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Original Article

Assessment of specific inflammatory responses in children with Entamoeba histolytica/dispar infections and how they relate to certain clinical and epidemiological features

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ABSTRACT

The goal of this study was to find out how certain inflammatory responses in children with E. histolytica and E. dispar infections. 230 subjects reported with diarrhea in General Pediatric Hospital, Kirkuk from September 2023 to January 2024. Experimental work was carried out at private Laboratories in Kirkuk, Iraq. 50 healthy children without any diseases were also taken as a control group. 230 samples were directly examined by using microscopic (wet mount) examination for parasite diagnosis. whereas, 91(39.57%) samples were found positive and 139(60.43%) samples were negative. The percentage of infected male patients was (53.8%%) while, the percentage of female patients was (46.2%%). The percentage of infected children in the age group less than one year was about (41.8%). Platelets, lymphocytes and granulocytes showed significant (P≤0.05) differences between E. histolytic and E. dispar patients compared to the control group. IgA showed significant (P≤0.05) reduction and C-reactive protein (CRP) showed significant (P≤0.05) elevation between E. histolytic and E. dispar patients compared to the control group. Based on the results of the current study, it was found that infection with E. histolytica/dispar leads to an increase in the immune cells.

Keywords: *E. histolytica, inflammatory reactions, IgA, Children*

Introduction

In 1903, Fritz Schaudinn gave the parasite the name E. histolytica because of its ability to cause tissue lysis [1]. Clifford Dobell stated in 1919 that two species of *Entamoeba, E. histolytica*, which produces cysts with four nuclei, and E. coli, which produces cysts with eight nuclei, are the parasites that infect the human gut. *E. histolytica* was considered an obligatory tissue parasite at the time by Dobell. When the parasite was initially produced on culture in 1925, they were subsequently compelled to modify this opinion [2]. *Entamoeba* are members of the class *Lobosa* and the phylum Sarcomastigophora [3]. The parasite *Entamoeba histolytica* can live in the human body as a vegetative trophozoite or as an infectious cyst [4]. Numerous investigations disclosed the frequency of amebiasis in different governorates within Iraq; the estimated prevalence of *E. histolytica/dispar* in Iraq ranged from 1.66% to 90.5% per study [5]. According to reports, 42.27% of appendicitis patients in Kirkuk City have E. histolytica/dispar [6]. It was discovered that 29.6% of hospital patients in Kirkuk had an *E. histolytica* infection [7]. The pathophysiology of *E. histolytica* is complex since the parasite's virulent molecules

and the host's immune system both contribute to the disease's development by damaging tissues that allow the parasite to enter systemic areas [8]. *E. histolytica* breaks down ingested cells through phagocytosis, cytolysis, and interaction with target cells, among other destructive processes. The cells twist the microvilli and open the Tight junctions (TJ) to increase the para-cellular permeability following contact with trophozoites in the epithelium [9]. Neutrophils were among the earliest immune cells to react to amebic attacks. The immune response to the intestinal tract serves as a supplementary line of defense against an *E. histolytica* infestation, even though the mucosal layer in the intestinal tract typically functions as the primary physical barrier against gut infections. Mucosal immunoglobulins, including secretory IgA, were the primary component of the gut defense system in humans [10]. According to a study, *E. histolytica*-caused diarrhea was associated with higher CRP levels. As a result, CRP can be utilized as a nonspecific immunological indicator to assess the infection's clinical presentation. The chemicals on *E. histolytica's* membrane that trigger mucosal-related macrophage stimulation are recognized by the innate immunity, and the concentration of CRP rises somewhat [11]. So, the current study was aimed to estimate some inflammatory responses in children infected with *E. histolytica/dispar*.

Materials & Methods

Patients

230 children (age: <1-15 years) reported with diarrhea in General Pediatric Hospital, Kirkuk from September 2023 to January 2024. Experimental work was carried out at private Laboratories in Kirkuk, Iraq. 50 healthy children without any diseases were also taken as a control group.

Stool collecting

Each participant had a 2 mg stool sample collected and put in a sterile container. After that, each specimen was examined under a microscope. Using 10x and 40x objective lenses, the examination was performed to determine the presence of cysts or protozoan trophozoites.

Ethical approval

According to the native ethics group, these studies were approved, and all patients who participated gave informed permission by their parents and provided information about the study's goal. Besides, the study is approving by the Medical Research Ethical Committee, Institutional Review Board of University, and Ministry of Health, Kirkuk Governorate Department, Training and Human Development, Knowledge Management and Research Division.

Measurements

- **Blood cells:** White blood cell and platelet sizes can be computed and determined using the impedance-based Swelab Alfa method. Using three initial WBC and platelet hydraulics, it prints the blood count data on thermal paper and shows the results on a liquid crystal display (LCD) with a histogram (swelab, Sweden).
- **CR protein:** IchromaTM CRP is a fluorescence immunoassay (FIA) that measures CRP quantitatively in human whole blood, serum, or plasma. It is used to estimate a patient's serum CRP levels. Sandwich immunodetection is used in this test; antigens in the sample bind to detector antibodies in the buffer to create antigen-antibody complexes, which then move onto nitrocellulose matrix to be collected by additional immobilized antibodies on the test strip.
- **Human IgA (Immunoglobulin A):** Sandwich-ELISA is the technique used with the IgA ELISA Kit (SUNLONG, China). This kit includes a Microelisa stripplate that has been pre-coated with an Endocab-IgA-specific antigen. The proper Microelisa stripplate wells are filled with standards or samples, which are then mixed with the designated antigen. Next, each Microelisa stripplate is well-filled with an antigen specific for Endocab that has been conjugated with horseradish peroxidase (HRP) and incubated. Free parts are removed by washing. Each well receives an addition of the TMB substrate solution. Only the wells containing HRP conjugated Endocab antigen and Endocab-IgA will initially appear blue before becoming yellow with the addition of the stop solution. At a wavelength of 450 nm, the optical density (OD) is determined using spectrophotometry.

Statistical analysis

The statistical analysis was performed using the SPSS version 21. Statistical test findings and bar graphs were expressed using Mean±SE. The unpaired t-test (Man-Whitney U) was performed to compare variable means between patients and healthy people.

Results and Discussion

Sample distribution

230 samples were directly examined by using microscopic (wet mount) examination for Entamoeba diagnosis (fig: 1). whereas, 91(39.57%) samples were found positive, and 139(60.43%) samples were negative, table 1.

Table 1. *Entamoeba histolytica*/ *dispar* microscopically positive samples

Procedures	Samples	Positive samples	
		No.	%
Direct examined (wet mount)	230	91	39.57

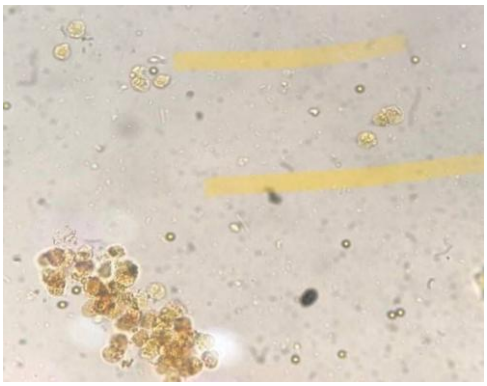

Figure 1. *Entamoeba* under microscope

Epidemiologic and clinical factors

The relation of gender with *E. histolytica* / *dispar* infection

The gender of patients showed non-significant (P≤0.05) differences. The percentage of infected male patients was 53.8% while, the percentage of female patients was 46.2% Table 2.

Table 2. Entamoeba histolytica/ dispar frequency in relation to the gender

Gender	Number of samples	Positive results	
		No.	**%**
Male	102	49	**53.8%**
Female	128	42	**46.2%**
Total	230	91	**39.6**
P-value		**0.195**	

The study's statistical analysis revealed that there was no significant difference ($p<0.05$) in the rate of Entamoeba species infection between males and females. In Malaysian Kirkuk city, comparable outcomes were documented [6]. Comparable results were noted in the Iraqi province of Al-Qadisiya, where 41.6% of infections with Entamoeba species were in females and 58.3% in males [13]. Male conduct at this age and increased exposure to harmful germs may be the cause of this. In a different study, 38,004 amoebiasis patients were included during the course of the 6-year study period (50.4% female and 49.6% male), indicating an equitable distribution by sex [14]. The coronavirus disease 2019 (COVID-19) pandemic in Iraq may have contributed to the lowest infection rate of any year during the study period, 2020, since people were fearful of hospitals and health facilities [15].

The relation of age with *E. histolytica / E. Dispar* infection

The current study showed the relationship between patients infected with the parasite E. histolytica by age groups less than (15 years). The percentage of infected numbers in the age group less than one year was about (41.8%). In contrast, the percentage of infected children in the age group 1-5 years was (29.7%), the age group between 6-10 years was (22.0%), the age group 11-15 years recorded 6.5%. The results of this study did not show any significant difference at the level of probability (0.05), as shown in table 3.

Table 3. The age of patients in current study

Age	Positive results	
	No.	%
<1	38	**41.8%**
1-5	27	**29.7%**
6-10	20	**22.0**
11-15	6	**6.5**
Total	91	**100.0**
P-value	**0.081**	

The statistical analysis's findings revealed no significant differences at the 0.05 level of probability. This is because the majority of infections in children under one year old are caused by mothers' lapses in basic personal hygiene, as well as the contamination of nursing supplies. Additionally, children under two years old have weakened immune systems and are more vulnerable to infection.. Repeated exposure to the parasite for young age groups (children) may stimulate their immune system and be more developed, leading to a decrease in infection rate [16]. According to the age highly significant differences ($p<0.05$) noticed between groups, <1 years was constituted the higher percentage, while 11-15 years was the lower percentage as shown in table (3). This difference may be due to the fact that the small children engage more in frequent hand to that mouth activity expedite infection transition. lt was in contrast to the study of , who showed that the infection rate were greatest in school children (11.6%) , in comprised to smaller ones [17] . showed that the infection rate in school children was 19.9% while in Kindergartens was (13.6%) in study of Al-Saeed and Issa [18]. The infection rate was highest in the age group 1–15 years, their attributed that maybe because parent are responsible for their hygiene. This result is in agreement with Hamad et al., [19] whom reported percentage 88 % in 1-12 years which may be attributed to defecation practices because these children groups are quite independent in latrine used and are more involved in both outdoor activities and feeding. The ≤ 1 year group had a low rate (8%) of infection comparing with those of E. histolytica. Also studies was significantly ($p<0.05$) highest in 1-10 years are similar to Entamoeba histolytica (60%), followed by 11-20 years (34%) and <1 year (27%), while showed lower infection rate (16%) in 41-50 age group [20].

The relation of diarrhea duration with *E. histolytica* / *dispar* infection

The current findings showed the relationship between patients infected with the parasite *E. histolytica/ dispar* and diarrhea duration. The percentage of infected numbers in the diarrhea duration less than 3 days was about 71.4%. In contrast, the percentage of infected people in the diarrhea duration 4-7 days was 20.9%, the diarrhea duration more than 8 days was 7.7%, as shown in table 4.

Table 4. The diarrhea duration of patients infected with *E. histolytica /*

Duration /day	Positive results	
	No.	**%**
<3	65	**71.4%**
4-7	19	**20.9%**
>8	7	**7.7**
Total	91	**100.0**
P-value	**0.001**	

The present results demonstrated that 71.4% of children had diarrhea for less than three days. These findings are in line with those of a previous study [6], which found that only 16 patients (7.27%) had diarrhea for more than ten days, while 132 patients (60%) had diarrhea for less than three days.

The relation of Type of feeding with *E. histolytica* / *dispar* infection

The present work showed the relationship between patients infected with the parasite *E. histolytica* and type of feeding. The percentage of bottle feeding was about 29.7% for children less than 2 years old. In contrast, the percentage of Mixed feeding was 41.7%, the edible food was 28.6%, The results of this study did not show any significant difference at the level of probability (0.05), as shown in table 5.

Table 5. Type of feeding in relation to *E. histolytica* / *dispar* infection

Type of feeding	Positive results	
	No.	%
Bottle	27	**29.7%**
Mixed	38	**41.7%**
Edible food	26	**28.6%**
Total	91	**100.0**
P-value	**0.091**	

The most significant risk factor for contracting *E. histolytica* infection was discovered to be nursing behavior, as instances of the illness, particularly those involving infants under a year old, exhibited a noticeably greater percentage of poor breastfeeding. This makes sense given that mature human milk and colostrum, that was produced during the first four days of breastfeeding, both significantly kill *E. histolytica* and shield breastfed infants from infection [21]. *G. lamblia* and *E. histolytica* are killed by bile salt-stimulated lipase in human milk, which achieves this deadly impact [22]. Furthermore, it is noteworthy that numerous research has confirmed the continued prevalence of bottle feeding and insufficient exclusive breastfeeding among *E. histolytica* cases. The findings of these research were concerning due to the remarkably low rate of exclusive breastfeeding. This falls well short of even the most stringent guidelines set by the World Health Organization, which urge exclusive nursing for 4-6 months. During the first six months of life, partial breastfeeding was the most common feeding pattern, and lactation length rapidly decreased [14]. The results also showed that Mixed and Edible foods cause E. histolytica / dispar infection in children. Studies worldwide have shown that fruits and vegetables can transmit protozoan cysts and oocytes of Giardia lamblia, Entamoeba coli, Entamoeba histolytica, Toxoplasma gondii [23].

Hematological parameters

Table 6 shows some hematological parameters significant (P≤0.05) differences between the study groups. Platelets showed significant (P≤0.05) differences between *E. histolytic* patients and *E. dispar* patients compared to the control group. There were also significant (P≤0.05) differences in the percentages of lymphocytes and granulocytes between *E. histolytic* patients and *E. dispar* patients compared to the control group.

Table 6. Some hematological parameters in E. histolytic / E. dispar patients

Parameter	*E. histolytic*		*E. dispar*		Control		P value
	Mean	±SD	Mean	±SD	Mean	±SD	
Platelets	366.70a	148.80	403.40a	108.90	272.30b	38.75	0.0004
WBCs	10.64a	4.94	7.70b	2.79	6.37b	1.39	<0.0001
Lymphocytes	3.82a	1.93	3.67a	1.36	3.82b	1.93	0.0133
Granulocytes	7.92a	4.69	4.93b	1.99	3.69b	1.71	0.0002

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

When compared to the healthy control group, patients infected with *E. histolytica* had a substantial increase ($P < 0.05$) in total leukocyte counts and differential type of granulocytes (Table 6). The study's data revealed a significant increase in WBCs, which could be explained by an increase in neutrophils, monocytes, lymphocytes, eosinophils, and basophils due to the development of protective immunity following infection with the pathogenic *E. histolytica* [24]. This protozoan's high density causes malabsorption of nutrients required for the synthesis of blood components [25]. The literature on the inflammatory response of human intestinal epithelial cells reflects the pathophysiology and protective immunity of leukocytes against protozoan parasites. Regarding parasite infection and the major impact of differential WBC count, lymphocytes demonstrated a high rate of both parasitic infections at all stages when compared to the control group. When compared to the control, the lymphocyte displayed a much higher rate of *E. histolytica*. Leukocytes demonstrated a substantial difference in all infection cases when compared to the control group. Granulocytes were higher in all infection types as compared to the control group. This is because, in an immunological response to the parasites, lymphocyte counts are increased during parasitic infection [26].

Immunological parameters

Table 7 shows some immunological parameters, a significant (P≤0.05) differences between the study groups were appeared. IgA showed significant (P≤0.05) decrease in *E. histolytic* patients compared to the control group. There were also significant (P≤0.05) elevated in the concentration of CRP between *E. histolytic* patients and *E. dispar* patients compared to the control group.

Table 7. Some immunological parameters in *E. histolytic* / *E. dispar infected* patients.

Parameter	*E. histolytic*		*E. dispar*		Control		P value
	Mean	±SD	Mean	±SD	Mean	±SD	
IgA	1.35b	0.28	2.46a	1.14	2.37a	0.67	<0.0001
CRP	12.37a	7.16	8.17a	4.28	4.68b	2.97	0.0146

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

A mucosal IgA antilectin antibody response is linked to immune protection against *E. histolytica* colonization, according to a study that looked into the relationship between protection from intestinal infection and systemic antibody or mucosal responses with the pathogen [27]. The current investigation found that a parasite infection led to a reduced in IgA levels. Additionally, a number of studies have shown that IgA is linked to resistance to a number of mucosal infections [28], [29]. The intestine's lining epithelial cells manufacture the antibodies that cause the rise in IgA because these cells' surfaces contain unique receptors that can bind to IgA, IgG, and IgM. Ninety percent of the cells in the thin layer of the duodenum and ileum create IgA. By creating these antibodies, these lymphocytes develop into plasma cells that produce local IgA antibodies. In mucosal humoral immunity, antibodies are essential because IgA may bind to pathogenic bacteria and neutralize poisons, preventing them from adhering to mucous surfaces. When mucous membrane injuries occur, a fall in serum levels and an increase in the number of immune antibodies are seen in the mucous sites due to their ability to transfer across the mucous membranes [30], Due to increased secretion and greater levels in the mucous lining of the intestine, which results in lower levels in blood serum, this explains the current study's findings about lower levels of IgA in the serum of patients infected with *Entamoeba histolytica.* In contrast, the current study's results of significantly elevated CRP in comparison to a healthy child were similar to those of previous studies that established CRP as a reliable indicator of inflammation and disease activity in amebiasis separately [31].

Conclusion

Based on the current study's findings, it was found that Entamoeba histolytica/dispar infection affects the immune system and directly affects the white blood cell counts of infected children. IgA and CRP are two of the most crucial markers that can be utilized for diagnosis.

Conflict of Interest

The authors declare that there is no competing of interests.

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