

Estimating of Some Interleukin Levels in Children with Amoebic Dysentery

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Abstract: Gastrointestinal protozoan parasites are one of the major and pervasive health issues that affect more than three billion people globally. *G. lamblia* and *E. histolytica* were the most common intestinal parasites found in children and newborns, who make up the bulk of patients exposed to illnesses. Therefore, the purpose of this study was to estimate certain interleukin levels in children who had amoebic dysentery. To find out if the child had an *E. histolytica* infection, a general stool investigation was performed. A total of 120 youngsters under the age of 15 attended the parasitology unit between April and June of 2024. The chosen children experienced stomach ache and diarrhea. The results showed that 39(32.5%) samples were found positive for *E. histolytica*. The gender of patients showed significant ($P \leq 0.05$) differences. The percentage of infected male patients at (29.1%) while, the percentage of female patients at (70.9%). The results found that the concentration of IgM (187.01 ± 17.75) and IgG (1532.5 ± 45.61) showed significant ($P \leq 0.05$) elevated between *E. histolytica* patients compared to the control group (57.59 ± 8.14 ; 729.4 ± 16.22 respectively). The results found that the concentration of IL-6 (6.04 ± 1.42) and IL-17 (18.74 ± 1.03) show significant ($P \leq 0.05$) elevated between *E. histolytica* patients compared to the control group (2.63 ± 0.45 ; 7.12 ± 0.29 respectively). According to the current

investigation's findings, females have a greater infection rate than males. However, this study found that children with *E. histolytica* who had healthy observation collection had considerably greater serum levels of IL-6 and IL-17.

Keywords: amoebic dysentery, interleukin, *E. histolytica*, IL-6.

Introduction

Acute gastroenteritis, which has a significant morbidity, mortality, and financial cost, continues to affect children worldwide. Over 500,000 children under five pass away from diarrheal illnesses every year, with low- and middle-income nations accounting for the majority of these deaths. Additionally, hospital stays and ER visits are frequently caused by diarrheal diseases [1–4]. When diarrhea lasts longer than two weeks, it might be either acute watery diarrhea without blood or acute bloody diarrhea or persistent diarrhea. Diarrhea is defined as having three or more bowel movements per day. Consistency is more important than frequency [5]. After breastfeeding, infants may occasionally pass semi-solid or yellowish feces, which is not diarrhea [6]. *Entamoeba histolytica* a protozoan parasite that can occur in either the cyst or trophozoite stage, is the cause of amoebic dysentery, an intestinal infection. The spherical *E. histolytica* cyst typically has a diameter of 10 to 16 μm and is encased in a retractable wall made of chitin. while it is mature, it has four nuclei; while it is immature, it has one nucleus, glycogen in a vacuole, and frequently chromatid bodies. The trophozoites are very motile and range in size from 20 to 40 μm in diameter [7, 8]. According to a number of studies, *Entamoeba histolytica* is one of the leading causes of death from parasitic infections, including schistosomiasis and malaria, and it infects 50 million people worldwide, resulting in 40,000 to 100,000 fatalities each year [9, 10]. Amebiasis is one of the most significant health issues in poor nations [11]. Environmental factors, including social, economic, demographic, and hygiene-related behaviors, have a significant impact on the prevalence of *Entamoeba histolytica* and have an impact on the spread and distribution of parasitic infections [12]. Numerous studies have found that age, place of residence, raw vegetable consumption, and drinking water quality are significant risk factors. Some researchers hypothesized that young newborns are unlikely to contract amebiasis very often because transmission is commonly linked to contaminated food and water. Malnutrition, immunosuppression, and young age are linked to more severe illness [13]. Unlike *E. histolytica*, host defense also depends on cell-mediated immune responses. In the initial phases of infection, intestinal epithelial cells (IECs) use the tolllike receptor (TLR)-2/4 to recognize and identify the carbohydrate recognition domain of the Gal/GalNAc lectin. This triggers NF κ B, which in turn causes the release of inflammatory cytokines, including IL-1, 6, 8, 12, IFN-, and TNF- α [14]. While IL-4 and TNF- have been connected to disease, IFN- has been connected to the removal of infection [15]. So, this investigation was aimed to estimating of some interleukin levels in children with amoebic dysentery.

Materials & Methods

Patients

To diagnosis for an *E. histolytica* infection, the child received a routine stool examination. The parasitology section received 120 visits from children under the age of 15 between April and June of 2024. Children that were chosen experienced stomach ache and diarrhea.

Samples collection

Fresh stool samples were taken and placed in a sterile, screw-disposable plastic container. For wet mount analysis, a piece of the material was treated directly. Serum samples were obtained from

patients whose *E. histolytica* test results were microscopically positive.

Blood collection

Using a sterile syringe, 5 ml of each the individual's venous blood was extracted, and the sample was placed in sterile gel tubes. The blood was separated by centrifuging it for five to ten minutes at 3000 rpm. After that, the serum was divided into four Eppendorf tubes and stored at -20 C in a deep freezer until it was needed [16-17].

Inclusion criteria

The study included all children aged 1month-12years who did not receive any treatment.

Exclusion criteria

Children over 12 years of age were excluded, and every child received treatment or suffered from other health problems.

Measurements

- **Immunoglobulin (IgG, and IgM):** As directed by the kit, blood immunoglobulin levels were measured using a quantitative sandwich enzyme-linked immunosorbent assay.
- **Interleukin-6 (IL-6):** The Human IL-6 ELISA Kit (No. EZHIL6, Sigma-Aldrich) is a sandwich enzyme-linked immunosorbent test (ELISA) that uses a 96-well strip plate that has been pre-coated with a capture antibody. This kit is designed to quantify human IL-6 precisely from serum.
- **Interleukin-17 (IL-17):** The Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kit for human IL-17 (No. RAB0262, Sigma-Aldrich) comes with a 96-well strip plate that has already been coated with a capture antibody. The precise measurement of human IL-6 from serum is the focus of this kit.

Statistical analysis

The independent t-test, spearman test, and ANOVA table were used to express significant changes at a probability threshold of 0.05 using the SPSS version of the computer program. The results were displayed as a $M \pm SE$ [18-19].

Results & Discussion

Sample distribution

The purpose of the study was to find out the prevalence of intestinal parasite infections were in kids less than fifteen. In order to test for parasite infections under a microscope, 120 stool samples from children who had diarrhea were taken. Children with gastrointestinal tract infections provided stool samples for the current study. In order to diagnose parasites, 120 samples were directly inspected utilizing microscopic (wet mount) examination. However, 81 (67.5%) samples were determined to be negative, whereas 39 (32.5%) samples were found to be positive, table (1)

Table (1): Distribution of study group according to results

Procedures	Samples	Positive samples	
		No.	%
Direct examined (wet mount)	120	39	32.5

According to the current study, the prevalence percentage of *E. histolytica* in Kirkuk city was 32.5%. This proportion was in line with the findings of Taher et al.'s study [20], which found that intestinal parasites were reported in 69 (31.7) people in Kirkuk city. Mahdi and Hussein's study [21], which found that the overall infection rate among children in Diyala city was 37.5%, was in agreement with the current study's findings. In contrast, the microscopical wet mount in the other investigation, conducted in Kirkuk city, revealed a 10.7% positive rate for *E. histolytica* / *E. dispar*

[22]. Additionally, earlier research conducted in Baghdad City (24.39%) [23] found a different outcome. The current study's percentage is lower than the total prevalence of *Entamoeba* infection, which was 65.7% (115/175) in a study done in Egypt [24] to determine the extent of *Entamoeba* infection in children. *Entamoeba histolytica* was the most common intestinal parasite, with an infection incidence of 27.81% across 356 of the 1280 stool samples analyzed in another study carried out in Jordan [25]. Evidently, 32.5% of the 230 stool specimens were microscopy-positive, which is consistent with a study by Uslu et al. [26] in Malaysia that found that 31.1% of stool specimens tested with trichrome staining contained *E. histolytica*.

The relation of gender with *E. histolytica* infection

There were significant ($P \leq 0.05$) variations in the patients' genders. The percentage of infected male patients at (29.1%) while, the percentage of female patients at (70.9%) (Table:2).

Table (2): the gender of patients in current study

Gender	Number of samples	Positive results	
		No.	%
Male	41	16	29.1%
Female	79	39	70.9%
Total	120	55	45.8%
P-value		0.001	

The current study's statistical analysis revealed a significant ($p < 0.05$) changes in the rate of infection by *Entamoeba* species between males and females, with females experiencing a higher rate of infection than males. Communities in rural Malaysia experienced similar outcomes [27]. Similar results were seen in the Iraqi province of Al-Qadisiya, where 41.6% of females and 58.3% of males were infected with *Entamoeba* species [28]. Male behavior at this age and more exposure to *Entamoeba* species infection sources than females, such as consuming tainted outdoor food or water more frequently and interacting with sick people, may be the cause of this.

Immunoglobulins

The table (3) showed some immunoglobulins parameters significant ($P \leq 0.05$) differences between the study groups. The results found that the concentration of IgM (187.01 ± 17.75) and IgG (1532.5 ± 45.61) showed significant ($P \leq 0.05$) elevated between *E. histolytica* patients compared to the control group (57.59 ± 8.14 ; 729.4 ± 16.22 respectively).

Table (3): the concentrations of some immunological parameters in *E. histolytic* / *E. dispar* patients compared with control group

Parameter	<i>E. histolytic</i>		Control		P value
	Mean	\pm SD	Mean	\pm SD	
IgM (mg/dl)	187.01 a	17.75	57.59 b	8.14	0.001
IgG (mg/dl)	1532.5 a	45.61	729.4 b	16.22	0.001

* Different letters indicate significant ($P \leq 0.05$) differences, while the same letters indicate non-significant ($P \leq 0.05$) differences.

Numerous infections are contracted by tainted food, drink, or both. In order to control the illness, T and B cells are activated, which helps to start immunity [29]. Specific blood immunoglobulin activation and estimate have been proven to be useful diagnostic and prognostic tools [30]. The elevated serum levels of IgG, IgM, and IgE documented in this investigation are comparable to those attained by certain employees [31]. It has long been recognized that whereas serum IgG levels rise for months or years, serum IgM levels rise initially and then decline with time [30]. *E. histolytica* has been reported to cause a greater rise in serum IgG levels, which may be because it contributes to tissue invasion and intestinal epithelial cell death, as well as migration that results in extraintestinal illness outside the intestine. A substantial systemic and local immune response

is triggered by all of these *E. histolytica* activities [31].

Immunological parameters

The table (4) shows some immunological parameters significant ($P \leq 0.05$) differences between the study groups. The results found that the concentration of IL-6 (6.04 ± 1.42) and IL-17 (18.74 ± 1.03) show significant ($P \leq 0.05$) elevated between *E. histolytica* patients compared to the control group (2.63 ± 0.45 ; 7.12 ± 0.29 respectively).

Table (4): the concentrations of some immunological parameters in *E. histolytic* patients compared with control group

Parameter	<i>E. histolytic</i>		Control		P value
	Mean	\pm SD	Mean	\pm SD	
IL-6	6.04 a	1.42	2.63 b	0.45	0.001
IL-17	18.74 a	1.03	7.12 b	0.29	0.001

* Different letters indicate significant ($P \leq 0.05$) differences, while the same letters indicate non-significant ($P \leq 0.05$) differences.

Interleukin-6 serum levels showed a considerable rise in children with intestinal parasite infections; this finding could mean that both intestinal protozoan parasites colonize the digestive tract and infect their hosts by consuming cysts. They adhere to the intestinal epithelial surface of the colon in *E. histolytica* or the duodenum/ileum in patients with *G. lamblia*, and they trigger an immunological response that includes the generation of interleukin IL-6 by mast cells, T-cells, and dendritic cells [32]. Inflammation is indicated by an increase in certain cytokines, such as IL-6 [33]. This study's high IL-6 levels could be explained by the presence of inflammatory anemia in children infected with protozoa. This finding is consistent with another study that found that patients with rheumatoid arthritis who had anemia of chronic diseases (ACD) had significantly higher IL-6 concentrations than those in the iron deficiency anemia group [34]. The current study's findings regarding elevated IL-6 levels in children infected with *E. histolytic*/*E. dispar* were consistent with a study by Abd and Saleem [35], which found that the concentration of (IL-6) in both male and female *E. histolytica* infections was significantly higher ($P < 0.05$) than in the control group. Additionally, the study found a significant increase ($P < 0.05$) in the concentration of (IL-6) in both the total number of *E. histolytica* patients and the total control group. These findings concurred with those of another study [36], which discovered a correlation between elevated levels of IL-17 and a chronic *E. histolytica* infection. The current study contradicts prior research that indicates IL-17 had no impact during the *E. histolytica* infection [37]. The recruitment of neutrophils to sites, control of dendritic cell activity, and Th1 responses via cytokines and chemokines generated by IL-17 are all components of the mechanism of IL-17-mediated protection [38–39].

Conclusions

According to the current investigation's findings, females have a greater infection rate than males. However, this study found that children with *E. histolytica* had considerably higher serum levels of IL-6 and IL-17 as compared to healthy observation collection, with a significant difference.

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