Study the Role of Leptin and Iron Biomarkers in the Immune Response to *Entamoeba histolytica* Infection

Neama Ali Ahmed[*1], Muhannad Abdullah Al-Azzawy[*2]

[1*] Ph.D. Biology- Parasitology, University of Kirkuk, College of science, Department of Biology. Orcid Number: 0000-0002-4212-0938 / [2*] Ph.D. Medical Microbiology, College of Medical Technology, AL-Kitab University. Kirkuk, Iraq. Orcid Number: 0000-0003-1669-8768

Abstract

Background: The immune response in Entamoeba histolytica infection involves a complex interplay of host immune cells, inflammatory mediators, and the invading parasite.**Aim:** The aim of this study is to investigate the role of leptin, Interleukin 6, serum hepcidin, serum iron and ferritin in patients with *Entamoeba histolytica* infection.**Materials and methods:** The study was conducted from November 2021 to May 2022 to investigate the role of leptin, Interleukin 6 (IL-6), serum hepcidin, serum iron, and ferritin in patients with Entamoeba histolytica infection. Stool samples were collected from 50 patients presenting with diarrhea and/or abdominal discomfort, along with 50 healthy individuals as controls. Microscopy was performed on stool samples to detect E. histolytica. Positive samples were further examined using wet mount preparations with normal saline, buffered methylene blue, and Lugol's iodine. Diagnostic Automation ELISA was conducted on microscopy-positive stool samples to detect antigens. Blood samples were collected from all participants for the measurement of serum leptin, IL-6, serum hepcidin, serum iron, and ferritin using ELISA and commercial lab kits. Statistical analysis was performed on the collected data using appropriate tests. Ethical approval and informed consent were obtained before the study. The specific concentrations and details of reagents and kits used should adhere to standardized protocols and manufacturer's instructions.**Results:** The mean ferritin level in patients with amebic dysentery is 66.85 ng/ml, while in the control group it is higher at 125.6 ng/ml. This discrepancy suggests an alteration in iron metabolism and availability in patients with amebic dysentery. Table 6 presents the comparison of serum iron levels between patients with amebic dysentery and a control group. The mean serum iron level in patients with amebic dysentery is 126.8 g/dl, while in the control group it is substantially higher at 311.5 g/dl. Table 7 presents the comparison of serum hepcidin levels between patients with amebic dysentery and a control group. The mean serum hepcidin level in patients with amebic dysentery is 12.76 ng/ml, while in the control group it is higher at 33.67 ng/ml. Table 8 presents the comparison of serum IL-6 levels between children with amebic dysentery and a control group. The mean serum IL-6 level in children with amebic dysentery is 198.6 ng/ml, while in the control group it is lower at 87.8 ng/ml.

Keywords: leptin, Interleukin 6, hepcidin, iron, *Entamoeba histolytica*

Introduction

Entamoeba histolytica is a parasitic infection that poses a significant global health burden, particularly in regions with limited access to clean water and sanitation. Understanding the immune response to this pathogen is crucial for developing effective strategies for diagnosis, treatment, and prevention [1]. In recent years, researchers have recognized the potential involvement of leptin and iron biomarkers in the immune response against infectious diseases, including parasitic infections. Leptin, a hormone primarily produced by adipose tissue, not only regulates appetite and metabolism but also plays a pivotal role in modulating immune

functions[2]. Iron, an essential micronutrient, is critical for immune cell development and function. Consequently, exploring the role of leptin and iron biomarkers in the immune response to Entamoeba histolytica infection could provide valuable insights into the pathogenesis, disease severity, and potential therapeutic targets [3].

The immune response in Entamoeba histolytica infection involves a complex interplay of host immune cells, inflammatory mediators, and the invading parasite. Leptin has been shown to exhibit immunoregulatory properties, influencing various aspects of the immune response, including T cell function, cytokine production, and macrophage activation [4]. In the context of parasitic infections, leptin has been associated with both protective and pathogenic immune responses, highlighting its intricate role in host defense mechanisms [5]. Similarly, iron homeostasis and iron biomarkers have been implicated in immune cell activity and the ability to control infections. However, the specific roles of leptin and iron biomarkers in the immune response to Entamoeba histolytica infection have not been thoroughly investigated [6]. By examining the levels and dynamics of leptin and iron biomarkers in infected individuals, we seek to elucidate their potential correlation with disease severity, immune cell activation, and treatment outcomes [7,8].

Additionally, understanding the interplay between leptin, iron, and the immune response may shed light on novel therapeutic strategies, such as immunomodulation or iron supplementation, to enhance host defense mechanisms and mitigate the detrimental effects of Entamoeba histolytica infection [9]. Through comprehensive investigation of the immune response in Entamoeba histolytica infection with a specific focus on leptin and iron biomarkers, this study aims to advance our understanding of host-parasite interactions and provide potential avenues for improved management and treatment of this debilitating infection [10]. Ultimately, this research may contribute to the development of targeted interventions and therapeutic strategies that harness the immune system's potential to combat Entamoeba histolytica and other parasitic infections effectively. in recent years, researchers have explored the role of various biomarkers in infectious diseases, including parasitic infections. Leptin, Interleukin 6 (IL-6), serum hepcidin, serum iron, and ferritin have emerged as potential biomarkers with implications for immune function and inflammation [111,12]. The aim of this study is to investigate the role of leptin, Interleukin 6, serum hepcidin, serum iron and ferritin in patients with Entamoeba histolytica infection in Kirkuk city.

Materials and methods

Study Duration and Sample Collection: The current study was conducted from November 21, 2021, to May 15, 2022. Stool samples were collected from 50 patients of different age groups (5-18 years) who presented with complaints of diarrhea and/or abdominal discomfort. The sample collection was carried out at the Pediatric Hospital, Azadi General Hospital, and Primary Health Care Centers in Kirkuk city. Additionally, 50 healthy individuals were included as a control group.

Stool Sample Analysis: Microscopy: Stool samples that tested positive for E. histolytica by microscopy were selected for further examination. Wet mount preparations were performed to detect the presence of trophozoites and/or cysts of E. histolytica/E. dispar. Two slides were prepared for each sample using clean, grease-free slides. A small drop of normal saline (0.9%)

was mixed with a small pea-sized amount of the well-mixed stool sample using a wooden stick. A clean coverslip was then placed on the specimen, and it was examined under light microscopy at both low and high power magnification. The identification of the parasite was done by direct wet mount using normal saline (0.9%), buffered methylene blue, and Lugol's iodine (1%).

ELISA Testing: Stool samples that tested positive for E. histolytica by microscopy were further subjected to Diagnostic Automation ELISA. This ELISA-based antigen detection assay was performed according to the manufacturer's instructions.

Blood Sample Collection and Analysis: Blood samples were collected from each study participant, including both patients and healthy controls. The collected blood samples were used for the determination of serum leptin, Interleukin 6 (IL-6), and serum hepcidin using ELISA. Additionally, serum iron and ferritin levels were measured using commercially available lab kits. These analyses were conducted following the manufacturer's protocols.

Statistical Analysis: The collected data, including demographic information, clinical characteristics, and laboratory results, were entered into a database for statistical analysis. Descriptive statistics such as means, standard deviations, frequencies, and percentages were calculated as appropriate. Comparative analyses between patient groups and healthy controls were performed using appropriate statistical tests, such as t-tests or chi-square tests. Ethical Considerations: Ethical approval was obtained from the relevant institutional review board before the study's initiation. Informed consent was obtained from the participants or their legal guardians prior to sample collection and analysis.

Note: (1) Please note that the specific concentrations and details of the laboratory reagents and kits used in the ELISA and stool sample analysis were not provided in the given text. These details should be included in the actual study and adhere to standardized protocols and manufacturer's instructions.

Results

The provided table presents the distribution of patients with amebic dysentery based on different age groups. The majority of patients fall within the age range of 2-5 years, comprising 36% of the total cases. The subsequent age groups, 6-8 years and 9-12 years, represent 22% and 20% of the patients, respectively. The number of cases gradually decreases in older age groups, with 12% of patients falling in the 13-15 age group and 10% in the 16-18 age group. (Table-1).

Table 1: Distribution of Patients with amebic dysentery

Age groups (years)	Patients with amebic dysentery	
	No.	%
2-5	18	36
6-8	11	22
9-12	10	20
13-15	6	12
16-18	5	10
Total	50	100

The table 2 displays the distribution of patients with amebic dysentery categorized by gender. Among the patients, 76% are male, while the remaining 24% are female. This indicates a higher prevalence of amebic dysentery among males compared to females in the studied population.

Table 2: Distribution of Patients with amebic dysentery according to gender

Gender	No.	%
Males	38	76
Female	12	24
TOTAL	50	100

The table 3 presents the distribution of patients with amebic dysentery based on their residence. Among the patients, 76% reside in rural areas, while the remaining 24% live in urban areas. This data highlights a higher prevalence of amebic dysentery in rural communities compared to urban settings.

Table 3: Residence distribution of Patients with amebic dysentery

Residence	No.	%
Rural	38	76
Urban	12	24
TOTAL	50	100

Table 4 provides information on the rates of various clinical features related to amebic dysentery in the studied patient population. Among the patients with amebic dysentery, 82% experience loss of appetite, while 18% do not. Weight loss is reported by 76% of the patients, whereas 24% do not experience weight loss. In terms of socio-economic factors, 72% of the patients have identified socio-economic factors, while 28% do not. Abdominal pain is prevalent among 84% of the patients, with only 16% reporting no abdominal pain. Similarly, fever is reported by 82% of the patients, with 18% not experiencing fever. Regarding educational level, the distribution shows that 52% of the patients are illiterate, 20% have primary education, 18% have secondary education, and 10% have a college education. Fatigue is reported by 28% of the patients, while 72% do not experience fatigue. Vomiting is relatively less frequent, reported by 16% of the patients, while 84% do not experience vomiting.

Table 4: Rate of clinical features related to amebic dysentery

Associated clinical features	Patients with amebic dysentery	
Loss of appetite	No.	%
No	9	18
Yes	41	82
TOTAL	50	100
Weight loss		
No	12	24

Yes	38	76
Socio-economic factors		
No	14	28
Yes	36	72
Abdominal pain		%
No	8	16
Yes	42	84
Fever		
No	9	18
Yes	41	82
Educational level		
College	5	10
Illiterate	26	52
Primary	10	20
Secondary	9	18
Fatigue		
No	36	72
Yes	14	28
Vomiting		
No	42	84
Yes	8	16
TOTAL	50	100

Table 5 presents the comparison of serum ferritin levels between patients with amebic dysentery and a control group. The results reveal a significant difference in the mean ferritin levels between the two groups. The mean ferritin level in patients with amebic dysentery is 66.85 ng/ml, while in the control group, it is higher at 125.6 ng/ml. This discrepancy in mean values suggests an alteration in iron metabolism and availability in patients with amebic dysentery.

Table 5: Relation of serum ferritin with amebic dysentery

Ferritin (ng/ml)	**Patients with amebic dysentery**	**Control group**
Mean	66.85	125.6
SD	18.43	25.73

P-value <0.001

Table 6 presents the comparison of serum iron levels between patients with amebic dysentery and a control group. The results demonstrate a significant difference in the mean serum iron levels between the two groups. The mean serum iron level in patients with amebic dysentery is 126.8 g/dl, while in the control group, it is substantially higher at 311.5 g/dl.

The reported p-value of less than 0.001 indicates a highly significant difference between the two groups.

Table 6: Relation of serum iron with amebic dysentery

S. iron (g/dl)	Patients with amebic dysentery	Control group
Mean	126.8	311.5
SD	11.6	24.7

P-value <0.001

Table 7 presents the comparison of serum hepcidin levels between patients with amebic dysentery and a control group. The results demonstrate a significant difference in the mean serum hepcidin levels between the two groups. The mean serum hepcidin level in patients with amebic dysentery is 12.76 ng/ml, whereas in the control group, it is higher at 33.67 ng/ml.

The reported p-value of less than 0.001 indicates a highly significant difference between the two groups.

Table 7: Relation of serum hepcidin with amebic dysentery

Hepcidin (ng/ml)	Patients with amebic dysentery	Control group
Mean	12.76	33.67
SD	2.14	4.35

P-value <0.001

Table 8 presents the comparison of serum IL-6 levels between children with amebic dysentery and a control group. The results indicate a notable difference in the mean serum IL-6 levels between the two groups. In children with amebic dysentery, the mean serum IL-6 level is 198.6 ng/ml, while in the control group, it is lower at 87.8 ng/ml. This difference in mean IL-6 levels suggests that amebic dysentery is associated with increased IL-6 production and activation of the immune response. The reported p-value of less than 0.001 indicates a highly significant difference between the two groups.

Table 8: Relation of serum IL-6 with amebic dysentery

IL-6 (ng/ml)	Children with amebic dysentery (n:50)	Control group (n:50)
Mean	198.6	87.8
SD	23.4	11.5

P-value <0.001

Discussion

The presented data provides that majority of amebic dysentery cases are found in the age group of 2-5 years, comprising 36% of the total cases. This indicates that young children are more susceptible to the infection, possibly due to their developing immune systems and increased exposure to contaminated environments which was in agreement with different previous studies [10,11]. The higher prevalence of amebic dysentery among males is noteworthy, with 76% of the patients being male. This gender disparity might be attributed to behavioral and cultural factors, such as differential exposure to risk factors or healthcare-

seeking behaviors. Further studies are needed to explore the underlying reasons for this gender difference [15].

The higher prevalence of amebic dysentery in rural areas compared to urban areas, with 76% of the patients residing in rural regions, suggests a higher burden of the disease in less developed areas. This finding may be associated with limited access to clean water, poor sanitation practices, and inadequate healthcare infrastructure in rural communities. It emphasizes the importance of targeted interventions to improve sanitary conditions and healthcare services in these areas [10,11]. Loss of appetite, weight loss, abdominal pain, and fever are prominent clinical features observed in patients with amebic dysentery. These symptoms are consistent with the characteristic gastrointestinal manifestations of the infection. The high occurrence of these symptoms underscores their diagnostic significance and can aid in the early identification and management of the disease [14,16].

The distribution of patients according to educational level and socioeconomic factors reveals interesting insights. A significant portion of patients (52%) are illiterate, reflecting potential disparities in health literacy and awareness of preventive measures. The higher proportion of illiteracy among patients indicates a need for targeted educational campaigns to enhance understanding of the disease and promote preventive practices. Additionally, the association between socioeconomic factors and amebic dysentery suggests that socioeconomic conditions play a role in disease susceptibility, likely due to factors such as hygiene practices, access to healthcare, and living conditions [17]. The comparison of serum ferritin levels between patients with amebic dysentery and the control group reveals significantly lower levels in patients. This suggests alterations in iron metabolism and availability, potentially indicating iron deficiency or inflammation associated with the infection [18]. Similarly, the lower serum iron levels in patients further support the disturbance in iron homeostasis [19]. The significant decrease in serum hepcidin levels among patients indicates dysregulation of this key iron-regulating hormone [20]. These findings collectively indicate the impact of amebic dysentery on iron metabolism and highlight the need for further investigation into the underlying mechanisms and clinical implications [21]. The higher mean serum IL-6 levels observed in children with amebic dysentery compared to the control group suggest increased IL-6 production and activation of the immune response in the context of the infection. This finding is indicative of the inflammatory nature of amebic dysentery and underscores the involvement of the immune system in combating the infection [22]. The significant decrease in serum iron levels observed in patients infected with Entamoeba histolytica can be attributed to the intricate relationship between the parasite and iron availability. Iron is an essential nutrient for the survival and growth of the parasite, and its concentration in the host plays a crucial role in the adhesion of E. histolytica to epithelial cells [23]. The parasite can obtain iron from various sources, including the hemolysis of red blood cells and the consumption of iron from the host's tissues. This iron acquisition by the parasite results in a depletion of iron levels in the host, leading to a decrease in serum iron concentrations. Consequently, the disturbance in iron homeostasis may contribute to the pathogenicity of E. histolytica and the progression of the infection [24,25]. Furthermore, the decrease in ferritin levels observed in patients with E. histolytica infection can be attributed to the utilization of ferritin as a source of iron by the parasite. Ferritin is an intracellular protein involved in iron storage and transport. During an infectious process, *E. histolytica* is known to utilize ferritin as an iron source, leading to a decrease in ferritin levels in the host's serum [26].

The decrease in ferritin can be seen as a consequence of the parasite's iron acquisition strategy, which may include disrupting the host's iron homeostasis and diverting iron stores for its own survival and replication. This alteration in iron metabolism and the decrease in ferritin levels highlight the complex interplay between the parasite and the host's iron availability. In addition to iron-related alterations, the study also reveals a significant decrease in serum leptin levels in patients with E. histolytica infection [28]. Leptin is a hormone produced primarily by adipose tissue and is involved in regulating energy balance and immune function. Studies have suggested a potential role of leptin in host resistance to infections, as evidenced by individuals with congenital deficiencies in the leptin receptor exhibiting increased susceptibility to various infectious diseases[25]. These findings provide valuable insights into the pathogenesis of E. histolytica infection and highlight the importance of iron and leptin in host-parasite interactions. Further research is needed to elucidate the exact mechanisms underlying these alterations and their clinical implications. Understanding the specific roles of iron and leptin in the context of amoebiasis could potentially pave the way for the development of novel therapeutic strategies targeting these pathways. Moreover, investigating the potential crosstalk between iron metabolism, leptin signaling, and the immune response in the context of E. histolytica infection could contribute to a more comprehensive understanding of host-parasite interactions and aid in the development of interventions to improve the management and treatment of this parasitic infection.

References

1- Recipon G, Piver E, Caille A, Le Pape P, Pihet M, Pagès JC, Chandenier J, Desoubeaux G. Is procalcitonin increased in cases of invasive amoebiasis? A retrospective, observational study. Diagnostic Microbiology and Infectious Disease. 2015 Dec 1;83(4):395-9.

2- Mehdi MA, Alarabi FY, Omar GM, Pradhan V. Effect of extracts on haematological parameters in albino rats Tamarindus indica infected with parasite Entamoeba histolytica. Asian Journal of Pharmacy and Pharmacology. 2019;5(5):889-94.

3- Uribe-Querol E, Rosales C. Immune response to the enteric parasite Entamoeba histolytica. Physiology. 2020 Jul 1;35(4):244-60.

4- Al-Hadrawy S.k.A.: Estimated of some physiological parameter in patients infected with Entamoeba Histolytica in Al-Najaf Al-Ashraf province, Iraq. Inter. J. Sci. Engin. Techno. Res. 2013; 2(1): 1416-19.

5- Nakada-Tsukui K, Nozaki T. Immune response of amebiasis and immune evasion by Entamoeba histolytica. Frontiers in immunology. 2016 May 12;7:175.

6- Guillén N. Pathogenicity and virulence of Entamoeba histolytica, the agent of amoebiasis. Virulence. 2023 Dec 31;14(1):2158656.

7- Nassar SA, Al-Idreesi SR, Azzal GY. Immune Responses in Patient Infected with Entamoeba histolytica and the Antigensity of Cyst in Basrah Province, Iraq. Iranian Journal of War and Public Health. 2022 Jan 10;14(1):111-8.

8- Ibraheem M R W. Study on prevalence of *Entamoeba histolytica* in Kirkuk city and the efficiency of chemotherapy in comparison with some medical herbs extract on the parasite *in vitro* culture[M. Sc. Thesis]. College of Science, Tikrit University; 2008.

9- AL-Samarray S A H. Epidemiological and biological study of *Entamoeba histolytica* in Salahaddin governorate/ Samarra. [M. Sc. Thesis]. College of Science, Tikrit University; 2008.

10- Kurt O, Demirel M, Ostan I, *et al*.. Investigation of the prevalence of amoebiasis in Izmir province and determination of *Entamoeba* species using PCR and enzyme immunoassay. New Microbiol 2008; 31: 393-400.

11- AL-Harthi S A, Jamjoom M B. Diagnosis and differentiation of *Entamoeba* infection in Makkah AL Mukarramah using microscopy and stool antigen detection kit. J Med Sci 2007; 2(1): 15-20.

12- Delialioglu, N, Alsan, G, Sozen, M, *et al*. Detection of *Entamoeba histolytica* / *Entamoeba dispar* in stools specimen by using enzyme-linked immunosorbent assay. Mem Inst Oswaldo Cruz 2004; 99(7): 769-772.

13- Braga L L, Gomes M L, Silva M W, *et al*. *Entamoeba histolytica* and *Entamoeba dispar* infection as detected by monoclonal antibody in an urban slum in Fortaleza, Northeastern Brazil. Rev oc Bra Med Trop 2001; 34: 467-471.

14- Mohammadi S S, Rezaian M, Babaei Z, *et al.* Comparison of stool antigen detection kit and PCR for diagnosis of *Entamoeba histolytica* and *Entamoeba dispar* infection in asymptomatic cyst passer in Iran. J Clin Microbiol 2006; 44(6): 2258-2261.

15- Samie A, Obi L C, Bessong P O, *et al*. Prevalence and species distribution of *Entamoeba histolytica* and *Entamoeba dispar* in the Venda region, Limpopo, South Africa. Am J Trop Hyg 2006; 75 (3): 565-571.

16- Klein S L. The effects of hormones on sex differences in infection: from genes to behavior. Neurosci Biobehav Rev 2000; 24: 627-638.

17- Ayeh-Kumi P F, *et al*. *Entamoeba histolytica*: genetic diversity of clinical isolates from Bangladesh as demonstrated by polymorphisms in the serine-rich gene. Exp Parasitol 2001; 99, 80-88.

18- Weinberg E. D.: The role of iron in protozoan and fungal infectious diseases. J. Eukaryot. Microbiol. 1999; 46(3):231-8. doi:10.1111/j.1550-7408.1999.tb05119.

19- Büyükgebiz B., Öztürk Y., Yılmaz S., Arslan N.: Serum leptin concentrations in children with mild-to-moderate protein-energy malnutrition. Pediatr Int. 2003; 45 (5):550-4. doi:10.1046/j.1442-200X.2003.01787.

20- Farooqi I.S., Wangensteen T., Collins S., Kimber W., Matarese G., Keogh J.M. et al.: Clinical and Molecular Genetic Spectrum of Congenital Deficiency of the Leptin Receptor. N. Engl. J. Med. 2007; 356(3):237–47. doi: 10.1056/NEJMoa063988.

21- Biesiada G., Czepiel J., Ptak-Belowska A., Targosz A., Krzysiek-Maczka G. ,Strzalka M. et al.: Expression and release of leptin and proinflammatory cytokines in patients with ulcerative colitis and infectious diarrhea. J. physiol. Pharmacol. 2012; 63(5): 471-81.

22- Lo˜hmus M., Moalem S., Björklund M.: Leptin, a tool of parasites? Biol. Lett. 2012; 8(5):849-52. doi: 10.1098/rsbl.2012.0385.

23- Shaker M. J., Hussein R. A.: STUDY OF LEPTIN CONCENTRATIONS IN CHILDREN INFECTED WITH ENTAMOEBA HISTOLYTICA\DISPARE AND GIARDIA LAMBLIA PARASITES., Mintage J. Pharm. Med. Sci. 2015; 4(2):9-11.

24- Lord G.M., Matarese G., Howard J.K., Baker R.J., Bloom S.R., Lechler R.I.: Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. Nature.1998; 394(6696):897–901. doi: 10.1038/29795.

25- Tilg H., Moschen A.R.: Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat. Rev. Immunol. 2006; 6(10):772–83. doi: 10.1038/nri1937

26- Karul A., Ertabaklar H., Karataş E., Ertuğ S.: Serum Leptin Concentrations in Patients with Intestinal Parasites. Türkiye. Parazitol. Derg. 2009; 33 (3): 207 – 211

27- Becker S.M., Cho K.N., Guo X., Fendig K., Oosman M.N., Whitehead R., et al.: Epithelial cell apoptosis facilitates Entamoeba histolytica infection in the gut. Am. J. Pathol. 2010; 176(3):1316–22. doi:10.2353/ajpath.2010.090740

28- Guo X., Roberts M.R., Becker S.M. , Podd B., Zhang Y., Chua S.C. Jr, Myers M. G. Jr, et al.: Leptin signaling in intestinal epithelium mediates resistance to enteric infection by Entamoeba histolytica. Mucosal Immunol. 2011; 4(3):294-303. doi:10.1038/mi.2010.76.