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Estimate of Some Immune-Biomarkers in Children with Entamoeba Histolytica Infection

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Abstract: The current work was aimed to evaluate of some immune-biomarkers in children with Entamoeba histolytica infection. General stool examination was done to patients for detection of E. histolytica infection. A total 150 patients of less than 13 years, whom attended the parasitology section at period February to June 2023. The chosen patients were suffered from abdominal pain and diarrhea. The results showed that gender is not related to infection with E. histolytica, as there were no significant (P≤0.05) differences between the proportions of males and females. The IgG concentration of infected children (24.05±3.94 ng/L) indicated a significant (P≤0.05) rise compared to the healthy children (11.72±2.55 ng/L). IgA concentration of infected children (159.42±18.62 ng/L) indicated a significant (P≤0.05) rise compared to the healthy children (38.93±7.01 ng/L). IgM concentration of infected children (4.84±0.45 ng/L) indicated a significant (P≤0.05) rise compared to the healthy children (2.17±0.35 ng/L). The IL-4 concentration of infected children (29.47±4.04 pg/ml) indicated a significant (P≤0.05) rise compared to the healthy children (16.54±3.94 pg/ml). IL-6 concentration of infected children (183.25±21.37 pg/ml) indicated a significant (P≤0.05) rise compared to the healthy children (26.12±4.51 pg/ml). The TLR2 levels of infected children (24.05±3.94 ng/L) indicated a significant (P≤0.05) rise compared to the healthy children (11.72±2.55 ng/L). therefore, its concluded that E. histolytica lead to a high immune response that leads to an increase in levels of immune parameters in infected children.

Keywords: E. histolytica; Immunoglobulins; Interleukins; TLR2; Immunity.

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 E. histolytica in humans [1-3]. E. histolytica infection is the third parasite in the world that causes death, after schistosomiasis and malaria [4]. One intestinal main that causes amoebic dysentery in people is E. histolytica, which poses a threat to public health in tropical areas [5-7]. The World Health Organization (WHO) estimates that it causes between 100,000 and 40,000 fatalities per year [8–9]. Diarrheal diseases associated with E. histolytica have been reported to negatively affect

children's growth. The persistence of amebic infection-related morbidity and mortality despite the availability of excellent therapy suggests that efforts aimed at limiting or eliminating disease are futile [10]. With a two-stage life cycle, E. histolytica can be found in the environment as resistant infectious cysts and in the human colon as potentially harmful trophozoites. When the gut is infected with E. histolytica, the parasite invades it and causes inflammation and tissue damage. Parasites destroy and phagocytose immune cells, erythrocytes, and human epithelial cells during this invasive phase. The most typical route is known as commensal colonization, in which trophozoites live in the gut lumen and use phagocytosis to feed on enteric bacteria [11–12]. Particularly in amebiasis patients, lymphocyte proliferation and lymphokine production have been identified as hallmarks of the cell-mediated response. imply a function for the body's immune system cells. the generation of cytokines that cause inflammation, such as TNF-α, IL-1α, IL-6, IL-8, IL-12, and IFN-α [13–14].

Materials & Methods

Patients

Patients had a general stool examination to check for the presence of E. histolytica infection. 150 patients, all under the age of 13, visited the parasitology unit between February and June of 2023. The selected patients experienced diarrhea and stomach ache.

Samples collection

In a sterile, screw-disposable plastic container, fresh stool samples were collected. A portion of the specimen was processed straight for wet mount analysis. Patients whose E. histolytica test findings were microscopically positive had their serum samples taken.

immunoglobulin (IgG, IgA, and IgM)

A quantitative sandwich enzymelinked immunosorbent assay was used to assess the levels of serum immunoglobulin in accordance with the kit's instructions.

Interleukins (IL-4 and IL-6)

The procedures followed the guidelines provided by the business that supplied the ELISA kit (US. Biological USA).

TLR 2 detection by ELISA procedure

The ELISA kit (Cat. No. MBS26890/Mybiosource/Canada) was used to measure the human serum levels of Toll Like Receptor 2 (TLR-2) in accordance with the manufacturer's instructions.

Statistical analysis

The statistical package for social science (SPSS) program version 17 (SPSS INC., Chicago IL, USA) was used for all statistical analyses. The findings were displayed as mean ± SD. To analyze the findings and compare the two groups, ANOVA, t-test, the least significant difference (LSD0.05), and correlation coefficient (r) were employed. When $P < 0.05$, differences were deemed significant [15].

Results & Discussion

Samples distribution

150 patient samples were gathered for the current investigation (table 1). 94 (62.7%) of the total samples showed positive results for E. histolytica, according to the data. Of the total samples, 57 (or 37.3%) showed negative results for the E. histolytica investigation.

Table (1): Distributed of study samples according microscopically exam

Groups	No. (%) +ve	No. (%) -ve	Total No.(%)
E. histolytica	94(62.7%)	57(37.3%)	150(100.0%)

Socio-demographic characteristics

Gender of children

The gender of children, were non-statistically significant at (P≤0.05) between male and female. The percentage of male patients at (54.3%). Whereas, the percentage of female patients at (45.7%) (Table 2).

Table 2: Gender of children

Gender	**No**	**Percentage (%)**
Boys (Male)	51	54.3
Girls (Female)	43	45.7
Total	94	100%
P-value	---	0.192 NS
NS: Non-Significant.		

The results of the present investigation demonstrated that there is no correlation between gender and E. histolytica infection, since the proportions of males and females did not differ statistically. This conclusion was consistent with the findings of Shrestha [17] and Tasawar et al. [18], who demonstrated that intestinal parasite infection was not gender-specific. The reported disparities in parasite infection between males and females may be explained by a number of variables, including exposure rates, social behavior, environment, and food, even though clinical studies of people and field studies of non-human animals are suggestive [19].

Age of children

The age group (10-13 years) recorded the highest percentage (40.4%), followed by the group of 5-9 years), was (33.0%). While the lowest percentage showed in age group (≤1-4 years), which is (26.6%) and there is non- significant (P≤0.05) changes between age groups (Table 3).

Table 3: age of children

Age (year)	**No**	**Percentage (%)**
≤1-4	25	26.6
5-9	31	33.0
10-13	38	40.4
Total	94	100%
P-value	---	0.174 NS
NS: Non-Significant.		

The present investigation demonstrated that there is no correlation between age and E. histolytica infection, as no discernible differences were seen between the patient age groups. While Sa'el, [22], Al-Saeed & Issa, [23], and Ouattara et al., [24] demonstrated that the infection was significantly correlated with age, these results disagreed with Kia et al., [20] and Mohammed et al., [21] who demonstrated that there were no significant differences between age groups.

immunoglobulin (IgG, IgA, and IgM)

The IgG concentration of infected children (24.05±3.94 ng/L) indicated a significant (P≤0.05) rise compared to the healthy children (11.72±2.55 ng/L). IgA concentration of infected children (159.42±18.62 ng/L) indicated a significant (P≤0.05) rise compared to the healthy children (38.93±7.01 ng/L). IgM concentration of infected children (4.84±0.45 ng/L) indicated a significant (P≤0.05) rise compared to the healthy children (2.17±0.35 ng/L), as shown in the table (4).

Table (4): immunoglobulin concentrations in studied groups

Parameter	Healthy children (25)	Infected children (94)	P-Value
IgG ng/L	11.72±2.55	24.05±3.94*	0.0005
IgA ng/L	38.93±7.01	159.42±18.62*	0.0003
IgM ng/L	2.17±0.35	4.84±0.45*	0.0001

Diarrheic subjects in this study have elevated IgG specific to E. histolytica. Thus, it should be regarded that the serum IgG found in this investigation would represent immune memory rather than illness, and that the agent's intestinal interaction could trigger boosting [25]. Nonetheless, diarrheal sera were shown to have elevated IgM reactivity. This could be explained by the pathogen having recently infected someone. Because of their agglutination, trapping within immune complexes, and clearance inside the mucous blanket, IgA antibodies block enteric pathogens from coming into touch with the intestinal epithelial surface [26]. The main mucosal secretions that contain IgA are tears, saliva, colostrum, and secretions from the prostate, gastrointestinal system, genitourinary tract, and respiratory epithelium. Blood contains very little IgA, as shown in samples that do not have diarrhea. On the other hand, diarrheal serum IgA reactivity level was shown to be higher than IgG in the flow cytometry analysis. Consistent with the current findings, Shetty et al. [27] found notably elevated IgG and IgA levels in the sera of individuals suffering from invasive amebiasis. Elevated serum IgA levels could indicate an invasive infection.

Interleukins (IL-4 and IL-6)

The IL-4 concentration of infected children (29.47±4.04 pg/ml) indicated a significant (P≤0.05) rise compared to the healthy children (16.54±3.94 pg/ml). IL-6 concentration of infected children (183.25±21.37 pg/ml) indicated a significant (P≤0.05) rise compared to the healthy children (26.12±4.51 pg/ml), as shown in the table (5).

Table (5): Interleukins concentrations in studied groups

Parameter	Healthy children (25)	Infected children (94)	P-Value
IL-4 pg/ml	16.54±3.94	29.47±4.04*	0.001
IL-6 pg/ml	26.12±4.51	183.25±21.37*	0.0003

When one Th2 cytokine, specifically IL-4, was measured in the blood of intestinal amoebiasis patients, the findings showed that majority of the patients had higher levels of IL-4 in their sera than the control group, a finding that was also supported by [28–29]. IL-4 is one of the cytokines that Th2 cells produce, and it functions as a cofactor in the activation of humoral immunity by stimulating the proliferation and differentiation of B and T cells [30]. Children infected with intestinal E. histolytica showed significantly higher serum levels of interleukin-6; this finding may suggest that the parasites colonize the digestive tract after infecting their hosts through cyst ingestion. In individuals suffering from E. histolytica, they adhere to the intestinal epithelial surface of the duodenum or ileum and trigger the synthesis of interleukin IL-6 by T-cells, dendritic cells, and mast cells as part of an immunological response [31]. There is evidence of inflammation when some cytokines, such as IL-6, are elevated [32]. IL-6 is a pleiotropic pro-inflammatory cytokine that stimulates the transcription of the hepcidin gene [34], hence inducing the synthesis of acute phase proteins, including hepcidin, in hepatocytes [33].

TLR2 levels

The TLR2 levels of infected children (24.05±3.94 ng/L) indicated a significant (P≤0.05) rise compared to the healthy children (11.72±2.55 ng/L), as shown in the table (6).

Table (6): TLR2 levels in studied groups

Parameter	Healthy children (25)	Infected children (94)	P-Value
TLR2 ng/L	11.72±2.55	24.05±3.94*	0.0005

A growing body of evidence suggests that distinct TLR agonists might influence the immune system's reaction to other TLR ligands, leading to modified inflammatory reactions [35–36]. The immune system is exposed to a variety of stimuli during infection, and their interactions may intensify or lessen disease. In this investigation, we assessed the TLR2 expression levels in kids infected with E. histolytica.

Conclusions

E. histolytica plays a role in causing various infections in the digestive system, which in turn causes a high immune response that leads to an increase in levels of immune parameters in infected children.

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