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The Interaction between The Immune Response and Interleukins as a Characterizing Landscape of The Host Response

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ABSTRACT

Aim of research: This research focus on defining the immune environment of amoebiasis through whereas IL-8 and IL-25 cytokines affect host defenses toward *Entamoeba histolytica* infection .

Materials and Methods: The study involved 44 confirmed patients together with 44 healthy controls as subjects during a 5-month research period in multiple healthcare facilities between October 2024 and February 2025. Structured interviews alongside medical record examinations produced sociodemographic data which enabled ELISA-based blood analysis of cytokines .

Results: The study results indicated that IL-8 concentrations were notably increased among patients at 223.34 pg/mL while controls maintained levels at 82.51 pg/mL according to statistical analysis (p=0.001). The research data demonstrated increased IL-25 PG/mL levels in patients at 1107.97 pg/mL which was higher than the levels observed in controls at 496.70 pg/mL thus demonstrating shared importance of these cytokines in the immune response to infection .

Conclusion: The examined patient population demonstrates acute inflammation through elevated IL-8 measurements and demonstrates adaptive immunity through elevated IL-25 levels while infected with *E. histolytica*. The discovered elevated levels of cytokines confirm that they function as vital biomarkers to determine disease severity and warrant additional investigation to enhance knowledge about the paths amoebiasis uses to interact with human hosts.

Keywords: *Amebiasis, Interleukin-8, Interleukin-25, Immune response*

1. INTRODUCTION

Entamoeba histolytica causes the disease known as amoebiasis which affects numerous developing nations worldwide as a major healthcare problem. The pathogenic organism causes severe gastrointestinal sickness which leads to high rates of sickness and death among patients. The aggressive nature of *E. histolytica* develops through its immune system evasion abilities together with its persistent inflammatory response which leads to problematic immune activity and tissue destruction. The precise understanding of immune system-Parasite relationship enables scientists to develop better therapeutic interventions (Guillén, 2023.(

IL-8 and IL-25 establish their influence as important immunological agents within *E. histolytica* infections. IL-8 serves as an essential chemokine that enables the recruitment of neutrophils to infected sites during amoebic infections thus causing tissue-damaging acute inflammation (Khalaf & Hafez, 2021). Medical research shows that IL-8 levels rise during infection because it drives inflammatory responses that may result in tissue destruction (AKh & FAb, 2024). The immune system depends on IL-25 or IL-17E to develop Th2 cell responses and increase mucosal protection against parasites. Research shows that IL-25 measurements in patients with amoebiasis rise significantly which indicates that this cytokine operates in adaptive immune responses of the host (Khalaf & Hafez, 2021.(

The scientific community acknowledges the significance of these cytokines but scientists still need to comprehend their precise infection roles and interactions mechanisms when dealing with *E. histolytica*. Academic research faces two main obstacles because immune responses differ between populations and scientists require thorough investigation of cytokine profiles during both acute and chronic amoebiasis stages. Research reveals the need to establish connections between sociodemographic characteristics and immunological responses because this approach would generate a thorough understanding of immune conditions during amebiasis.

The presence of epithelial tissue damage caused by amoebic dysentery reflects an acute inflammatory response, indicating the interaction between the parasite and the host's immune system in the gastrointestinal tract (Oleiwia & Hassan, 2024).

1.1 Aim of research

The research aims to define amoebiasis immune patterns through cytokine IL-8 and IL-25 evaluations of *Entamoeba histolytica* host immune reaction. Specific objectives include:

The analysis determines and examines the quantities of IL-8 and IL-25 for patients with *Entamoeba histolytica* infections in contrast to samples from normal subjects.

Investigate how IL-8 and IL-25 function during immune inflammatory and adaptive responses that occur during *E. histolytica* infections.

This evaluation examines how rising cytokines connect with clinical results which demonstrate disease seriousness as well as possible medical complications occurring within infected people.

2. METHODOLOGY

2.1. Study Design

This study investigates IL-8 and IL-25 levels in patients who have received diagnosis of *Entamoeba histolytica* protozoan infection. Research involved confirming *E. histolytica* infection through stool examination or serological testing to enroll 44 patients as subjects while using the same number of healthy people who served as controls. The research conducted from October 2024 up until February 2025 utilized healthcare facilities Al-Sader Medical City and Al-Hakeem General Hospital and Al-Najaf Al-Ashraf Hospital and Al-Furat Al-Awsat Hospital and the Gastroenterology and Liver Hospital. All groups of patients from both male and female genders participated in the study.

2.2. Study Population

2.2.1. Inclusion Criteria

Adults aged 18 and above having *E. histolytica* infection fell under the patient group regardless of their infection severity ranging from being asymptomatic to experiencing mild or severe symptoms. A control group composed of typical populace participants who lacked gastrointestinal health issues or recent infections was formed.

2.2.2. Exclusion Criteria

Participants excluded from the research had either autoimmune diseases or chronic inflammatory conditions plus ongoing immunosuppressive treatment.

2.2.3. Demographic Data

The researchers obtained demographic data by combining structured interviews with medical record reviews among participants for age, sex information, occupational background and residential details. The research team evaluated this information for identifying probable risk variables correlated with *E. histolytica* infections.

2.3. Cytokine Measurement

2.3.1. Sample Collection

The investigators collected blood through venous draws from the antecubital vein of their study cohort. The samples entered into clotting then proceeded to centrifugation at 4000 RPM for 10 minutes to extract serum. The researchers stored the serum at -80°C before proceeding with the experiments.

2.3.2. Cytokine Assays

For measuring IL-8 and IL-25 cytokines in serum we used enzyme-linked immunosorbent assay (ELISA) BT LAB/ China kits which required following manufacturer guidelines. The detection threshold of both examinations reached levels below 5 pg/mL. The experiments consisted of two duplicate readings which were averaged to establish experimental accuracy.

2.4. Statistical Analysis

Multiple statistical tests will be utilized to analyze the immune conditions in amoebiasis within the presented study. Sociodemographic data will be summarized by descriptive statistics while the chi-square test will analyze sex distribution patterns between patient and control groups. The comparison of IL-8 and IL-25 mean cytokine levels between groups will be done with the independent samples t-test and Levene's test will determine variance homogeneity before utilizing Welch's t-test when the assumption fails. The study will analyze correlations between cytokine concentrations and clinical results

while using confidence intervals to determine mean difference changes.

2.5. Ethical Considerations

Three approval institutions supported the research including the Medical Laboratory Services Division at the College of Health and Medical Technologies together with the Najaf Health Department and the Training and Development Center. All participants granted their written consent for the study while following ethical guidelines for research.

3. RESULTS AND DISCUSSION

3.1. Demographic Data of study groups

A comparison of demographic traits occurs in Table (1) between 44 *Entamoeba histolytica* parasite-infected patients and 44 control patients.

Table (1): Demographic Data of patients and control groups

Variables	Patients(44)		Control(44)		P.value
	Freq.	%	Freq.	%	
Age (Years)					
10-19	9	20.5	8	18.2	*0.535
20-29	6	13.6	7	15.9	
30-39	13	29.5	8	18.2	
40-49	6	13.6	10	22.7	
50-59	4	9.1	3	6.8	
60-69	6	13.6	7	15.9	
≤ 70	0	0.0	1	2.3	
Sex					
Male	33	75.0	27	61.4	**0.172
Female	11	25.0	17	38.6	

The patient group had significant representation rates in both the 10-19 (20.5%) and 30-39 (29.5%) age ranges but the control group displayed balanced age distribution according to the p-value of 0.535. A higher percentage of men (75.0%) infected with tuberculosis existed in the patient group than the control group (61.4%); this data failed to reach statistical significance (p=0.172).

3.2. Comparison of IL-8 concentration levels in the study groups

The research data shows that infected patients demonstrate IL-8 concentrations at 223.34 pg/mL while the control participants show levels at 82.51 pg/mL. Standard deviation among infected patients at 158.70 indicates the diverse IL-8 concentration levels throughout their group. The submitted data demonstrated a statistically substantial difference between patient and control groups (t=4.952, p=0.001) which produced a mean difference of 140.83 pg/mL with a 95% confidence interval extending from 84.30 to 197.36 pg/mL .

The patient group demonstrated higher IL-8 results (mean = 1.194) than the control group (mean = 0.581) according to testing. A significant difference emerged statistically (p=0.001, t=6.806) as indicated by the results with a mean distinction of 0.613 between groups along with a confidence interval spanning from 0.434 to 0.792 .

The test results from Levene's Test confirm that variances between these groups are equal for IL-8 test results hence the t-test remains appropriate for mean comparison.

Table(2): Descriptive statistics about IL8 Quantitative measurements alongside IL8 results derived from evaluating study group differences.

	Groups	N	Mean	Std. Deviation	Std. Error Mean
IL-8 Quantitative	Patients	44	223.336834	158.6962172	23.9243549
	Control	44	82.509886	101.9473011	15.3691339
IL-8Test Results	Patients	44	1.194284	.4465342	.0673176
	Control	44	.580859	.3974779	.0599220

Immunological parameters		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig.	Mean Difference	Std. Error Difference	95% Confidence Interval	
									Lower	Upper
IL-8 Quantitative	Equal variances assumed	3.229	0.076	4.952	86	0.001	140.826	28.435	84.298	197.355
IL-8 Test Results	Equal variances assumed	0.698	0.406	6.806	86	0.001	0.613	0.090	0.434	0.792

Levels of IL-8 show marked elevation within infected patients than controls at a statistically decisive level. Two previous research findings by Haque et al. (2023) and Serrano-Luna et al. (2023) support IL-8's function in attracting neutrophils to infection sites during amoebic infections . IL-8 operates as a vital component which *E. histolytica* uses to evade the immune response according to Stanley (2023). Levene's test confirms equal quantitative variable variances across groups so results remain valid while qualitative variable variations indicate response complexity .

Studies by Al-Shaibani (2020) together with Shlash (2016) proved that infected patients had elevated cytokine levels thus demonstrating consistent results. Cytokine results from Al-Quraishi (2024) match those from this study about the role of cytokines across medical scenarios showing enlarged IL-8 levels align with Gonzalez et al. (2023) who demonstrated *E. histolytica* infection depends on these cytokines .

3.3. Comparison of IL-25 concentration levels in the study groups

Data in Table (3) shows patients possess mean IL-25 levels at 1107.97 pg/mL above the control group where levels stand at 496.70 pg/mL. Higher IL-25 standard deviation values among patients amounting to 703.76 pg/mL indicate that immune responses to the infection vary to some extent between subjects .

A t-test analysis becomes appropriate to evaluate mean differences because the Levene's Test finds no significant disparity in sample variances (F=2.782, p=0.099). Data from the t-tests demonstrate a significant difference regarding IL-25 quantitative measurements with t=4.807 and p=0.001 to validate the mean difference of 611.27 pg/mL as statistically relevant. The experimental findings demonstrate IL-25 functions as a major element of the immune response when the body encounters *E. histolytica* infection.

The IL-25 test results between patients and controls demonstrated a major increase in values where patients displayed a mean of 1.264 while controls presented 0.740 with a t-value of 6.176 and p-value of 0.001 to indicate this significant mean difference of 0.524. These study results are shown to be dependable by the confidence intervals calculated for the IL-25 quantitative and test results combined.

Table (3): Present data about IL25 Quantitative and IL25 results when comparing study groups.

	Groups	N	Mean	Std. Deviation	Std. Error Mean
IL-25 Quantitative	Patients	44	1107.973177	703.7640068	106.0964160
	Control	44	496.701300	465.1437513	70.1230589
IL-25 Test Results	Patients	44	1.264236	.4186874	.0631195
	Control	44	.739980	.3765528	.0567675

Immunological parameters		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig.	Mean Difference	Std. Error Difference	95% Confidence Interval	
									Lower	Upper
IL-25 Quantitative	Equal variances assumed	2.782	0.099	4.807	86	0.001	611.271	127.175	358.454	864.089
IL-25 Test Results	Equal variances assumed	0.885	0.350	6.176	86	0.001	0.524	0.084	0.355	0.693

IL-25 shows increased levels in patients who have *Entamoeba histolytica* infection according to both quantitative analysis and the results of the laboratory tests as measured against control group data. Research indicates that IL-25 locates itself as a key player in directing immunological reactions toward behavioral responses toward pathogenic parasites. Al-Zayyadi et al. (2023) performed assessments of comparable immune variables among subjects from Al-Najaf to underscore the importance of assessing IL-25 cytokines in *E. histolytica* infection responses. The study provides evidence supporting a relationship where higher IL-25 levels create a link between stronger immune response and enhanced inflammatory activity as an active protective mechanism of the immune system against the infection.

The research conducted by Al-Shaibani (2020) on blood sample analysis of infected patients shows results that match the elevated IL-25 data observed in this review. The findings indicate *E. histolytica* infection creates variations in blood measurements that affect systemic immune responses. The systemic immune response includes multiple cytokines such as IL-25 that causes T helper type 2 (Th2) responses thus revealing an adaptive *E. histolytica*-targeting mechanism.

The work by Al-Quraishi (2024) about TNF-α effects adds essential insights to understand how different cytokines interact during *E. histolytica* infections. Immune regulation together with inflammation depend on TNF-α function while TNF-α and IL-25 interactions generate vital information about immune response protection versus pathogenesis. The elevation of IL-25 in patients may facilitate secondary complications because it indicates a crucial trio of immune markers (TNF-α, IL-25 and others) for measuring disease severity, based on the research of Zayyadi et al. (2023) and Al-Quraishi (2024.(

The research by Das et al. (2023) reviews the intricate pattern of immune system modifications that occurs during amoebiasis. The exploration of IL-10 in relation to other cytokines presented by Das et al. (2023) improves understanding of why elevated IL-25 appears in this research analysis .

IL-25 functions as a pro-inflammatory agent alongside IL-10 which functions as an anti-inflammatory cytokine to mold the immune environment that affects both pathogen elimination and maintenance of host tissue health (Das et al. 2023). Hameed and Khalaf (2022) contribute essential evidence regarding *E. histolytica* serology testing among different patient demographics for the detection and management of amoebiasis infections.

3.4. Comparison of Immunological Parameters Between Patients and Control Group

The data in Table (4) demonstrates that infected individuals feature elevated IL-8 concentrations amounting to 223.33 ± 158.69 vs. 82.50 ± 101.94; p = 0.001 for quantitative and 1.19 ± 0.44 vs. 0.85 ± 0.39; p = 0.001 for result measures thus correlating to inflammation levels.

Quantitative (1107.97 ± 703.76 vs. 496.70 ± 465.14; p = 0.001) and result measures (1.26 ± 0.41 vs. 0.73 ± 0.37; p = 0.001) evaluated the integral function of IL-25 in combating *E. histolytica* .

Table (4): Comparison of descriptive statistics mean ± standard deviation for interleukins between study groups

Immunological Parameters	Patients(44)	Control(44)	P.value
	Mean ± Std	Mean ± Std	
IL8 Quantitative	223.33 ± 158.69	82.50 ± 101.94	0.001
IL8Result	1.19 ± 0.44	0.85 ± 0.39	0.001
IL25 Quantitative	1107.97 ± 703.76	496.70 ± 465.14	0.001
IL25 Result	1.26 ± 0.41	0.73 ± 0.37	0.001

Laboratory tests measuring interleukin concentrations show meaningful changes in immune responses between patients who have *Entamoeba histolytica* infection and people without the parasite. The infected group exhibited elevated IL-8 levels that contrasted with control levels indicating typical acute inflammation from parasitic infections according to Haque et al. (2023) regarding IL-8 functions in amoebic neutrophil recruitment along with inflammation. Illustrious IL-8 elevations indicate prolonged inflammatory reactions that lead to *E. histolytica*-associated tissue damage and resulting symptoms which affect disease conditions and their severity.

The research showed that IL-25 levels increased substantially in the patient group to 1107.97 ± 703.76. The small attention given to IL-25 makes its elevated levels in patients stand out as a significant discovery. The research by Martínez-Hernández et al. (2023) shows IL-25 supports the development of mucosal immunity. An adaptive immune response attempting to enhance defenses against the parasite could be indicated by these elevated measurements because it demonstrates IL-25's role in the complex relationship between *E. histolytica* and the immune response.

CONCLUSIONS

Findings from studies of amoebiasis immune response reveal that patients infected with *Entamoeba histolytica* display higher levels of immune response cytokines IL-8 and IL-25 than healthy controls. The acute inflammatory response that originates from elevated IL-8 levels helps attract neutrophils to infection areas but simultaneously higher IL-25 levels suggest adaptive immune mechanisms intended to strengthen mucosal resistance. High levels of these cytokines which persist in the system potentially have negative effects on disease progress and may lead to complications or organ damage. The identification of IL-8 and IL-25 proves essential for determining infection severity thus researchers must investigate these cytokines and their immunological functions in amoebiasis treatment.

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