Cytokines and Kidney Function Biomarkers in Entamoeba Histolytica-Infected Patients

Hadi Hussein Mohammed and Huda Mawlood Taher
Department of Biology, College of Education for Pure Science, University of Kirkuk, 36013 Kirkuk, Iraq
{epbm23007, huda.mawlood}@ uokirkuk. edu.iq

Keywords: Entamoeba Histolytica, IL-1β, IL-8, IFN-γ, Renal Biomarkers, Amebiasis.

Abstract: This study investigates the immune response and kidney function in individuals infected with Entamoeba histolytica in Kirkuk, Iraq. Samples were obtained from hospitals and private laboratories between October and December 2024. Among 220 stool samples analyzed for E. histolytica cysts and trophozoites, 130 were excluded due to chronic or concurrent infections. The remaining participants were categorized into two groups: 70 infected individuals and 20 healthy controls. Blood samples were assessed for cytokine levels (IL-1β, IL-8, IFN-γ) and kidney function biomarkers (Creatinine, Urea, BUN). Statistical analysis using SPSS revealed significant elevations ($p \leq 0.05$) in IL-1β, IL-8, Creatinine, Urea, and BUN levels in infected individuals, suggesting immune activation and potential kidney function impairment. However, no significant differences were noted in IFN-γ levels or the BUN/creatinine ratio. These findings highlight the impact of parasitic infections on immune regulation and kidney health, underscoring the need for improved diagnostic and therapeutic strategies in endemic regions.

1 INTRODUCTION

Entamoeba histolytica, a tissue-invasive protozoan, is considered one of the anaerobic intestinal parasites responsible for approximately 50 million infections worldwide, with a mortality rate exceeding 1,00,000 annually [1]. The parasite invades the intestinal mucosa, causing amoebic colitis and potentially liver abscesses [2]. Amoebic infection is the third leading cause of parasitic-related deaths globally and in Iraq after malaria and schistosomiasis [3]. The infection is widespread in Kirkuk [4] and in developing countries such as Bangladesh, India, tropical African nations, and certain regions of Mexico and Brazil [5]. It is increasingly observed among the populations of both developing and developed countries, including the United States and European nations, due to international travel and migration from endemic areas [6]. Most parasitic infections tend to be asymptomatic. However, common symptoms associated with them include abdominal pain, discomfort, vomiting, and bloody diarrhea [7]. In severe cases, the infection can lead to complications such as gastroenteritis, malnutrition, and malabsorption. Children are more susceptible to intestinal protozoan infections, primarily due to the poor social and economic conditions of individuals, which is considered the main factor contributing to the spread of intestinal parasites [8]. When Entamoeba histolytica infects the host, the parasite binds to the epithelial layer of the colon and the mucosal layer through the secretion of Gal/GalNAc lectin by the parasite's trophozoite cells. After attachment, the parasite begins to degrade the epithelial cells using hydrolytic enzymes and surface proteins, particularly cysteine proteases[9]. This leads to the infection of intestinal cells and the destruction of extracellular matrix components. The parasite enters the initial defence against parasitic conditions, particularly the mucosal gut layer [10]. Interleukin-1 beta (IL-1β) is a basic inflammatory cytokine that contributes to the regulation of inflammation and immune system responses [11]. A group of stimuli, including lipopolysaccharides (LPS), TNF-α, and other signaling molecules, stimulates activated macrophage cells and various immune cells to produce it [11], [12]. E. histolytica leads to amoebiasis diseases, leading the colon epithelial cells to produce IL-8. Amoebic elements and substances released post-cell lysis may facilitate this process by improving the ejection of IL-8 mRNA [12].

The inflammatory reaction and tissue damage in gastrointestinal amoebiasis are mainly started by IL-8, which is vital in drawing neutrophils to the site of disease [13], [14]. Consequently, IL-8 acts an essential part in the pathophysiology of amoebiasis by connecting the virulence of E. histolytica with the immune response [15]. The aim of this study was to assess the effect of amino dysentery infection on some immune variables, such as Beta-1 Beta (IL-1β), Interleukin-8 (IL-8), and Interferon Gamma (IFN-γ). In addition, the study aims to assess the kidney function in individuals with Amoebic infection by measuring the levels of urea, creatinine, urea nitrogen in blood (BUN), and BUN/Creatinine.

2 MATERIALS AND METHODS

2.1 Study Population and Sample Collection

Initial study samples (stool) collected from 220 individuals during the period from October 2024 to December 2024 of both genders (aged 18-65 years) who visited Kirkuk hospitals and private laboratories in Kirkuk.

2.1.1 Inclusion Criteria

The study included 90 participants after excluding 130 cases based on predefined criteria. Inclusion required: (1) signed informed consent, (2) varying gastrointestinal symptoms (diarrhea, abdominal pain, dysentery), and (3) microscopic confirmation of Entamoeba histolytica cysts or trophozoites. All demographic and clinical data were meticulously documented before analysis.

2.1.2 Exclusion Criteria

Participants were excluded from the study if any of the following criteria matched:

1) Diagnosis of chronic metabolic disorders (diabetes, chronic renal insufficiency).
2) The presence of synchronous infection (bacterial, viral, or parasitic confirmed by laboratory tests).
3) Using antibiotics or immunosuppressants within 4 weeks before collecting samples.

These criteria aimed to reduce confusing variables in analysis of cytokines and kidney function. The final number of samples suitable for analysis was stabilized to 90 valid samples. Samples are kept in sterile containers with tightly closed covers to maintain the safety of the sample and maintain moisture. A medical syringe was used to take 90 samples of blood, which were then placed in the test tubes with gel. After that, Samples were centrifuged at 3000 ×g for 15 min at 4°C (Eppendorf 5804 R, Germany) with a balanced rotor configuration. Serum was aliquoted and stored at -20 °C (Thermo Scientific) for ≤2 weeks, avoiding freeze-thaw cycles..

2.2 Microscopic Examination

To determine the cystic and trophozoite stages in samples of stool, the samples were examined both directly and through a light microscope. The samples were divided into two groups: 70 samples from parasite-infected patients and 20 samples from healthy individuals. To make sure participant diversity and more accurately reflect the disease's local prevalence in the area, samples were taken from both public hospitals and private laboratories. A wooden stick was used to take a faeces sample, that was then put on a glass slide with a drop of regular saline. After that, a cover slip was set at an angle to keep air bubbles from developing. The microscope was used to examine the slide, first at a small magnification (10X) and then at high magnification. The exact same steps were employed for making a second slide, and one droplet of Lugol iodine stain—which was previously prepared using Luna's method (1968)—was added. This was accomplished through the addition of 10 grammes of iodine crystals after five grammes of potassium iodide had been dissolved in 1000 millilitres of distilled water. After slowly stirring the mixture until it was dissolved, it was filtered and put in sterile, tightly-sealed containers. The Entamoeba histolytica (tissue-invasive protozoan) parasite's cyst stage was coloured by the pigment. The samples were taken from several parts of the sample in order to improve the possibility of locating it.

2.3 Laboratory Analyses

2.3.1 Cytokine Measurement (ELISA)

The Enzyme-Linked Immunosorbent Assay (ELISA) method was employed using an ELISA reader and specific kits provided by the Chinese company Sunlong for the serum samples in the study. In this test, specific antibodies targeting key Cytokines (Interleukin-1 beta, Interleukin-8, and Interferon gamma) were used. These antibodies coated the

surface of 96 wells in a microtiter plate, as per the manufacturer's instructions (Sunlong,China). After completing the steps according to the guidelines, the color change was measured using an ELISA reader at a wavelength of 450 nm.

2.3.2 Renal Function Tests

Serum creatinine levels in individuals with infection were measured using the Jaffe Kinetic test (22, 2002). In this method, creatinine in the serum sample reacts with picric acid in an alkaline solution (alkaline picrate) from the reagent, resulting in the formation of an orange-colored complex. The amount of creatinine in the test samples was calculated based on the intensity of the color developed over a fixed period. The color intensity was measured using the fully automated Cobas C311 analyzer to detect creatinine levels in serum [16], [17]. The urea levels in the serum were analyzed using urease to produce ammonium and carbonate. Subsequently, L-glutamate was generated through reactions between ammonium and 2-oxoglutarate in the presence of glutamate dehydrogenase and the coenzyme NADH. During this reaction, 2 moles of NADH were oxidized to NAD+ for each mole of urea hydrolyzed. The rate of decrease in NADH concentration was directly proportional to the urea concentration in the serum sample, which was determined photometrically using the fully automated Cobas C311 analyzer [16], [17]. The blood urea nitrogen (BUN) was calculated according to the formula provided by [16], as follows:

$$BUN = Urea/2.14.$$

2.4 Statistical Analysis

The results were analyzed statistically by SPSS to determine significant differences between groups using the independent t-test. Subsequently, the magnitude and type of correlation between variables were assessed using the Pearson correlation coefficient [18].

3 RESULTS AND DISCUSSION

3.1 Microscopic Examination

The microscopic examination results in the current study, as shown in Table 1, revealed an infection rate of 31.818% (70 samples) from a total of 220 stool samples examined microscopically. These samples were collected from patients visiting the govermental Hospital in Kirkuk and private clinics between October 2024 and December 2024.

Table 1: Infection rate with E.histolytica by microscopic examination in the study groups.

Microscopic Examination	Positive Samples	Negative Samples
220	70 (31.818%)	150 (68.18%)

The cyst stage is characterized by a round or oval transparent body containing three to four nuclei and a chromatoid body, as shown in Figure 1. The trophozoite stage is characterized by an irregular protoplasmic mass with protrusions extending in all directions. It contains a single nucleus with a central nucleolus that regulates and controls the cell's functions, as shown in Figure 2.

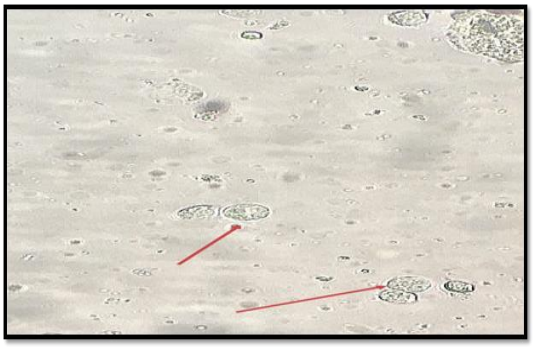

Figure 1: Cyst stage of Entamoeba histolytica stained with Lugol's iodine under 40X magnification.

The infection rate of this study was recorded at 30.22%. The findings also agreed with [19] in Dhi Qar, [20] in Kalar, who reported infection rates of 29.9%, 31.6%, respectively. These results are higher than those recorded by [21] in Kirkuk,, who reported an infection rate of 27.7%.

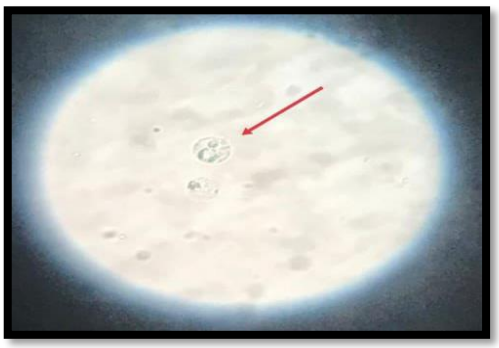

Figure 2: Trophozoite stage of Entamoeba histolytica stained with Lugol's iodine under 40X magnification.

3.2. Cytokine Profile

3.2.1 IL-1β Level

The results presented in Table 2 showed a significant increase ($P \leq 0.05$) in the level of Interleukin 1-beta (IL-1β) in individuals infected with Entamoeba histolytica (18.42 ± 3.98 ng/ml) when compared to healthy or uninfected individuals (14.81 ± 3.61 ng/ml)

Table 2: IL-1β level in the study groups.

Parameters Groups	Interleukin IL-1β ((ng/ml	Sig.
Control (Healthy) N =20	14.81 ± 3.61 B	0.012
Patients (Infected) N = 70	18.42 ± 3.98 A	

The results are presented as mean ± standard deviation, where (N) indicates the sample size. Different letters denote significant differences ($p \leq 0.05$), while similar letters indicate no significant differences.

These results are in agreement with the study by [22], which indicated a significant increase in this variable in infected individuals compared to healthy controls. These results can be explained by the inflammatory response triggered by the invasion of the parasite into body tissues. Inflammatory reactions and tissue damage result from the protozoa's stimulation of the host epithelial cells in order to release pro-inflammatory Cytokines, like interleukin-1 beta (IL-1β). Additionally, amoebic proteins possess a capacity to induce caspase-1 activity and activate proIL-1β, the precursor form of IL-1β, each of which increase inflammation [23]. Numerous vital processes are involved in the creation of amoebic proteins related with the pro-IL-1β precursor of interleukin-1 beta, To become its active state, pro-IL-1β must be cleaved by caspases, specifically caspase-1, after being created as a precursor that remains inactive [8]. Multiple triggers, for example pathogen-associated molecular patterns (PAMPs), lack in host cells yet present in specific pathogens, can cause this reaction [24]. In E. histolytica sickness, interaction between the amoeba and host immune cells may produce pro-IL-1β by inflammatory signal pathways [8].

3.2.2 Interferon Gamma Level

There are not any significant variations ($P < 0.05$) in the concentrations of gamma interferon between people infected (123.16 ± 6.32 ng/ml) with the Entamoeba histolytica (tissue-invasive protozoan) E. histolytica, according to the results shown in Table 3 when compared to healthy or uninfected individuals (128.24 ± 12.26 ng/ml).

Table 3: Interferon Gamma level in the study groups.

Parameters Groups	IFN-ϒ (ng/ml)	Sig.
Control (Healthy) N =20	128.24 ± 12.26 A	0.58
Patients (Infected) N = 70	123.16 ± 6.32 A	

The results obtained line up with a number of earlier investigations that showed no discernible difference in gamma interferon levels between infected and healthy controls. Moreover, amoebic infections appear to possess no impact on gamma interferon levels. This result is consistent with studies by [11] and [12], in which a group of individuals received a vaccine which contained the parasite's infectious stage. Studies have showed that although individuals produced more gamma interferon in response to the immunisation, there were no significant differences in gamma interferon levels between infected and non-infected individuals. Due of the particular immune system reaction mechanisms involved, amoebic infection may not have a major impact on gamma interferon (IFN-γ) levels. To study, when amoebic proteins occur, phagocytic cells infected with the parasite Entamoeba histolytica do not consistently produce more IFN-γ or produce more tumour necrosis factor (TNF-α) than when they are stimulated with lipopolysaccharide (LPS) [25]. There was no statistically significant relation between gamma interferon and interleukin-1 beta ($r = 0.17$, Sig = 0.27), or between gamma interferon and interleukin-8 ($r = 0.14$, Sig = 0.37), . There are many kinds of causes of this, including:

1) Temporal Variability: There is a time disparity in the secretion of these Cytokines. Gamma interferon appears early in the course of infection, whereas the interleukins mentioned are typically secreted in the later stages of infection [26]. This temporal difference may prevent a statistical correlation between their levels.

2) Sample Size: The small sample size could also reduce the statistical power to detect meaningful correlations, even if they exist.

3.2.3 Interleukin 8 Level

Patients showed significantly higher IL-8 levels (29.65 ± 5.50 ng/ml) compared to healthy controls (23.47 ± 6.12 ng/ml; p = 0.003) (see Table 4).

Table 4 Interleukin 8level in the study groups.

Parameters Groups	Interleukin 8 (ng/ml)	Sig.
Control (Healthy) N =20	23.47 ± 6.12 B	0.003
Patients (Infected) N = 70	29.65 ± 5.50 A	

These results are consistent with the study by [27]. which indicated that interleukin 8 levels are significantly higher in patients compared to healthy individuals. The elevated levels of interleukin 8 (IL-8) in amoebic infections, particularly those caused by Entamoeba histolytica, are primarily attributed to the direct stimulation of colonic epithelial cells by the parasite. This stimulation, either by the parasite itself or its components, leads to increased expression of the IL-8 gene in these cells via post-transcriptional mechanisms. Over time, this results in enhanced secretion of IL-8 [28]. Furthermore, the inflammatory response is amplified by Cytokines such as IL-1β, which activate NF-κB pathways, promoting IL-8 production and attracting immune cells to the site of infection [29]. The Pearson correlation coefficient indicates a strong positive correlation between interleukin 8 and interleukin 1 beta (r = 0.998, sig = 0.00) on one hand, while no statistically significant correlation was observed between interleukin 8 and gamma interferon (r = 0.14, sig = 0.38) on the other hand. This finding was explained earlier (in the interpretation of the results for interleukin 1 beta and interferon gamma).

3.3 Renal Function Test

The results presented in Table 5 [25] show a significant increase (P ≤ 0.05) in the level of urea in individuals infected with the Entamoeba histolytica (tissue-invasive protozoan) E. histolytica (41.99 ± 3.96) when compared to healthy or uninfected individuals (37.40 ± 4.35). These results are consistent with the study by [25], which indicated a significant increase in urea levels in infected individuals compared to healthy controls.

Table 5: Kidney Function biomarkers levels in study groups.

Group Parameters	Control N =20	Patients N = 70
Urea (mg/dL)	37.40 ± 4.35 B	41.99 ± 3.96 a
Creatinine (mg/dL)	0.18 B 0 . 8 8	1.01± 0.16 a
BUN (mg/dl)	80.04 ± 9.30 B	89.86 ± 8.47 a
BUN/Creatinie ratio	20.73 ± 4.57 A	19.70 ± 2.62 a

The elevated urea levels in the blood may be attributed to the breakdown and degradation of proteins due to kidney involvement in the disease, leading to an increase in urea concentration. One explanation for this is that the last product of protein metabolism is urea [9]. These findings are in line with a study by [30], which found that people infected with E. histolytica had lower urea levels. The results presented in Table 5 show a significant increase (P ≤ 0.05) in the creatinine level in individuals infected with the Entamoeba histolytica (tissue-invasive protozoan) E. histolytica (1.01 ± 0.16) when compared to healthy or uninfected individuals (0.88 ± 0.18).

These results are consistent with the study by (Mohammed et al. 2022). However, this study differs from a study conducted by [31], which reported a slight decrease in creatinine levels in infected individuals [32].The results presented in Table 5 show a significant increase (P ≤ 0.05) in the BUN level in individuals infected with the Entamoeba histolytica (tissue-invasive protozoan) E. histolytica (89.86 ± 8.47) compared to healthy or uninfected individuals (80.04 ± 9.30).

These results are consistent with the study by [32], which indicated an elevation in BUN levels during amoebic dysentery infection. The statistical results analyzed using the SPSS program showed a significant increase (p ≤ 0.05) in the levels of (IL-1β), (IL-8), creatinine, urea, and BUN. In contrast, no significant difference was observed in the levels of interferon gamma (IFN-γ) and the BUN/Creatinine ratio between the two groups. Urea levels may also be accompanied by rising IL-1β levels.

This suggests how the inflammatory response impacts renal function. The kidneys' decreased ability to remove metabolic waste as a result of an increase in inflammatory Cytokines (such IL-1β) might cause urea to build up in the blood. The correlation results also showed a strong positive correlation (r = 0.00)

between the levels of Interleukin-1β and Interleukin-8 (r = 0.998, sig = 0.00).

This can be interpreted by the fact that Entamoeba histolytica stimulates the production of Interleukin-8 in colon cells as part of the inflammatory response during infection. At the same time, the presence of Interleukin-1β enhances the secretion of Interleukin-8, contributing to the inflammatory environment associated with amoebic dysentery [23], [29].

The statistical analysis of the BUN/Creatinine ratio values showed no significant differences between the patient group (20.73 ± 4.57) when compared to the control group (19.70 ± 2.62). The BUN/Creatinine ratio is an important indicator for assessing kidney function, with normal values typically ranging from 10:1 to 20:1. However, in the case of amoebic infection, no specific or well-documented ratios exist. According to prior studies, modifications to this ratio could be a symptom of underlying medical disorders such as dehydration or cut kidney function, each of which can be greatly impacted by severe amoebic infections [33].

IL-1β and creatinine indicate a slight positive correlation of roughly 0.45. Putting it another way, there may be a relationship between high IL-1β and creatinine levels. The inflammatory impact of the amoeba parasite on renal function may account for this. The infection's immunological and inflammatory effects can make it harder for the kidneys to remove waste. IL-1β and urea share an a little positive link, with a correlation value of 0.38. While urea and IL-1β have a fairly beneficial relationship, rising blood

A Pearson correlation value of 0.51 shows a substantial connection between IL-1β and BUN. Given the strong positive relationship between blood urea nitrogen (BUN) and IL-1β, it is possible that elevated BUN levels are linked to raised IL-1β levels. This result indicates that a decrease in kidney function may be associated to the inflammatory response triggered by the amoeba parasite. BUN levels can rise as a result of acute inflammation brought on by high IL-1β levels that impair the kidneys' ability to clear out waste.

The inflammatory response carried on by the amoeba infection may affect renal function, as indicated by the moderate major positive correlations observed between IL-1β, creatinine, urea, and BUN. High levels of IL-1β are probably causing inflammation in the kidneys, leading to decreases in their capacity to filter waste and raising blood levels of creatinine, urea, and BUN.

4 CONCLUSIONS

Statistical analysis using SPSS revealed significant elevations ($p \leq 0.05$) in IL-1β, IL-8, Creatinine, Urea, and BUN levels in infected individuals, suggesting immune activation and potential kidney function impairment. However, no significant differences were noted in IFN-γ levels or the BUN/creatinine ratio. Given there were notable increases in interleukin 1 beta and interleukin 8 levels, which indicate the activation of the inflammatory response, we reach our conclusion that individuals infected with the Entamoeba histolytica (tissue-invasive protozoan) have an exaggerated immune response. Significant rises in creatinine and urea levels additionally show the infection's detrimental effects on renal function. However, there were no appreciable variations in the BUN/Creatinine ratio or gamma interferon levels between infected and healthy people. These findings emphasise how crucial it is to assess organ function indicators and immunological markers when trying to create efficient diagnosis and treatment plans.

REFERENCES

[1] R. Fotedar, D. Stark, N. Beebe, D. Marriott, J. Ellis, and J. Harkness, "Laboratory diagnostic techniques for Entamoeba species," Clinical Microbiology Reviews, vol. 20, no. 3, pp. 511–532, Jul. 2007, doi: https://doi.org/10.1128/CMR.00004-07.

[2] S. Gupta, L. Smith, and A. Diakiw, "Amebiasis and amebic liver abscess in children," Pediatric Clinics, vol. 69, no. 1, pp. 79–97, 2022.

[3] M. Tanyuksel and W. A. Petri, "Laboratory diagnosis of amebiasis," Clinical Microbiology Reviews, vol. 16, no. 4, pp. 713–729, Oct. 2003, doi: https://doi.org/10.1128/CMR.16.4.713-729.2003.

[4] H. M. Taher, A. H. Mohamed, and S. S. Yaseen, "Prevalence of intestinal parasitic infections among children in Kirkuk City, Iraq," Revista Bionatura, 2022. [Online]. Available: https://revistabionatura.com/files/18-2_5303169525380815898.pdf

[5] M. Espinosa-Cantellano and A. Martínez-Palomo, "Pathogenesis of intestinal amebiasis: from molecules to disease," Clinical Microbiology Reviews, vol. 13, no. 2, pp. 318–331, Apr. 2000, doi: https://doi.org/10.1128/CMR.13.2.318.

[6] X. Que et al., "A surface amebic cysteine proteinase inactivates interleukin-18," Infection and Immunity, vol. 71, no. 3, pp. 1274–1280, Mar. 2003, doi: https://doi.org/10.1128/IAI.71.3.1274-1280.2003.

[7] D. R. Pillai and K. C. Kain, "Recent developments in amoebiasis: the Gal/GalNAc lectins," Microbes and Infection, vol. 2, no. 14, pp. 1775–1783, Nov. 2000, doi: https://doi.org/10.1016/S1286-4579(00)01330-7.

[8] S. C. Lee, D. W. Dickson, W. Liu, and C. F. Brosnan, "Induction of nitric oxide synthase activity in human astrocytes by interleukin-1β and interferon-γ," Journal of Neuroimmunology, vol. 46, no. 1–2, pp. 19–24, Jul. 1993.

[9] P. Morán et al., "Amoebiasis: advances in diagnosis, treatment, immunology features and the interaction with the intestinal ecosystem," International Journal of Molecular Sciences, vol. 24, no. 14, p. 11755, 2023.

[10] K. Nakada-Tsukui and T. Nozaki, "Immune response of amebiasis and immune evasion by Entamoeba histolytica," Frontiers in Immunology, vol. 7, May 2016, doi: https://doi.org/10.3389/fimmu.2016.00175.

[11] X. Guo et al., "Protection against intestinal amebiasis by a recombinant vaccine is transferable by T cells and mediated by gamma interferon," Infection and Immunity, vol. 77, no. 9, pp. 3909–3918, Sep. 2009, doi: https://doi.org/10.1128/IAI.00487-09.

[12] C. P. Ivory, "Cytokine modulation in parasitic infections," Parasite Immunology, vol. 29, no. 5, pp. 223–231, 2007.

[13] H. M. Taher and A. H. Mohamed, "The effect of Entamoeba histolytica infection on levels of adiponectin and histamine in children," Bahrain Medical Bulletin, vol. 44, no. 2, 2022.

[14] H. H. Mohammed and H. M. Taher, "Analysis of liver function in patients with intestinal parasitic infections: a comparative study of AST, ALT, and ALP levels," Central Asian Journal of Medical and Natural Science, vol. 6, no. 2, Mar. 2025, doi: https://doi.org/10.17605/cajmns.v6i2.2767.

[15] S. Saidin, N. Othman, and R. Noordin, "Update on laboratory diagnosis of amoebiasis," European Journal of Clinical Microbiology and Infectious Diseases, vol. 38, no. 1, pp. 15–38, Jan. 2019, doi: https://doi.org/10.1007/s10096-018-3379-3.

[16] R. M. McLaughlin and R. E. Fish, "Clinical biochemistry and hematology," in The Biology of the Laboratory Rabbit, Elsevier, 1994, pp. 111–127, doi: https://doi.org/10.1016/B978-0-12-469235-0.50013-0.

[17] A. H. B. Wu, "A selected history and future of immunoassay development and applications in clinical chemistry," Clinica Chimica Acta, vol. 369, no. 2, pp. 119–124, Jul. 2006, doi: https://doi.org/10.1016/j.cca.2006.02.045.

[18] C. Heumann, M. Schomaker, and Shalabh, Introduction to Statistics and Data Analysis. Cham, Switzerland: Springer, 2016, doi: https://doi.org/10.1007/978-3-319-46162-5.

[19] Z. S. Hadi, "A study of prevalence of intestinal parasitic infection in Shatrah district/Thi-Qar governorate," Journal of Education and Pure Science, University of Thi-Qar, vol. 1, no. 5, pp. 28–39, 2011.

[20] S. M. A. Ali and M. A. Qader, "Nutritional status of children infected with Giardia lamblia and Entamoeba histolytica infections in Kalar town, Iraq," Tikrit Journal of Pharmaceutical Sciences, vol. 7, no. 2, pp. 157–166, 2011.

[21] L. A. Mohsin, S. S. Hamad, and S. M. Rahim, "The effect of infection with the Entamoeba histolytica on oxidative stress status in Kirkuk hospital patients," Journal of Pharmaceutical Negative Results, vol. 13, pp. 3191–3195, 2022.

[22] D. J. Ibrahim, H. Q. Mohammed, and H. N. Abd, "Molecular and immunological study for Entamoeba histolytica in pediatric patients," Biochemical and Cellular Archives, vol. 22, no. 1, 2022.

[23] M. M. Bakr, H. M. Taher, F. K. Hussein, and A. H. Mohamed, "Study the effective role of metronidazole nanoemulsion for the treatment of skin lesions in mice induced by Entamoeba histolytica," South Asian Research Journal of Biology and Applied Biosciences, vol. 6, no. 1, pp. 1–7, Jan. 2024, doi: https://doi.org/10.36346/sarjbab.2024.v06i01.001.

[24] P. M. Exconde et al., "The tetrapeptide sequence of IL-18 and IL-1β regulates their recruitment and activation by inflammatory caspases," Cell Reports, vol. 42, no. 12, p. 113581, Dec. 2023, doi: https://doi.org/10.1016/j.celrep.2023.113581.

[25] F. K. Hussein, A. J. Mahmoud, and B. J. Yousif, "Estimation of immunoglobulin A, immunoglobulin G, and immunoglobulin M antibody levels in laboratory mice Balb/c infected with Entamoeba histolytica and treatment with aqueous extracts of Cyperus rotundus and Thymus serpyllum," Polytechnic Journal, vol. 10, no. 1, pp. 126–129, Jun. 2020.

[26] A. Al-Hamish, "The role of interleukins in early and late immune responses to parasitic infections," Journal of Parasitology Studies, vol. 15, no. 2, pp. 45–56, 2024.

[27] M. Abas, S. A. Nadir, H. Fadhil, and N. Tektook, "A prevalence of Entamoeba sp. in children with diarrhea," International Journal of Medical Research, vol. 1, no. 1, pp. 9–13, 2024.

[28] A. Chou and R. L. Austin, "Entamoeba histolytica infection," in StatPearls [Online], StatPearls Publishing, 2023. Available: https://www.ncbi.nlm.nih.gov/books/NBK557718/

[29] K. A. Kh. and S. F. Ab., "Immunological and cytokine profile (IL-25 and IL-35) in patients with Entamoeba histolytica infection in Southern Iraq," Archives of Razi Institute, vol. 79, no. 2, p. 315, 2024.

[30] R. S. Bray and W. G. Harris, "The epidemiology of infection with Entamoeba histolytica in The Gambia, West Africa," Transactions of the Royal Society of Tropical Medicine and Hygiene, vol. 71, no. 5, pp. 401–407, Jan. 1977.

[31] A. Bamanikar and P. Soni, "Renal function alterations in individuals with Entamoeba histolytica infection," International Journal of Clinical Medicine, vol. 19, no. 1, pp. 16–21, 2024.

[32] J. Royo et al., "Elevated plasma interleukin-8 as a risk factor for mortality in children presenting with cerebral malaria," Infectious Diseases of Poverty, vol. 12, no. 1, p. 8, Feb. 2023, doi: https://doi.org/10.1186/s40249-023-01059-2.

[33] S. M. Zia Ziabari et al., "Blood urea nitrogen to creatinine ratio in differentiation of upper and lower gastrointestinal bleedings: a diagnostic accuracy study," Archives of Academic Emergency Medicine, vol. 7, no. 1, p. e30, 2019.