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Serum Interleukin-6, Interleukin-8, and Interleukin-10 Levels in Intestinal Amebiasis: Association with Clinical Symptom Severity and Intestinal Inflammation

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ABSTRACT

Objective: *This study aimed to investigate the epidemiological and immunological aspects of Entamoeba histolytica and to evaluate the relationship between the severity of intestinal clinical symptoms and serum levels of interleukin-6, interleukin-8, and interleukin-10 in infected patients.* ***Method:*** *This study was conducted from October 2023 to January 2025, collecting 180 samples, 90 of which positive for amoebiasis and 90 control. Diagnosis was performed using microscopy and Coproantigen Antigen testing. Cytokines were measured using Sandwich ELISA, and statistical analysis was performed using t-test, ANOVA, and Pearson correlation coefficient.* ***Results:*** *This study illustrates that males had a higher infection rate compared to females 55.6% and 44.4% respectively, and that individuals under 15 years of age were the most susceptible to amoebiasis, with an infection rate of 38.9%. The study showed a significant increase in the levels of all cytokines (interleukin-6, interleukin-8, and interleukin-10) in patients compared to the control group. Interleukin-8 also exhibited a high level in all clinical symptoms, particularly in cases of bloody diarrhea, with a level of 145.30 ± 18.40 pg/ml compared to other clinical symptoms ($r = 0.684$, $P < 0.001$). The results indicated a strong positive correlation between interleukin-8 and clinical symptoms given that interleukin-8 is a key driver of the inflammatory process in amebic dysentery. This explains the marked increase in bloody diarrhea, accompanied by an elevated IL-10 level to limit mucosal damage.* ***Novelty:*** *These cytokines serve as biomarkers for monitoring disease severity and for the balance of immune and inflammatory processes.*

INTRODUCTION

Entamoeba histolytica parasite is one of the most important pathogenic intestinal parasites, posing a significant global health challenge, particularly in developing regions [1]. Amoebiasis is an intestinal infection, manifesting with symptoms including watery diarrhea or amoebiasis. It can also progress to extraintestinal infections when the parasite enters the bloodstream and reaches other vital organs, most notably the liver, causing what is known as an amoebic liver abscess [2]. The pathogenic mechanism of this parasite involves not only direct cell destruction but also disruption and modification of the host's immune and neurological pathways [3]. The invasion of tissues by the parasite causes an immune response, leading to the release of a group of cytokines, including IL-6, IL-8, and IL-10. These cytokines have been shown to have a dual and important role [4]. These substances attract immune cells to the site of injury, activating the acute inflammatory response and, on the other hand, balancing this inflammation to limit widespread tissue damage [5]. This underscores the importance of these cytokinetics in the immune response of the host. Therefore, the significance of this study lies in the relationship between the severity of clinical symptoms of amoebiasis and the levels of these three

cytokinetics. These cytokinetics serve as a biomarker of clinical severity, Although conventional tests, such as microscopy and stool antigen testing, confirm *E. histolytica* infection, they do not reflect the extent of tissue damage or assess the severity of the patient's intestinal inflammatory response. Examination of serum cytokines (IL-6, IL-8, and IL-10) provides significant added clinical value as it serves as a host "immune fingerprint" directly correlated with disease severity. Identifying these immune markers can help predict disease progression and severity and optimize therapeutic interventions based on the host's response.

MATERIALS AND METHODS

Study Design and Ethical Considerations

The current study was conducted from October 2023 to January 2025 on patients across multiple clinical centers in Najafprovice. The study involved examining 180 suspected samples, of which 90 tested positive for *E. histolytica*. 90 other samples were collected from healthy individuals who did not carry this parasite and did not suffer from any symptoms, and these were classified as the control group. Verbal consent was obtained from all participants in this study, and from the parents of the children, after explaining its nature and objectives. The confidentiality of the patients' personal data was strictly maintained, and the study was limited to collecting stool and blood samples for scientific research purposes only. **Patients with autoimmune diseases, other intestinal diseases, or dual parasitic/bacterial infections were excluded to ensure the accuracy of the results for this study.**

Clinical Assessment and Data Collection

A structured questionnaire was administered to each patient to document demographic data and some key clinical symptoms associated with *E. histolytica* infection (patient code, sex, and age), as well as the main clinical symptoms associated with the infection, including the presence and type of diarrhea (watery or bloody), abdominal pain, fever, and laboratory findings.

Laboratory Diagnostic Methods for the Parasite

Diagnosis of *E. histolytica* infection was performed on stool samples in two stages*:*

1-Microscopic Examination

Conventional microscopic examination: A direct stool swab was used, coated with 0.9% saline and Lugol's iodine to determine trophozoite and cysts of the parasite. It was then examined under a light microscope at 10x and 40x magnification lenses [6].

2-Coproantigen Detection

Test was an immunoassay for specific detection of the parasite's antigens in stool. kits from **Biopanda Reagents Ltd, UK**, were used, and the test was performed according to the manufacturer's protocol to ensure accurate diagnosis, the diagnostic sensitivity reached 97.6% and the specificity 98.8%, ensuring a high level of diagnosis for the parasite.

Immunological Assay

For cytokine quantification and After confirming the test was positive, 5 ml of venous blood was withdrawn from patient, blood was allowed to clot at room temperature, and the serum was obtained using a centrifuge at 3000 rpm for 10 minutes. Which was stored at -20°C until the immunological tests were performed. Cytokinetic levels IL-6, IL-8 and IL-10 were assessed according to the protocol provided with using commercial Sandwich ELISA kits **(Servicebio, China)** in accordance with the manufacturer's protocols,Optical density was measured using an ELISA microplate reader at a wavelength of 450 nm.

Statistical Analysis

The statistical analysis of the data from this study was performed using SPSS version 26. The t-test was used to compare the patient and control groups, and one-way ANOVA was used to evaluate statistical variations across different clinical symptom categories. Differences were considered statistically significant at $P < 0.05$ and highly significant at $P < 0.01$.

RESULTS AND DISCUSSION

Results

The results of the current study, indicate that 90(50%) out of 180 participants positive for amoebiasis (Table 1).

Table 1. Distribution of *E. histolytica* parasite in the study samples in AL-Najaf province (n=90).

Study Groups	Frequency (No.)	Percentage (%)
Patients	90	50%
Control	90	50%
Total	180	**100%**

and illustratesthat males were more likely to contract amoebiasis (55.6%) compared to females (44.4%) (Table 2).

Table 2. Distribution of E. histolytica parasite prevalence by gender and age of infected patients in AL- Najaf province (n = 90).

Variables	Category	Frequency (No.)	Percentage (%)
gender	Male	50	55.6%
	Female	40	44.4%
Total		**90**	**100%**
Age Groups (Years)	< 15 years	35	38.9%
	15 – 30 years	30	33.3%
	31 – 45 years	25	27.8%
Total		**90**	**100%**

Immunological analysis, as illustrates in (Table 3, 4, 5) revealed a significant increase in serum cytokines in amoebiasis patients compared to control group , IL-6 levels was significantly elevated in the patients compared to control group (48.52 ± 12.30 (pg/ml) and 5.12 ± 1.45(pg/ml), respectively) (Table 3).

Table 3. Comparison of serum IL-6 levels between amoebiasis patients and the control group in Najaf province (n = 90).

Group	No.	Mean ± SD (pg/ml)	P-value
Patients	90	48.52 ± 12.30	0.001 (HS)**
Control	90	5.12 ± 1.45	
Total	180		

HS: Highly Significant (P < 0.01).

** : Highly Significant difference at (P < 0.01).

Similarly, level of IL-8 was significant increase in the patient group (115.70 ± 25.40 (pg/ml)), compared to control group, (12.35 ± 3.10 (pg/ml), with a probability value of P = 0.000 (Table 4).

Table 4. Comparison of serum IL-8 levels between amoebiasis patients and the control group in Najaf province (n = 90).

Group	No.	Mean ± SD (pg/ml)	P-value
Patients	90	115.70 ± 25.40	0.000 (HS)***
Control	90	12.35 ± 3.10	
Total	180		

HS: Highly Significant P = 0.000

*** : Highly Significant difference at (P < 0.001).

Table 5 shows a significant increase in IL-10 levels in the patient group, with a mean of 22.15 ± 5.80 (pg/ml) compared to the control group, which had a mean of 8.40 ± 2.15 (pg/ml), p-value < 0.05.

Table 5. Comparison of serum IL-10 levels between amoebiasis patients and the control group in Najaf province (n = 90).

Group	No.	Mean ± SD (pg/ml)	P-value
Patients	90	22.15 ± 5.80	0.005 (S)*
Control	90	8.40 ± 2.15	
Total	180		

S: Significant (P < 0.05).

* : Significant difference at (P < 0.05).

Table 6 of the results illustrates a clear variability in cytokine levels among patients with amebiasis. Based on the type and severity of clinical symptoms, the results indicate that the highest clinical elevations were concentrated in IL-8 levels across all symptoms. The highest level was recorded in cases of bloody diarrhea (145.30 ± 18.40 pg/mL), while in watery diarrhea it was 92.10 ± 12.25 pg/mL. Abdominal pain levels reached 120.50 ± 15.60 pg/mL, and fever levels reached 105.20 ± 14.30 pg/mL, reflecting a state of acute enteritis.

Furthermore, Pearson correlation analysis confirmed a statistically significant positive correlation between IL-8 levels and the severity of clinical symptoms ($r = 0.684$, $P < 0.001$). Serum interleukin-6 and interleukin-10 also showed positive correlations with clinical symptoms ($r = 0.412$ and $r = 0.305$ respectively) (Table 6).

Table 6. The relationship between the levels ofcertain cytokinetic markers IL-6, IL-8, and IL -10 and the clinical symptoms in patients with amoebiasis (n=90).

Clinical Symptoms	No. of Patients	IL-6 (Mean ± SD) pg/ml	IL-8 (Mean ± SD) pg/ml	IL-10 (Mean ± SD) pg/ml
Bloody Diarrhea	40	58.20 ± 10.15 (a)	145.30 ± 18.40 (a)	25.10 ± 4.20 (a)
Watery Diarrhea	50	40.15 ± 8.60 (b)	92.10 ± 12.25 (b)	19.80 ± 3.15 (b)
Abdominal Pain	75	52.40 ± 11.30 (a,b)	120.50 ± 15.60 (c)	23.40 ± 5.10 (a,b)
Fever	35	65.80 ± 9.45 (c)	105.20 ± 14.30 (b,c)	21.15 ± 3.90 (a,b)
F-value (ANOVA)	—	14.32	35.81	8.45
P-value	—	< 0.001 (HS)	< 0.001 (HS)	0.002 (S)
Pearson Correlation (r)	—	r = 0.412(p = 0.003)	r = 0.684(p < 0.001)	r = 0.305(p = 0.015)

Discussion

The results of the current study, as illustrates in Table 1, indicate a widespread prevalence of *E. histolytica* infection among the participants in the region [7]. The study also noted an increase in parasitic and microbial infections in the Middle Euphrates region due to pollution [8]. This confirms that childhood remains a significant health challenge in the region [9]. This marked increase in positive cases is attributed to continuous exposure to the parasite and the monitoring of parasitic loads in clinical settings, highlighting the need to study cell kinetics to understand how the immune system responds to this spread. The demographic distribution, as illustrates in Table 2, indicates that males are more susceptible to intestinal amebiasis due to environmental and occupational factors compared to females, consistent with [10]. The study also reveals a significant increase in incidence among children under 10 years of age. This increased susceptibility in early childhood is mainly attributed to the incomplete development of the immune system in young children, which aligns with another study conducted in Iraq [11]. Therefore, we confirm through these data that infection with this parasite in the region is usually closely related to behavioral habits and physiological

differences in the host's susceptibility. The study, as shown in Table 3, reveals elevated IL-6 levels in infected patients. This increase is attributed to the body's strong inflammatory response to the invasion of intestinal tissue by *E.histolytica.* This finding is consistent with similar studies that confirmed elevated IL-6 levels in patients with acute gastroenteritis [12]. It also aligns with another study that recorded a significant increase in IL-6 levels in children with acute diarrhea [13]. Furthermore, this parasite has been shown to regenerate intestinal and monocyte cells, leading to the production of large quantities of IL-6 to activate the immune defenses of infected individuals. This explains the elevated cytokine levels.. The study also indicated elevated IL-8 levels in infected patients, as illustrates in Table 4. This finding reflects the vital role of this cytokine as a chemotactic attractant for immune cells to the site of intestinal infection [14]. This elevation may be due to tissue damage caused by amoebic dysentery, particularly in cases of severe amoebic diarrhea [12]. It was also confirmed that colon cells secrete large amounts of IL-8 upon attachment of any parasite as a reaction, leading to neutrophil polarization and exacerbation of acute inflammation [15]. Elevated levels are directly related to the body's defense mechanism against the parasite and are considered a crucial indicator in understanding the pathogenesis of the infection. The study recorded a significant increase in IL-10, as shown in Table 5, which may be attributed to a regulatory immune response in the patient. Other studies have investigated the role of IL-10 in infectious and parasitic intestinal infections. They indicated that the body secretes IL-10 as a defense mechanism to reduce the damage caused by inflammation resulting from the *E histolytica* parasite, thus protecting intestinal tissue from severe damage [16]. It was also confirmed that this increase in patients is related to the immune system's attempt to control chronic infection and limit the acute inflammatory response [17]. Interleukin-10 is considered an immune regulator, and its levels rise in conjunction with inflammatory cytokines to prevent complete damage to intestinal tissue [18]. Although this was observed in a large number of cases, it reflects an inflammatory state in which the host body attempts to expel the parasite. It has been suggested that there is a link between the type and severity of clinical symptoms and the levels of immune cytokines in patients with gastrointestinal tract infections [19]. The severity of intestinal symptoms has been associated with elevated IL-6 levels during colon regeneration due to parasite proteins [20]. IL-6 is a regulator of systemic responses, such as fever, in cases of invasive parasitic infections. Furthermore, the severity of clinical symptoms in *E. histolytica* infection reflects the level of immune expression of inflammatory cytokines [21]. The significant increase in IL-8 may be attributed to the fact that, with increased cellular activity, there is a greater influx of cells that secrete enzymes causing erosion of the intestinal lining in the host. Therefore, the predominance of IL-8 in patients with amebiasis is due to its primary role as a chemoattractant.

CONCLUSION

Fundamental Finding : The results illustrates a significant increase in the levels of the cytokines IL-6, IL-8, and IL-10 in individuals with amebiasis compared to the control

group. A strong correlation was also found between the severity of clinical symptoms and the level of inflammatory response, with bloody diarrhea showing the highest increase in IL-8 levels, followed by abdominal pain and then fever, compared to the other cytokines. The study demonstrated a strong positive correlation between the severity of clinical symptoms and levels of inflammatory cytokines, particularly IL-8 (r=0.684, p. **Implication :** These are considered clinical biomarkers. The findings suggest that inflammatory cytokines, particularly IL-8, may serve as potential clinical biomarkers for assessing the severity of intestinal symptoms in individuals with amebiasis. **Limitation :** The study is limited to the assessment of serum IL-6, IL-8, and IL-10 levels and their association with clinical symptom severity in individuals with amebiasis. It does not establish the diagnostic accuracy or prognostic value of these cytokines as biomarkers. **Future Research :** Future research should validate the diagnostic and prognostic value of IL-6, IL-8, and IL-10 in larger patient populations. Further studies should also investigate changes in cytokine levels during treatment and recovery to determine their potential for monitoring disease progression and predicting clinical outcomes.

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