healthy child. In order to detect the whole blood count, 2 ml were added to the EDTA tube. To initiate the clotting process, 3 ml of the remaining blood was placed in a gel tube and allowed to stand at room temperature for half an hour. After that, the material was centrifuged for five minutes at 3000 rpm to separate the serum for biochemical testing.

Hematological Tests

Whole blood count: The swelab Alfa (Swedan) automatic hematology analyzer was used to measure the platelet count, hemoglobin level (Hb), and white blood cell count in human blood. A mere 30µl of fully preserved blood in an EDTA tube was pipetted into a cuvette and introduced into the apparatus. The normal range of white blood cell count was 3.5 -10.0 10^3/UL with a hemoglobin level (11.5 -15.5 g/ dl), and a platelet count (150 -400 10^3/UL).

Biochemical Test

Kidney function (urea, and Creatinine), CRP and ferritin were measured by Accent 200 (Poland) automatic biochemical analyzer. To begin the clotting process, only 3ml of blood were placed in a gel tube and allowed to stand at room temperature for 30 minutes. After that, the sample was centrifuged for five minutes at 3000 rpm to separate the serum for the biochemical test. The normal range of serum urea is 15-40 mg/dl, serum Creatinine is 0.6 -1.2 mg/dl, acceptable fasting blood sugar levels may be higher at 80-130 mg/dl.

Electrolytes

Sodium, potassium, and chloride were measured by EX-Ds JOKOH (Japan) automatic electrolyte analyzer. The normal range of serum sodium is 136 -155 mmol / L, serum potassium is (3.5 -5.3 mmol/ L), and serum chloride is (95 -105 mmol / L)

Ethical Approval

The study was carried out in compliance with moral guidelines derived from the Helsinki Declaration. The local ethics committee examined and approved the permission form, subject information, and study protocol.

Statistical Analysis

Computer applications SPSS version 21 and GraphPad Prism version 8 were used to statistically analyze the data. Bar graphs and statistical test results were presented as Mean±SE. The Man-Whitney U test, or unpaired T-test, was employed to compare the parameter means between the patient and control groups.

Result & Discussion

Sample Distribution

In the current study, stool specimens were collected from children with gastrointestinal tract infection. 240 samples were directly examined by using light microscopic with wet mount to screen the prevalence of a variable parasitic form. Yet, 129 (53.8%) samples were found positive while others, 111(46.2%) samples were negative, table (1).

Table 1. Distribution of study group according to results

Procedures	*Samples*	*Positive samples*	
		No.	%
Direct examination (wet mount) for parasite	240	129	53.8

Table (2) shows different methods used in the current study, which included the direct method, reporting 89(68.9%) out of a total of 240, while the results using the ELISA method revealed 48(37.2%) of the total children with diarrhea. Considering the results of the sensitivity and specificity test, it was found that the ELISA method was more sensitive (97.9%) for the specificity (98.8%) compared to live microscopy, and the sensitivity was (56.1%) for the specificity (57.8%).

Table 2. Methods of diagnosis for *E. histolytica*

Types of parasite	*Methods of diagnosis*	
	Microscopic by ?	ELISA
E. histolytica	89(68.9%)	48(37.2%)
Sensitivity %	56.1%	97.9%
Specificity %	57.8%	98.8%

While *E. dispar* is found commensally and is non-invasive, *Entamoeba histolytica*, one of the two species of Entamoeba with similar morphology that infect humans, causes invasive intestine and extra intestinal illnesses. Microscopically, there is no way to distinguish between *E. dispar* and *E. histolytica* due to their identical morphology. However, the ELISA technique can be used to identify the antigens of *E. dispar* and *E. histolytica* [8]. Tables (4-3) summarize the result which reveals that 89(68.9%) were positive by microscopy. On the other hand, using Bio-Pharma *E. histolytica* ELISA assay, as a gold stander test, the antigen was detected in only 48(37.2%) sample.

Hematological Parameters

The results of the current study showed that there is a significant (P≤0.05) difference in some hematological parameters of patients with both *E. histolytica*. However, hemoglobin concentration demonstrated significant (P=0.001) reduction in patients with *E. histolytica* (9.39±1.84) compared with the control (12.74±1.05). The percentage of PCV demonstrated a significant (P=0.001) reduction in patients with *E. histolytica* (39.12±2.49) compared with the controls (27.51±3.62).

Table 3. Some of hematological parameters in studied groups

Paramters		*Groups*	*No.*	*Mean±SD*	*t-test*	*P value*
Hematological parameters	Age (year)	Control	50	2.72±1.89	0.21	0.935
		E. histolytica	48	2.61±2.35		
	Hb (g/dl)	Control	50	12.74±1.05	8.55	0.001
		E. histolytica	48	9.39±1.84		
	PCV (%)	Control	50	39.12±2.49	8.92	0.001
		E. histolytica	48	29.85±2.71		

* Same letters mean there is non-significant (P≤0.05) differences, different letters mean there are significant (P≤0.05) differences.

These findings are in line with those of [9], who found that anemia was common in those who had certain intestinal parasite infections. Furthermore, a study [10] discovered that gastrointestinal parasite infections affected 37% of anemic individuals. Also, anemia and parasitic illnesses were reported to be related in the provinces of Baghdad and Mosulby [11, 12], respectively. Every prior study that included participants infected with gastrointestinal parasites revealed a high frequency of anemia. Based on the current study's findings, anemia was primarily reported in children under the age of one to two years. The results from other countries [13, 14] are consistent with the higher incidence of anemia in children under the age of three.

Kidney Functions

The results of the current study showed that there is a significant (P≤0.05) difference in kidney function parameters of patients with both *E. histolytica*. In addition, the levels of urea significantly (P=0.031) elevated patients with *E. histolytica* (39.73±6.03) compared with control (15.93±4.17). The levels of creatinine showed non-significant (P=0.141) differences in the serum of patients with *E. histolytica* (0.85±0.17) compared with controls (0.61±0.14).

Table 4. Kidney function parameters in studied groups

Parameters		*Groups*	*No.*	*Mean±SD*	*t-tes*	*P value*
Kidney function	Age (year)	Control	50	2.72±1.89	0.21	0.935
		E. histolytica	48	2.61±2.35		
	Urea mg/dl	Control	50	0.61±0.14	5.17	0.031
		E. histolytica	48	39.73±6.03		
	Creatinine mg/dl	Control	50	0.61±0.14	0.74	0.141
		E. histolytica	48	0.85±0.17		

*Same letters mean there is non-significant (P≤0.05) differences, different letters mean there is significant (P≤0.05) differences.

The liver produces urea as a waste product during the digestion of proteins, and the glomeruli in the kidneys ordinarily remove urea from the body by ultrafiltration. The breakdown product of creatine phosphate in muscle, which the body produces at a fairly steady pace, is creatinine, an important sign of renal health [14].

The results of the current study indicated that the level of B. Urea in patients with diabetes who were carriers of intestinal parasites were significantly higher when compared to the control group. These results are consistent with the results of the study conducted in Iraq [15] which found that the level of B. Urea for patients with Diabetic patients carrying intestinal parasites were mmol/L 5.48±1.63 vs. 4.98±1.37 and at a probability level of 0.01.

Inflammatory Parameters

The results of the current study showed that there is a significant (P≤0.05) difference in some inflammatory parameters of patients with both *E. histolytica*. Also, the counts of WBC demonstrated significant (P=0.001) elevations in patients with *E. histolytica* (13.94±3.72) compared with control (6.49±2.51). The levels of CRP significantly (P=0.001) elevated in the serum of patients with *E. histolytica* (58.93±10.23) compared with the controls (3.19±1.41). Ferritin concentration significantly (P=0.001) reduced in serum of people with *E. histolytica* (8.83±0.72) compared with the controls (18.04±1.65).

Table 5. Some of inflammatory parameters in studied groups

Parameters		*Groups*	*No.*	*Mean±SD*	*t-tes*	*P value*
Inflammatory parameters	Age (year)	Control	50	2.72±1.89	0.21	0.935
		E. histolytica	48	2.61±2.35		
	WBC	Control	50	6.49±2.51	8.36	0.031
		E. histolytica	48	13.94±3.72		
	CRP (mg/dl)	Control	50	3.19±1.41	6.81	0.001
		E. histolytica	48	58.93±10.23		
	Ferritin (µg/mL)	Control	50	18.04±1.65	9.11	0.001
		E. histolytica	48	8.83±0.72		

*Same letters mean there is non-significant (P≤0.05) differences, different letters mean there is significant (P≤0.05) differences.

The total number of white blood cells rises or falls below the normal range because it shows how the body defends against this disorder due to immune cell nomination of basophils and lymphocytes in infection areas or neutrophil collapse brought on by the parasite's virulence factors [16]. Additionally, a study [17] mentioned that the presence of the parasite may have caused alterations in white blood cells, with lymphocytes and granule cells in particular acting as the body's immune response to the parasite. The current study concurs with a work [18], who discovered a statistically significant difference in CRP levels between the *E. histolytica* positive cases and control group. This could be because infants are more likely to be exposed to *E. histolytica* at this early age through contaminated food or water, as well as behavioral factors linked to children's propensity to explore their surroundings through touch and taste behaviors that raise the risk of contracting the infection. Since serum ferritin is a measure of total body iron reserves and is used to diagnose iron insufficiency in the absence of comorbid conditions, ferritin is regarded as an early and highly specific marker [19]. Ferritin levels in children infected with *E. histolytica* were shown to be lower in this study than ferritin levels in the control group. Children with *E. histolytica* infection had a considerably higher frequency of low ferritin levels (63.82%) than the control group, where the prevalence was lower (29.4%). The trophozoite of *E. histolytica* is able to use ferritin as an iron source, which is probably why children infected with this parasite have low ferritin levels [20]. These findings corroborated those of another Iraqi study, which found that ferritin levels were lower in children infected with *E. histolytica* and greater in non-infected children [11].

Electrolytes

The results of the current study showed that there is a significant (P≤0.05) difference in the levels of some electrolytes in serum of patients with both *E. histolytica* and the levels of Potassium significantly (P=0.016) reduced in serum of patients with *E. histolytica* (4.13±0.35) compared with the controls (4.93±0.21). The levels of Sodium significantly (P=0.026) reduced in serum of patients with *E. histolytica* (122.81±5.25) compared with the controls (143.12±4.94). The levels of chloride significantly (P=0.001) decreased in the serum of patients with *E. histolytica* (89.31±3.17) compared with the control (102.83±3.51), as in table (6).

Table 6. Some of some electrolytes in studied groups

Paramters	*Groups*	*No.*	*Mean±SD*	*t-tes*	*P value*
Age (year)	Control	50	2.72±1.89	0.21	0.935
	E. histolytica	48	2.61±2.35		

Electrolytes	Potassium (mmol/L)	Control	50	4.93±0.21	5.73	0.016
		E. histolytica	48	4.13±0.35		
	Sodium (mmol/L)	Control	50	143.12±4.94	4.99	0.026
		E. histolytica	48	122.81±5.25		
	Chloride (mEq/L)	Control	50	102.83±3.51	7.84	0.001
		E. histolytica	48	89.31±3.17		

*Same letters mean there is non-significant (P≤0.05) differences, different letters mean there is significant (P≤0.05) differences.

The results of potassium showed that there is a significant decrease in the patient with *E. histolytica* compared with the control group for potassium level. Regarding the levels of potassium, a study [21] recorded no significant changes in potassium with parasitic infection. Yet a study [22] was in agreement reporting that hypokalemia was observed in patients with diarrhoea. This established the presence of ex-amine potassium channels, such as related mediators from E. histolytica-induced host cell death, and the barrier of potassium evasion that prevented *E. histolytica* from causing cytotoxicity. The action of human potassium ducts indicates a decrease in the intercellular potassium concentration in the host cell when combined with an increase in extracellular potassium concentration. This is a direct action of the parasite that evades potassium; after its presence, potassium produced by *E. histolytica* is required for human cell death and inflammation. When amoebic colitis symptoms include diarrhea, the significance of potassium ion transport to *E. histolytica* cytotoxicity with inflammation is suitable [23, 24]. The finding that infectious parasite disorders can cause hyponatraemia implies that the condition itself is not the cause of hyponatraemia, but rather the severity of the ailment. Second, as numerous investigations with hospitalized adult patients have shown, hyponatraemia is a well-known marker of disease severity and a predictor of mortality regardless of the cause [25].

Conclusions

Based on the results of the current study, Entamoeba histolytica has a direct effect on blood parameters, as it can cause anemia in children and cause a high immune response in patients. Also, due to diarrhea resulting from infection with the parasite, it leads to a loss of electrolytes and a decrease in their levels in the blood serum of infected children, along with high levels of urea.

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